# Natural history and population ecology of a rare pierid butterfly, *Euchloe ausonides insulanus* Guppy and Shepard (Pieridae)

Amy Michelle Lambert

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# Abstract

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Amy Michelle Lambert

Chairperson of the Supervisory Committee:
Kern Ewing
School of Forest Resources

The island marble butterfly, *Euchloe ausonides insulanus* Guppy and Shepard 2001(Pieridae) is one of the most restricted butterfly endemics in the continental United States. While much research has been devoted to understanding the species level biology of the large marble, *Euchloe ausonides* Lucas 1852 (Pieridae), relatively little is known about the biology of the subspecies, *E. ausonides insulanus*. This thesis focuses on the biology, natural history and population ecology of *E. ausonides insulanus*. Conservation and management issues related to the biology and population ecology of *E. ausonides insulanus* are discussed in the context of my research findings.

Chapter 1 summarizes the first comprehensive field study of the biology, morphology and behavior of each immature stage (egg, larva and pupa) of *Euchloe ausonides insulanus*. There are many morphological and behavioral similarities between the species (*E. ausonides*) and subspecies (*E. ausonides insulanus*) however, this study revealed several key differences. The most distinct morphological difference between species and subspecies is the coloration and pattern of stripes of larval stages instars III and IV. The white spiracular stripe subtended by yellow-green subspiracular stripe and green-yellow ventral areas are notably different from stripe coloration and pattern described for *E. ausonides*. In addition, the species is known to pupate directly on the host plant whereas *E. ausonides insulanus* larvae will wander up to 4 meters in search of a pupation site. Knowledge of the “wandering” behavior of *E. ausonides insulanus* provides managers with information to design conservation buffers for overwintering pupae.

In Chapter 2, I investigate the relationship between egg-laying patterns and host plants *Brassica rapa* L. var. *rapa, Sisymbrium altissimum* L., and *Lepidium virginicum var. menziesii* (DC) Hitchc. I have two overall research objectives; 1) I explore how adult biology may influence egg-laying patterns using descriptive studies that focus on adult phenology, mating behavior, egg phenology and egg dispersion and 2) I further explore egg-laying patterns related to host plant traits, density and patch size. My results indicate that females prefer to oviposit on plants that are taller and have a greater number of racemes among all three host plant species. In *B. rapa*, the presence of eggs was also highly dependent on plant phenology. In the host plant density study, egg loads were highest in areas where host plants (*B. rapa* and *S. altissimum*) occurred at low densities. The host plant patch study showed that medium size, moderately dense patches received the highest number of eggs per square meter and that dense (>1 plant/m$^2$) host plant patches received the lowest numbers of eggs per square meter. Understanding the relationship of oviposition site selection to host plant traits and host plant density is important because if *E. ausonides insulanus* preferentially lays eggs on plants of particular size or arrangement (e.g., large plants on the edges of dense host plant patches) then larvae may be limited to such plants. This study aims to help researchers predict the occurrence of eggs and larvae among host plants and host plant patches and design host plant habitat that maximizes oviposition site selection by *E. ausonides insulanus*.

In Chapter 3 I quantify larval survival and mortality that may contribute to the rarity of *E. ausonides insulanus*. This study is the first to provide insights into the key role of immature stages in the demography of *E. ausonides insulanus*. The objectives of this study were to 1) assess whether survivorship differed among the three host plant species (one native and two non-native host plant species), 2) assess which factors cause mortality (e.g., predation and deer herbivory) of immature stages and 3) determine which immature stages (egg, instars I-V larval stages) are most vulnerable to different sources of mortality. My results indicate that high egg mortality on host plants of *B. rapa* and *S. altissimum* was mainly attributed to predation and deer herbivory. Predation was the greatest source of egg and larval mortality throughout the four year study period; 47% of all eggs tracked. Predation by spiders was observed most often although social paper wasps (Family Vespidae, *Polistes* spp.) were also observed to predate on larvae. Deer herbivory reduced *E. ausonides insulanus* abundance by indirectly reducing availability of oviposition sites and by direct consumption of eggs and larvae. Over the course of four years of study 26% of all eggs tracked were eaten by deer. This study also showed that the only known native host plant, *L. virginicum* var. *menziesii* supported the highest percent survivorship from the egg stage to larval instar IV but that *L. virginicum* var. *menziesii* habitat was susceptible to offshore storms and tidal flooding that likely contributed to an observed local population extinction of *E. ausonides insulanus* from one research site over the course of the four year study.

In Chapter 4, I explore the use of an alternative native host plant in an effort to enhance prairie remnants to support rare butterfly populations. *Turritis glabra* L., tower mustard, a potential native host plant, was selected for research. This study experimentally tested restoration treatments to foster establishment of *T. glabra* in introduced grasslands, compared plant traits of *T. glabra* and *B. rapa* as they related to *E. ausonides insulanus* oviposition site selection (based on research described in Chapter 2), tested whether *E. ausonides insulanus* would oviposit on *T. glabra* and tested whether *T. glabra* could support egg and larvae development. The findings indicate that *T. glabra* may be a good candidate for native host plant introduction. However, more study is warranted to confirm whether *T. glabra* can support the development of larvae under field conditions. The comparative study indicated that *T. glabra* was significantly shorter in height that *B. rapa* and may have contributed to the low number of eggs observed on *T. glabra* in areas of *B. rapa*. I also found that the establishment of *T. glabra* requires disturbance and seed input and that the exclusion of deer may be necessary to the long-term establishment of *T. glabra*.

Finally, in Chapter 5, I discuss key ecological issues related to the conservation and management of *E. ausonides insulanus* including potential impacts of climate change, host plant patches dynamics, disturbance, topographic and habitat heterogeneity and significant mortality factors that likely contribute to overall population abundance. Numerous processes can lead to extinction and many of the processes discussed in this thesis (e.g., disturbance, host plant availability etc.) can operate at different temporal and spatial scales. My findings show that a combination of factors likely influence overall low population numbers and local population extinctions related to patch dynamics in *E. ausonides insulanus*. Thus, managers should consider multiple management strategies to maintain and increase abundance of *E. ausonides insulanus* at American Camp including further experimental research to better understand the ecological mechanisms that contribute to overall population abundance.

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# Chapter 1

# Biology of the Immature Stages of *Euchloe ausonides insulanus* Guppy and Shepard (Lepidoptera; Pieridae) on San Juan Island, Washington

## INTRODUCTION

The island marble butterfly, *Euchloe ausonides insulanus* Guppy and Shepard 2001(Pieridae) is distinct from populations of the large marble, *Euchloe ausonides* (Pyle 2002) and is one of the most restricted endemic butterflies in the continental United States (Pyle 2006). It also has a remarkable history in that it is one of the few species in the world that was rediscovered long after it was thought to be extinct. Since it's rediscovery in 1998 (Fleckenstein and Potter 1999), the biology of *E. ausonides insulanus* has been of interest to agencies working to conserve and protect this rare species. The island marble is currently a candidate for state listing (WDFW 2010) and is a conservation priority for US Fish and Wildlife Service and the National Park Service (Pyle 2006).

The biology of immature stages of *E. ausonides insulanus* is as equally fascinating as the butterfly's rediscovery. Guppy and Shepard (2001) summarized the natural history of *E. ausonides insulanus*; however, no detailed field research of the biology of immature stages of *E. ausonides insulanus* has been conducted. This paper summarizes the first comprehensive field study of the biology, morphology and behavior of each immature stage (egg, larva and pupa) of *E. ausonides insulanus*.

Numerous researchers have described aspects of the species level biology of the large marble, *Euchloe ausonides* Lucas 1852 (Pieridae) (Coolidge and Newcomer 1908, Tietz 1972, Scott 1973, Opler 1974, Scott 1974, 1975, Shapiro 1981c, 1984, Courtney 1986, Bridges 1988). In comparison, relatively little is known about the biology of the subspecies, *Euchloe ausonides insulanus*. Opler (1974) studied the life history and morphology of immature stages of *E. ausonides* in great detail. One can assume that there are similarities in life history between the species (*E. ausonides*) and subspecies (*E. ausonides insulanus*). However, because *E. ausonides insulanus* populations are insular, physically separated from populations of *E. ausonides* by oceanic and continental geography, behavioral and phenotypic differences are likely to occur. This study documents observations of immature stages of *E. ausonides insulanus* during four years of field study from 2005 to 2008. Research documents (i) biology and morphology of eggs and larvae, (ii) development time of egg, larvae and pupae and (iii) larval ecology and behavior of *E. ausonides insulanus*.

## History and distribution

*Euchloe ausonides insulanus* was historically known as an unnamed subspecies of *Euchloe ausonides* until the subspecies was formally described by Guppy and Shepard in 2001. Based on location records of fourteen specimens collected between 1861 and 1908, *E. ausonides insulanus* ranged from the southeastern lowland of Vancouver Island along Georgia Strait, to Nanaimo, and on Gabriola Island, British Columbia, Canada (Shepard 2000). *Euchloe ausonides insulanus* was presumed extinct (extirpated from historically known locations) until it was rediscovered at American Camp, San Juan Island National Historic Park, San Juan Island, Washington, United States in 1998 (Fleckenstein and Potter 1999). Surveys for additional populations of *E. ausonides insulanus* have been ongoing since 2002 (Miskelly 2004, Hanson et al. 2009). An extensive survey for *E. ausonides insulanus* on nearby islands and coastal areas in northern Washington was conducted by Washington Department of Fish and Wildlife in 2005 (Miskelly and Potter 2005). During this time, *E. ausonides insulanus* was observed in small numbers on Lopez Island but no additional populations outside of San Juan and Lopez Islands were found (Miskelly and Potter 2005). Since 2005, exploratory surveys have focused on the San Juan Islands and additional populations of *E. ausonides insulanus* on San Juan and Lopez Islands have been documented (Miskelly and Fleckenstein 2007, Hanson et al. 2009, Miskelly and Potter 2009). However, populations of *E. ausonides insulanus* had not been observed outside of San Juan and Lopez Islands, suggesting that the subspecies has a very narrow distribution (Miskelly and Potter 2005, Miskelly and Fleckenstein 2007, Hanson et. al. 2009, Miskelly and Potter 2009).

## Study site

This study was conducted at American Camp, San Juan Island National Historical Park on the southern end of San Juan Island, Washington, USA. *Brassica rapa* L. var. *rapa*, *Sisymbrium altissimum* L., and *Lepidium virginicum var. menziesii* (DC) Hitchc., are the sole known host plants for *E. ausonides insulanus*. Host plants are defined as those plants that support development of larvae through the final instar under field conditions. *Brassica rapa* and *S. altissimum* are introduced host plant species. *Lepidium virginicum var. menziesii* is a native plant species. Grasslands, sand dunes and tidal lagoon/shoreline support habitat unique to populations of *E. ausonides insulanus* and each plant community includes specific host plants and pupation sites (Table 1.1). Generally, host plant species do not overlap in distribution and therefore limit the development of immature stages of *E. ausonides insulanus* to one host plant species.

## METHODS

### Biology and morphology of eggs and larvae

The biology and morphology of eggs and larvae were studied at eight sites at American Camp (Appendix A). Eggs and larvae were examined at four sites containing *B. rapa*, three sites having *S. altissimum* and one site containing *L. virginicum* var. *menziesii*. Observational data were collected over the course of four years from 2005-2008, but not all study sites were surveyed every year (Appendix A).

To observe the life history sequence and larval behavior of *E. ausonides insulanus*, host plants were searched for eggs and all eggs found were tracked through egg and larval development. Plants occupied by eggs were marked with flagging tape. Color coded wire was placed at the base of branching stems and ink markings on buds and flowers were used to locate eggs and larvae as they developed. The position of eggs and larvae on each host plant was recorded making it possible to track life stages and the length of time of development between stages. For each host plant, the cohorts of eggs and larvae were surveyed at the same time, as development proceeded from egg to late-instar larva (i.e., instar V), or until all larvae disappeared or were observed dead. Eggs were inspected at five-day intervals. Early-instar larvae (I-III) were inspected at three-day intervals and late-instar larvae (IV-V) were inspected several times a day. Inspection date varied because larva became increasingly mobile as they matured. Morphological characteristics of eggs and larvae were recorded (i.e., color and arrangement of stripes) using a hand lens and photo-documentation. The general characters of setae (hairs) and pinaculae (flat hardened plates on the surface of the skin from which hair grows) were described for the purpose of identifying larval stages under field conditions for future research by conservation managers. Diagnostic drawings of setae and pinaculae (or chalazae) for all larval stages (instars I-V) of *E. ausonides* (Opler 1974) were used as baseline for field observations.

Observations of larval behaviors such as molting, feeding and “wandering” to pupation sites were documented. Fifth-instar larvae were monitored on their host plants and attempts were made to follow larvae that left their food plant in search of a pupation site. Detailed accounts of “wandering” and behaviors leading to the formation of the pupa (e.g., ‘back-bending’) were recorded (n = 3). Once larvae selected a pupation site, their location was flagged to allow relocation.

### Development time of eggs and larvae and larval size (instars I – V)

Development time of eggs and larvae (instars I-V) were studied at four sites at American Camp (Slope South of Redoubt, Old Town Lagoon Grassland, Dunes and South Beach, Jakle's Lagoon; Appendix A) in 2005 and 2006. The size of larvae at each developmental stage was measured in 2005. Eggs and larvae were examined at two sites containing *B. rapa*, one site having *S. altissimum* and one site containing *L. virginicum* var. *menziesii*. Methods for tracking eggs and larvae were the same as described above except in this study only newly deposited greenish-white eggs (deposited by females < 48 hours before) were tracked in the egg stage.

The mean number of days for each stage (eggs, instars I-V) was calculated from the day the first stage was observed to the day the next stage was observed. Stages include egg to instar I (egg stage), instar I to instar II (instar I), instar II to instar III (instar II), instar III to instar IV (instar III), instar IV to instar V (instar IV) and instar V to prepupal larva (instar V) (see Table 1.2 for sample size of each stage). Sample sizes differed between stages because survival from one stage to the next varied. Only those individuals that were observed from the beginning of each stage and subsequently followed to the next stage of development were included in the analysis.

In most cases, the intervals in which surveys were conducted for eggs and early instar larvae (instars I-II) did not capture the exact time of transition from one stage to the next. For example, eggs were inspected at five-day intervals and two consecutive surveys did not always match the actual time needed for eggs to complete development. A standard procedure was used to account for the discrepancy between survival intervals and actual development time. The mean number of days was calculated taking the average of the shortest and the longest intervals (numbers of days) between surveys. For example, when tracking one individual, if instar I was observed on surveys dates 5/13 and 5/16 (4 day interval) and instar II on survey date 5/19 (6 day interval from first survey, 7th day not counted because larva was observed as instar II) development time from instar I to instar II averaged 5 days:

> 4 days (total number of days= survey 1 + survey 2) + 6 days (total number of days= survey 1+ survey 2 + survey 3 - 1 day) / 2 = 5 days

In rare cases where larva developed faster than the survey timeframe (i.e., larva molted twice between survey dates), the mean number of days between two instars was calculated.

Size is another diagnostic characteristic used to distinguish among larval instars. The length of larva (mm) was measured using a centimeter ruler (see Table 1.3 for sample sizes). Larvae were not handled or manipulated while taking measurements and efforts were taken to prevent contact or disruption of larval movement and feeding activities.

To test if there was a difference between development time at the egg stage and larval instars I-V among years (2005 and 2006), t-tests were conducted. Test assumptions such as independence, normality and equal variance were met prior to the test.

### Searching behavior of late-instar larvae

To examine searching behavior used in locating secondary food plants (subsequent to defoliating the original host plant or becoming physically dislodged from the original host plant), a controlled experiment testing search behavior of late-instar larvae (IV-V instar) was conducted at American Camp from June 15 – 19, 2005. Samples of host plants in the same phenophase and with equal raceme size were collected from *B. rapa*, *S. altissimum* and *L. virginicum* var. *menzeisii*. Three samples (one of each host plant species) were randomly placed in fifteen 5-quart disposable paint buckets. Plants were placed upright and secured in holes at the bottom of buckets. Shallow aluminum pans filled with water were placed below each bucket. The stem of each host plant was placed at equal distance at the bottom of each bucket. To control for larval movement in the direction of upright objects other than plant stems, a rock approximately 3cm in diameter was placed at the bottom of each bucket. To observe host locating behavior in the host plant canopy, plant inflorescences were intertwined above the rim of the bucket to allow movement between plant species.

Late-instar larvae were salvaged from a restoration site scheduled to be burned for purposes of invasive species management and relocated to a safe host plant habitat after the experiment. Larvae were removed from plants of *S. altissimum* and immediately transferred to experimental buckets. Larvae were released at the center of each bucket on June 15 at 7:45am. Initial contact with host plants and/or rock was recorded. To examine cues larvae might use for locating host plants, larval behavior (i.e. feeding, resting and searching) was noted for all surveys including surveys conducted in the first 5, 30, 120 minutes and 30 hours. A total of 16 surveys were conducted over the course of 5 days (n = 240 observations).

To test if the number of larvae to contact the nearest vertical object after release at the center of the bucket was independent of host plant species chi-square tests were performed for the time intervals (5, 30, 120 minutes and 30 hours).

## RESULTS AND DISCUSSION

To provide an overall account of the biology of immature stages of *E. ausonides insulanus*, a comprehensive description of morphology and behavior at each stage is presented in order of the butterfly’s life history (egg, larval instars I-V and pupa).  Life history stages are grouped according to shared characteristics in biology and morphology. Descriptive groups include: eggs and instars I-II, instars III and IV, instar V and wandering phase, selecting a pupation site and pupa.

The earliest stages of the life cycle, eggs and instars I – II larvae almost exclusively fed on the inflorescence (buds and flowers) of the host plant and are especially vulnerable to predation and desiccation. Larval instars III and IV displayed similar trends in larval morphology (e.g., grey-green stripe located in the dorsal area) and fed on inflorescences, stems and leaves of the host plant. Larvae were relatively mobile in instar III and IV stages and occasionally moved to adjacent food plants in search of food. Larvae that survived to instar V fed on fruits in addition to inflorescences, leaves and stems and were easily discernable because of their large size and coloration of stripes (e.g., white spiracular stripe is well-defined). In the later stages of development, instar V larvae changed color and size and “wandered” from the food plant to select a suitable pupation site. Prior to pupation, larvae attached themselves to the pupation site with silk in preparation for the final molt. Pupae were pale brown, long and slender and tapered to a point.

The development time of *E. ausonides insulanus* from egg to pupa was 38 days. Development time varied among stages. Eggs took the longest amount of time to develop (10.75 ± 2.18 days) and instar II larvae developed in the shortest amount of time (4.67 ± 2.05 days). There was no difference in mean development time at each stage between years for instars I, III, IV (*t* test, $t = 1.91$, $P = 0.234$; *t* test, $t = 0.71$, $P = 0.477$; *t* test, $t = 1.57$, $P = 0.122$, respectively) (Table 1.2). However, a statistical difference was detected at the egg stage and instar II stage (*t* test, $t = -3.91$, $P = 0.0001$; *t* test, $t = -3.62$, $P = 0.0003$, respectively); egg and instar II stages developed more rapidly in 2005. The difference may be attributed to variation in temperature between 2005 and 2006, especially during the month of May during early stages of egg and larval development. For example, in 2005 the mean temperature was 13.3°C compared to 11.7°C in 2006 (Friday Harbor Airport, Federal Aviation Administration). The effect of temperature on the growth and foraging patterns of larvae has been well documented in Lepidoptera (Scriber and Slansky 1981, Taylor 1981, Stamp and Bowers 1992, Casey 1993). For example, Courtney (1986) found that *Pieris rapae crucivora* larvae grew more quickly in full sunshine compared to *Pieris napi nesis* or *Pieris melete* located on food plants growing in partial shade. Seasonal variation in temperature is also likely to have contributed to variation in larval growth in *E. ausonides insulanus*.

### Eggs and early stages of larval development (instars I and II)

Eggs of *E. ausonides insulanus* were observed to be columnar in shape and red in color (Figure 1.1.A) as with other inflorescence-feeding *Euchloe* species in North America (Shapiro 1981c, Scott 1992). Eggs were approximately 1 mm in height and had approximately 15 vertical ridges that adjoined at the top of the egg (Figure 1.1.A). When the egg was initially oviposited by the female the color of the egg was greenish-white (Figure 1.1.B). The greenish-white color of the egg developed an orange tinge in 24 – 48 hours

(Figure 1.1.C). Mean duration (in days) of egg development was 10.75 ± 2.18 (mean ± SD, n = 120; range: 6 – 15.5) (Table 1.2). The egg continued to change color as it developed. The egg changed color from bright orange to deep red to brown (Figure 1.1.A – 1.1.C and Figure 1.2.A). In the final stages of development, prior to hatching, there was a distinct black band at the distal end of the egg (Figure 1.3.A). The black coloration was the head of the soon to be emerging larva. Coloration changes documented in this study were consistent with other species of *Euchloe* (Shull 1907, Coolidge and Newcomer 1908, Opler 1974, Clench and Opler 1983, Opler and Krizek 1984).

Occasionally, plants of *B. rapa* produced single orange buds (located among inflorescences of tightly clustered green buds) which were similar in size, shape and color to *E. ausonides insulanus* eggs (Figure 1.1.D). Buds of *L. virginicum var. menziesii* were also similar to the morphology of *E. ausonides insulanus* eggs (Figure 1.2.A). Host plants may mimic egg morphology to deter females from laying eggs which may lead to lower rates of caterpillar herbivory. Similar observations of host plant mimicry have been observed in other *Euchloe* species. For example, Shapiro (1981c) observed callosities on the leaves of *Streptanthus glandulosus,* Tamalpais jewel flower, to look similar to eggs of *Pieris sisymbrii*, California white, butterfly. In this species, females typically lay one egg per plant and subsequent females (con or heterospecific) avoid laying eggs on a plant that is already occupied (i.e., “egg-load assessment”), a phenomenon also observed in *E. ausonides insulanus*. In a subsequent study, Shapiro (1981a) experimentally removed callosities from *S. glandulosus* and found that females were more likely to oviposit on plants without callosities and that *P. sisymbrii* larvae completely consumed the host plant. Based on the results of the study, Shapiro hypothesized that mimicry may be a mechanism to reduce defoliation and increase plant fitness (Shapiro 1981c). Similarly, plant mimicry may play a role in reducing *E. ausonides insulanus* herbivory of host plants, especially isolated plants of *L. virginicum var. menziesii* that were observed to be easily defoliated by late-instar larvae. Other species of *Euchloe* have also been shown to have significant defoliation impacts on native endemic host plants. For example, *Euchloe hyantis*, California marble butterfly, was observed to consume all plant parts leading to complete defoliation of *Streptanthus tortuosus,* shieldplant (Shapiro 1981b, Karban and Courtney 1987).

### *Location of oviposition sites*

The placement or location of eggs varied on host plants. Females preferentially deposited eggs on terminal flower buds (79.6%, n = 1048). The placement of eggs on or near inflorescences was consistent with other inflorescent-feeding *Euchloe* species (as opposed to leaf-eating species). For example, *E. ausonides, Euchloe olympia*, Olympia marble, and *Euchloe creusa*, northern marble, butterflies are also known to deposit eggs on terminal buds as well as feed on buds and flowers (Shull 1907, Coolidge and Newcomer 1908, Meiners 1938, Remington 1952, Opler 1974, Shapiro 1981c). Although eggs of *E. ausonides insulanus* were observed most often on terminal flower buds, eggs were also deposited on the pedicle of flower buds (11.8%, n = 156), axillary buds (6.2%, n = 81), leaves (1.5%, n = 20), and occasionally on stems (0.9%, n = 12) of host plants.

There is very little discussion in the literature to address why females oviposit on different parts of the host plant. For example, a significant percentage of *E. ausonides insulanus* eggs were observed not on terminal flower buds but on the pedicles of flower buds. It is interesting that Shull (1907) also observed eggs of *E. olympia* on the pedicle. It may be that females unintentionally deposit eggs on the pedicle because they mistaken the pedicle for a flower bud. At early stages in host plant development females may not be able to distinguish between the base of buds and pedicles and inadvertently oviposit eggs on pedicles (as opposed to buds). The pedicle is a small stem-like attachment that secures each flower bud to the base of the inflorescence (Figure 1.2.C). The pedicle increases in length as the host plant develops. When the host plant is young each inflorescence is composed of a tight cluster of buds and pedicles are very short. Females were observed to oviposit eggs between several buds on a single inflorescence and may preferentially select racemes with multiple buds. The occurrence of eggs laid on pedicles of *B. rapa* and *S. altissimum* was especially high on plants with inflorescences composed of 10 or more buds. Alternatively, the oviposition of eggs on pedicles of host plants, rather than on buds, may have prevented some egg mortality. If flower sepals and petals of the host plant matured faster than eggs hatched, eggs were susceptible to being shed with flower parts as the host plant matured (Figure 1.4.B). Unlike flower petals, the structure of pedicles remained constant as host plants matured providing the necessary foundation for egg development. On the other hand, larvae that emerged from eggs deposited on pedicles of fruits were more likely to starve in the absence of edible plant tissue (i.e., buds and flowers) (Figure 1.2.C).

Females oviposited less frequently on axillary flower buds perhaps because they were more inconspicuous, emerged later in the season and developed faster than eggs and larvae matured. Growth of axillary flower buds was responsive to both deer herbivory and increased precipitation. Axillary flower buds emerged as a result of removal of the terminal raceme usually caused by deer herbivory and through the stimulation of new growth by precipitation late in the growing season. In *B. rapa* severe deer herbivory produced additional side-branches and new buds on axillary racemes (Figure 1.4.A). The new flush of growth increased the number of oviposition sites (i.e., clusters of flower buds or inflorescences) available to gravid females. However, removal of the terminal raceme also increased the rate at which buds, flowers and fruits grew, thus, shortening the amount of time eggs and larvae had to feed and develop (e.g., eggs are susceptible to falling from the host plant if flower petals senescence faster than eggs hatch; Figure 1.4.B).

In circumstances where precipitation occurred late in the season and stimulated new growth on mature host plants, new flower buds emerged from axillary racemes that otherwise had only mature fruits (Figure 1.4.C).  In many cases, axillary flower buds had fewer buds on each raceme (< 5 in a cluster) compared to terminal inflorescences (10 − 25 buds). For these reasons, early instar larvae from eggs laid on axillary flower buds were more likely to desiccate because they were unable to penetrate mature fruits after consuming the few palatable buds that were available.

Eggs were occasionally found on leaves and stems of *B. rapa* and *S. altissimum* (Figure 1.2.B). The oviposition of eggs on parts of the host plant other than inflorescences was rare but has also been observed in several related species. Coolidge (1925) reported that eggs of *Euchloe (hyantis) lotta*, desert marble butterfly, were occasionally found on leaves and stems. Opler (1974) also observed eggs of *E. hyantis* on the lower surface of clasping leaves of *Streptanthus polygaloides*, milkwort jewelflower. In another study, Courtney and Courtney (1982) reported that individual plants of *Hesperis matronalis*, Dame’s violet, located on the periphery of host plant patches received disproportionately high numbers of *Anthocharis cardamines*, orange tip butterfly eggs, many of which were deposited on lower stems and leaves. Courtney (1986) suggested that females may use less favorable sites for oviposition (i.e., leaves and stems) when the density of conspecific eggs are high on a single host plant. Although this trend was not observed in *E. ausonides insulanus* (more than 50% of plants with eggs on stems and leaves only had 1- 2 eggs), females were observed to alter the location of oviposition based on a combination of factors including host plant condition, oviposition timing, fecundity (i.e., egg complement in the abdomen) and searching behavior (Lambert unpub. data). On several occasions eggs were observed on leaves and stems of plants located in close proximity to one another which suggests that females may oviposit on stems or leaves more than once. Based on these observations it is possible that *E. ausonides insulanus* site selection (inflorescence verses leaves) may be a learned behavior. Papaj (1986) found that females of *Battus philenor,* pipevine swallowtail, learned to alight on the leaves of host plant species based on the leaf shape of host plants in which young females were exposed. It may be that young females of *E. ausonides insulanus* naively oviposited eggs on leaves and stems while they were learning to discriminate between different parts of the host plant.

### *Hatching from egg to instar I*

Eggs hatched mid-to-late May when terminal buds on host plants began to flower. During this time, all three species of host plants overlapped in flowering phenology (Table 1.1), adults were active, and larval resources were abundant. Upon emerging from the egg, many larvae were observed to feed on the egg shell (Figure 1.3.B). However, 31% (n = 873) of newly emerged larvae did not immediately feed on the egg shell (Figure 1.5.A) and instead were observed feeding on buds and flowers several centimeters from the empty egg case (Figure 1.2.C; Figure 1.3.C). This pattern suggests that consuming the egg shell may not be a requirement of larval development but instead may be a facultative response to nutritional deprivation (e.g., in the absence of buds and flowers). The larvae of many species of Lepidoptera are known to feed on the egg shell shortly after emerging from the egg case. This behavior is not surprising considering that eggs shells are thought to be comprised almost entirely of protein. For example, more than 95% of the weight of the egg shell of silkmoths, *Bombyx mori* and *Antheraea spp.* consist of chorion proteins (Kawasaki et al. 1971, Kafatos et al. 1977). Proteins are essential for larval growth and substantial protein reserves carry over from larval feeding to support adult reproduction (Telfer and Kunkel 1991, Telang et al. 2002).

In addition to nutritional benefits, egg cannibalism is thought to be a secondary explanation for egg shell consumption, although egg cannibalism was not observed in *E. ausonides insulanus* over the four year study period. Courtney (1986) suggests that for some pierid species (e.g., Yamamoto 1981) eating the egg shell may be an ancestral behavior linked to egg cannibalism and that egg cannibalism may be an infrequent and accidental occurrence. Alternatively, Brower (1961) hypothesized that egg cannibalism may be an ancestral trait in many butterfly species.

Egg cannibalism has been reported for several pierids (Ford 1945, Jones and Ives 1979, Yamamoto 1981, Courtney and Duggan 1983, Courtney 1986) and is thought to be common in *Euchloe* species in the inflorescence guild (Courtney 1986). For this reason, it is unusual that egg cannibalism was not observed in *E. ausonides insulanus*. The lack of egg cannibalism may be due to the distribution of eggs over a large number of robust host plants and/or lack of competition with other pierids. Additionally, the low number of eggs oviposited on host plants reduces competition with conspecifics and may reduce the occurrence of cannibalism (Courtney and Shapiro 1986, Porter 1992). Interestingly, in studies by Shull (1907) and Coolidge and Newcomer (1908) *E. ausonides* and *E. Olympia* were found not to consume the egg shell but were observed to eat other eggs and larvae when larvae were in captivity.

Shortly after larvae emerged from their egg shell they sought plant tissue that could be easily consumed (buds and flowers) or they risked desiccation. For this reason, first instar larvae were rarely observed to leave the inflorescence (bud and flower cluster) where they hatched from the egg. However, larvae that hatched from eggs that were laid on tough vascular portions of leaves and stems (or pedicles) were observed to move in order to find edible plant material (Figure 1.3.C). Larvae at this early stage were only able to travel a few centimeters from where they emerged.  Desiccated early instar larvae were frequently observed on stems, leaves or seedpods where host plants were in an advanced senescent stage (i.e., no young plant material available) or early instar larvae were unable to reach young plant material (e.g., buds and flowers).

Early instar larvae depend on edible plant material upon emergence. *Euchloe ausonides insulanus* larvae were observed to chew a small hole or "pinhole" in the surface of plant material at the onset of feeding on buds and flowers (Figure 1.5.A and 1.5.B). "Pinholes" were observed prior to finding larvae hidden in bud and flower clusters and may be used as an indicator of the presence of newly emerged larvae. Larvae may create "pinholes" in buds to access highly nutritious plant material such as developing ovaries and stamens or seeking protection at a time when they are especially vulnerable. Early instar larvae of other species of *Euchloe* also depend on edible plant material upon emergence and will search to find suitable plant material. For example, larva of *E. hyantis* were observed to chew holes through the leaves of *Steptanthus polygaloides* to find the edible flowering portions of the plant without feeding on tough leaves (Opler 1974). Similarly, Sherman and Watt (1973) and Hayes (1980) found that other species of pierid butterflies (i.e., *Colias* sp.), including *Colias alexandra,* Queen Alexandra's sulphur butterfly, chew "pinholes" in the surface of leaves.

Larvae feeding on flower buds were relatively cryptic and hidden (Figure 1.5). Many first instar larvae were observed in flowers or underneath clusters of buds. The coloration of the first instar was golden-yellow with a distinct black head (Figure 1.5.A). The head remained black until the second molt. This trait is characteristic of the genus (Opler 1974). The first instar grew to a mean length of 1.97 ± 0.80 mm (n = 238) (Table 1.3; Figure 1.5.B). Mean development time in the field was 5.66 ± 1.96 (n = 275; range: 2–13) days from hatching to first molt (Table 1.2). First instar larvae turned dark grey-brown and hirsute prior to molting (Figure 1.5.C).

### Instar II

Second instar larvae grew to a length of 4.96 ± 1.25 mm within 4.67 ± 2.05 (n = 186; range 2 – 11) days (Table 1.2 and 1.3; Figure 1.6.C). They had a golden-yellowish-green body with a black head (Figure 1.6). Primary setae (hairs) and pinaculae (flat hardened plates on the surface of the skin from which hair grows) became more visible in the second instar (Figure 1.6.A).

Second instar larvae had a textural look and were more variable in color compared to first instar larvae (Figure 1.6). Larvae primarily fed on buds and flowers at early stages in development. In circumstances where buds and flowers were unavailable, larvae attempted to feed on developing fruits (Figure 1.2.C; Figure 1.6.B and 1.6.C). Movement in the first two instars was minimal (1 −2cm). In many cases, larvae only moved among buds and flowers on one inflorescence.

The head capsule was shed separately from the skin and sometimes at different times. Asynchronous molting of the second instar made second instars in late stages of development and third instar larvae in early stages of development difficult to discern in the field. Some larvae were observed to have black head capsules characteristic of second instar larvae but stripe patterns on the body suggestive of third instar larvae (Figure 1.7.A). In these cases, the size of the head capsule relative to the size of the body, stripe coloration and larval length was used to distinguish the difference between instars. For example, in Figure 1.7.A the small black head capsule of the second instar larva remained attached to the body although the body capsule had been shed. The disproportionately small head capsule compared to the size of the body indicated that it was third instar larva that had recently molted. In addition, the body length (9 mm) and grey-green and yellow-green stripes on the dorsal and subdorsal areas of the body helped to distinguish third instar larvae from second instar larvae. This is the first study to document asynchronous molting in *Euchloe* species.

### Instars III and IV

In general, third and fourth instar larvae grew rapidly, reaching a length of 8.76 ± 2.38 mm in 4.92 ± 1.97 (n = 121; range 2 – 11) days and 15.96 ± 4 .00 mm in 5.85 ± 2.64 (n = 49; range 3 – 12) days, respectively (Table 1.2 and 1.3). Based on diagnostic characteristics presented by Opler (1974), *E. ausonides insulanus* larvae are similar to *E. ausonides* in coloration, size of primary setae and extent of pinaculae at instars III-IV stages. Setae increased in size and number and pinaculum remained the same proportion to the size of larva throughout larval development. The coloration and pattern of stripes of *E. ausonides insulanus* in the last three instars differed from observations of *E. ausonides* summarized by Opler (1974) based on study by Coolidge and Newcomer (1908). For example, the spiracular stripe of instar III and IV larvae was white in *E. ausonides insulanus* as opposed to yellow described for *E. ausonides* by Opler (1974). The white stripe along the spiracles was also found in instars III and IV in populations of *E. ausonides* in British Columbia (Guppy and Shepard 2001).

Differences in color pattern among species were found in other *Euchloe* species (Opler 1974). Morphological differences between larvae of *E. ausonides* and *E. ausonides insulanus* may be genetically based or attributed to phenotypic traits. For example, the fact that the white spiracular stripe was present in instars III and IV (Guppy and Shepard 2001) in British Columbia populations of *E. ausonides* and absent in California populations described by Opler (1974) may indicate that the presence of the white spiracular stripe may be a phenotypic plastic trait. In another study, phenotypic traits were observed in swallowtail butterflies. For example, pupal color in swallowtails changed depending on the color of pupation site and photoperiod experienced by the larva (Hazel and West 1979, 1983, Stefanescu 2004).

Stripe coloration and arrangement were the same for instars III and IV although the prominence of stripe color varied (Figure 1.7 and 1.8). Larvae were grey-green in dorsal areas and yellow-green in subdorsal areas followed by a thin grey-green supraspiracular stripe. This color pattern was most often observed when examining the larva from above (Figure 1.7.B). Following a grey-green, yellow-green stripe pattern, the area surrounding the spiracles was distinctly white. The white stripe was most prominent when viewing the larva from a side angle (Figure 1.8.A). The white stripe was lesser in the third instar although the color white was discernable in areas that encircled the spiracles (Figure 1.7.C). Following the white stripe, a thin yellow-green subspiracular stripe faded into the color green-yellow in ventral areas (Figure 1.8.B). In contrast to first and second instar larva, third instar larva had a green-brown-black head (Figure 1.7.B). The head capsule turned increasingly dark and smaller in proportion to the body as the larva neared molting. Third instar larvae shed the head capsule separately from the cast of the body (Figure 1.7.D). However, fourth instar larvae shed both the head and molt of the body at the same time. The cast of the head was indistinguishable from the rest of the body. Larva molted from the front of the body and the molted head was lifted upward as the newly emerging fifth instar exited the exoskeleton (Figure 1.10.A).

Movement increased between plant racemes as larvae grew larger and became more mobile. By late third instar, larvae are able to feed on three or four inflorescences and travel to two or three branching racemes. Third instar larvae were observed to feed on buds, flowers and newly developing fruits but rarely leave the original host plant. In contrast, fourth instar larvae moved along stems, between branches (Figure 1.8.B) and even between adjacent host plants in search of food especially if the original host plant had matured and the larva was unable to consume the tougher vascular portions of the plant. The fourth instar was the most active and mobile stage, although larvae tended to stay in the upper reaches of host plants, moving from one plant to another when the top of two or more host plants were in contact. Several individuals were observed to move from one plant to another with assistance by light wind. Wind blew host plants closer together allowing larva to “reach” for branches that came into contact. Grass blades were also used as bridges between adjacent host plants. Depending on the host plant habitat and environmental conditions, larvae may forage over an area as large as one square meter.

Fourth instar larvae fed on developing fruits but also ate buds, flowers, petioles, young stems and leaves of host plants. In 2005, a single fourth instar larva was recorded feeding on a fruit of *L. virginicum* var. *menziesii*. The larva consumed both fruit and pedicel in 19 minutes, stopping only twice; 4 minutes at the point when the fruit was ¾ consumed and 3.5 minutes between consuming the fruit and feeding on the pedicel (Figure 1.9). After feeding, larvae were observed to move toward the stem to begin a resting phase. Larvae assumed a position “stemward” or toward the position of the fruit from the stem along the pedicel (*B. rapa* and *S. altissimum*) (Figure 1.8.A). If larvae were resting on the stem of *L. virginicum* var. *menziesii*, the head was usually oriented upward. This resting position was parallel to the stem of the plant and relatively cryptic and may serve as an avoidance mechanism from visual predators or simply be a good position on the plant to avoid incidental disturbance or dislodgement.

Occasionally larvae became dislodged or fell from the host plant. For example, when heavy winds caused host plants to brush against nearby plants (e.g., *Cirsium arvense*, Canada thistle) late instar larvae were displaced and fell to the ground. Additionally, larvae were observed to crawl off the original host plant in search of a second food plant when the original host plant had been completely defoliated or when plant tissues were too tough to consume (e.g., senescent plant). To examine the searching behavior required for locating a second host plant, a controlled experiment was conducted to test the searching behavior of late-instar larvae. Larvae were released into a bucket that contained racemes of three different host plant species and a non-host plant object (i.e., rock). Larvae proceeded directly from the point of release to the nearest vertical object independent of host plants ($x^2 = 0.333$, df = 3, n = 15, $P = 0.954$) (Table 1.4). For example, larvae initially crawled to the top of the rock and began searching for the nearest object to climb. In two cases, larvae climbed the walls of the bucket.

Not long after larvae were released all inorganic objects were abandoned and host plants were located. The majority of larvae located host plants in less than 30 minutes from the time of release with the exception of 4 larvae that did not move from the point of release on the first day (Table 1.4). The delay in movement was likely caused as a result of molting. The sequence of data in Table 1.4 represents the amount of time larvae spent locating host plants. All 15 larvae located host plants by the second day (30 hours). This study suggests that larvae search for host plants from the ground will crawl up the nearest vertical object regardless of the type of vegetation or structure.

In addition to examining searching behavior, this experiment also tested food plant preference. Significant differences in host plant preference were detected 30 hours from the release of larvae ($x^2=8.4$, $P< 0.015$). Results suggest that larvae preferred *S. altissimum* to *B. rapa* or *L. virginicum* var. *menzeisii*. Larvae were present on *S. altissimum* for more than 50% of the 240 observations. Twenty-six percent of larval observations were recorded on *B. rapa* and 4% on *L. virginicum* var. *menzeisii*. The results however may be confounded because larvae were predisposed to *S. altissimum* prior to the experiment. Plants of *S. altissimum* supported the development of eggs and larvae before larvae were removed and relocated to experimental hosts. Early exposure to secondary compounds may have preempted late instar larvae to prefer their host of origin. Although larvae were observed feeding, resting and searching on all three species of host plants over the course of the experimental study (5 days), larvae were not observed to occupy more than one host plant species in the field. Host plant resources were partitioned by habitat type and larvae do not have the dispersal capabilities of traveling distances greater than a couple meters.

### Instar V and wandering phase

Early fifth instar larvae fed on plant material voraciously often consuming whole fruits, pedicles and stems until the food plant was completely defoliated (Figure 1.11.A). Late in the season, larvae avoided over-mature fruits and senescent plant material and often left the primary host plant in search of more nutritious food plant material. Fifth instar larvae were notorious for disappearing from their food plant when not carefully observed.

The color and pattern of stripes on the fifth instar changed over the course of development and was a good indicator of the stage of early and late instar development. In the earliest stages of development the larvae were grey in dorsal areas, yellow in subdorsal areas followed by a thin light grey supraspiracular stripe. The white spiracular stripe was well-defined and subtended by a thin yellow subspiracular stripe. Ventral areas were green-yellow. The head was greenish-yellow-grey. Pinaculae were black-glossy and setae were sparse and inconspicuous (Figure 1.10.B and 1.10.C).

Upon emerging from the molt, early fifth-instars characteristically had disproportionately large bulbous heads in comparison to the length and width of the body (Figure 1.10.A). Larvae were particularly conspicuous and vulnerable to predators such as spiders and wasps in early stages, especially in the initial molting phase when larvae were immobile and defenseless. Over several days, the size of the body increased as larvae fed. When the head and body become proportionally similar the instar was several days into development (Figure 1.10.C). Fifth-instar larvae were substantially larger than the fourth-instar (Figure 1.10.B); mean length was 25.62 ± 5.25 mm (Table 1.3; Figure 1.10.C). Slight changes in stripe pattern and coloration occurred in late stages of fifth-instar development. Stripe color and pattern were grey in dorsal areas, alternating yellow and white in subdorsal areas, followed by grey supraspiracular stripe and thin white spiracular stripe (not as well-defined as in the earlier stages of fifth-instars). Most importantly, the white spiracular stripe was subtended by an alternating yellow and white subspiracular stripe and ventral areas were whitish-green (Figure 1.10.D). In the late stages of fifth-instar development, pinaculae were well defined and visually noticeable.

In late stages of fifth-instar development, larvae were almost double the size of fourth-instar larvae. Fifth-instar larvae ceased feeding, crawled down the stem of the host plant and positioned their head downward. In the final stages of development, larvae were relatively immobile and appeared somewhat swollen and shriveled. The head capsule was grey which was smaller relative to the body. The coloration of the body was lighter in hue and the sharp contrast between stripes became less apparent although the pinaculae were well-defined and clear (Figure 1.11.A). Shull (1907) described the color of the body of *E. olympia* during this phase as having a “purplish tinge”. During this time of transition, sedentary larvae were observed to “wait” for 16 – 48 hours prior to crawling off the host plant in search of a pupation site. The process of crawling in search of a suitable pupation site has been described as “wandering” (Shull 1907, Feltwell 1982). The cues for initiating wandering were not determined although photoperiod and temperature are suspected to jointly influence movement.

Development time of the fifth-instar to the “wandering” phase was 6.33 ± 0.58 (n = 3; range 6 – 7) days (Table 1.2). By late June, most larvae completed development and began “wandering” in search of a pupation site, although fifth instar larvae were observed on host plants as late as July 12th. Unlike other *E. ausonides* species (Opler 1974), pupation dis not occur on the host plant but instead in surrounding vegetation.

Several larvae were observed crawling from their host plants in search of a pupation site and detailed accounts of “wandering” behavior were recorded. Two larvae were observed in 2005; one on June 18th in tidal lagoon habitat and the second on June 19th in grassland habitat. The third larva was observed June 25, 2006, in a restoration management area dominated by early successional grassland species.

In tidal lagoon habitat, larva “wandered” approximately 4 meters crawling across *Salicornia virginica* L., pickleweed (Figure 1.11.B), *Juncus spp*., rush and *Elymus glaucus*, blue wild-rye, and pupated on the woody base of perennial-subshrub *S. virginica*. Larvae in the grassland habitat and the restoration management area wandered 2.1 and 0.5 meters, respectively. The larva in the high density grassland habitat crawled across grasses and forbs such as *Holcus lanatus*, velvetgrass, *C. arvense*, *Vica sativa*, garden vetch, *Bromus rigidus*, ripgut brome, and *Elymus repens*, quackgrass. In the restoration management area larva crawled across low density grasses and forbs such as *Teesdalia nudicaulis*, barestem teesdalia, *H. lanatus*, *Rumex acetosella*, sheep sorrel and *Plantago lanceolata*, narrowleaf plantain. In the grassland habitat site the larva pupated on the lower stalk of the senescent grass *H. lanatus* (Figure 1.11.C) and in the restoration management area larva pupated on a dry stem of *T. nudicaulis* (Figure 1.12.A).

In all three locations, larvae were observed crawling in the lower to mid-canopy vegetation (50 −160 cm from the ground). Larvae exploited grass and rush stems (*H. lanatus*, *E. glaucus*, and *Juncus sp*.) in order to move across varied and diverse vegetation forms. The combination of wind blowing grasses and the weight of the larva bending flexible stems of grasses created directional pathways for larvae to walk 25–30 cm across vegetation at heights within the lower to mid-canopy. Essentially, larvae used grass stems as structural ‘bridges’. When ‘bridges’ were unavailable, larvae moved up and down stems of grasses and across leaves of vegetation until locating an area on the stem or leaf that was in contact with an adjacent plant. Larvae in search of a pupation site were often observed to lift their head and thorax from the stem and move their head side-to-side (i.e., “head-waving”). “Head-waving” was described by Jones (1977) to illustrate the movements of *Pieris rapae*, small white (or cabbage white) butterfly larvae that were manipulated (starved) under controlled laboratory conditions. Based on my field studies, the mechanism for the behavior is likely associated with searching or ‘reaching’ for adjacent plant material from which to move or change direction in pursuit of an appropriate pupation site. Overall, larvae did not move in any one particular direction during the “wandering” phase. However, larvae were reluctant to turn more than 90° at any time during movement. This suggests that larvae move in a linear direction but that directionality also depends on the structure and arrangement of vegetation available to the larvae in the lower to mid-canopy.

The size and arrangement of vegetation may also influence the amount of time larvae wander. The “wandering” phase lasted 2 hours and 5 minutes in tidal lagoon habitat and 53 minutes in grassland habitat. Since the grassland habitat was denser and structurally diverse it may be that larvae did not have to search as long for the appropriate pupation site. Shull (1907) found that *E. Olympia* wandered into dense bunchgrasses from open sand dunes to pupate and suggested that dense grasses protect larvae from both environmental (e.g., wind and temperature) and predation factors. In addition, it may also be that larvae that have access to more nutritious resources (i.e., larger host plants in grasslands habitat) may grow larger and move at a faster pace. Jones (1977) found that the speed at which pupating larvae travel was correlated to body size. Crawling closer to the ground, larvae are susceptible to predation, especially spiders. All three larvae that were tracked while moving from the food plant to pupation site encountered spider webs. One larva was trapped for 30 seconds before escaping a spider web. Two larvae came in direct contact with spiders; one did not stop moving while the other ceased movement for several minutes. Burger et. al. (1978) found the chemical secretion in fifth-instar larvae of *Papilio demodocus*, citrus swallowtail butterfly, were different than that of younger larvae. It may be similar chemicals are secreted by late fifth-instar larvae of *E. ausonides insulanus* to deter predators while “wandering” in search of a pupation site.

### Pupation site selection

Once larvae selected a pupation site they prepared for the formation of a pupa. Two distinct behaviors were observed to be associated with this phase, ‘back-bending’ and ‘head-turning’. The first observation of ‘back-bending’ concluded the “wandering” phase and commenced behavior leading to the sedentary “prepupal” stage. Larvae spent 69.33 ± 8.08 (n = 3; range 62 – 78) minutes preparing for the formation of a pupa.

‘Back-bending’ was observed when a larva lifted the head and thorax from contact with the stem and leaned backwards away from the stem in an upright position. Each ‘back-bend’ was repeated 8-10 times in succession and occurred several times on different stems before the larva selected a pupation site. The purpose of ‘back-bending’ may be to determine the spatial requirements needed for a pupation site prior to investing time and resources into constructing the silken framework necessary for attaching the pupa. In all three types of habitat, larvae selected slender dry stems positioned at a 60° angle located in the lower canopy of moderately dense vegetation.

‘Head-turning’ may also serve a similar function as ‘back-bending’. ‘Head-turning’ was described as the movement a larva make when turning its head from side to side while crawling vertically between stems of the pupation site. Web-like silk was observed on the surface of stems shortly after ‘head-turning’ activities began which suggests that the ‘head-turning’ behavior is likely related to silk formation. Shull (1907) also observed ‘head-turning’ behavior in *E. olympia* associated with silk formation. Pieridae are known to create a flat thick surface of silk that they can then attach the back pair of prologs that situate the pupa (called the cremaster) (Scott 1992). Following the selection of a pupation site and production of silk, the mature larvae positioned themselves upright (anterior upwards) and attached themselves by a silk girdle that surrounded the middle of the pupa.

### Pupal stage

Shortly after larvae attached to the stem of woody vegetation they become sedentary and “prepupal”. The bodies of pre-pupa larvae turned brown in color and the abdomen and prolegs became thick and compacted but the head remained elongated. After the final molt, the pre-pupa larvae assumed a thin, sub-cylindrical shape and harden into immobile waxy pupae. Pupation took approximately 48 hours. The pupa was slender and cylindrical (branch-like) characteristic of the genus (Guppy and Shepard 2001) (Figure 1.12.A). The posterior end to the mid-section was aligned with the stem by the silk girdle. The ventral sides of pupae were straight and did not curve outward. The anterior ends of pupae were suspended from a silk girdle at approximately 20° angle and the head tapered to a round point. The overall color of the pupae was light paper-brown marked with thin fading bands of darker shades of brown and grey. Spiracles were well-defined by linear black points occurring parallel to the body in areas along the abdominal section and in curving rows at the base and upper portions of the wings. Wide dark-grey to black longitudinal stripes were located on both sides of the mid-axillary line extending the length of pupae and were easily recognizable (Figure 1.12.A). The accompanying thin longitudinal brownish grey streaks observed in *E. ausonides insulanus* are also a defining characteristic of the species (Edwards 1874 as cited by Guppy and Shepard 2001). The length of pupae was approximately 17- 20 mm. Pupae were cryptically colored during the winter and resembled the stems of senescent vegetation. *Euchloe ausonides insulanus* overwintered as pupae until the following spring. One pupa in the restoration management area was in diapause for 334 days (11 months) and the adult eclosed from the pupa on May 24th, 2007.

## CONCLUSION

There are many morphological and behavioral similarities between the species (*E. ausonides*) and subspecies (*E. ausonides insulanus*), however this study revealed several key differences. The most distinct morphological difference was the coloration and pattern of stripes of larvae in instars III and IV. The white spiracular stripe subtended by yellow-green subspiracular stripe and green-yellow ventral areas were notably different from stripe coloration and pattern described for *E. ausonides* by Opler (1974). An important behavioral difference between the species and subspecies was the subspecies engaged in a "wandering" phase prior to pupation. Opler (1974) observed the species to pupate directly on the host plant whereas *E. ausonides insulanus* will wander up to 4 meters in search of a pupation site. *Euchloe ausonides insulanus* was observed to pupate on vegetation surrounding host plants (i.e., non-host plants).

The behavioral and morphological attributes may be important for managing *E. ausonides insulanus* populations. For example, morphological traits are important for biologists to identify differences in instars and track mortality and survival at different stages. The knowledge of the "wandering" behavior of *E. ausonides insulanus* provides managers with information to design conservation buffers for overwintering pupae. Understanding the biology and developmental stages of *E. ausonides insulanus* may also help to inform the conservation and management of this narrow endemic species.

**Table 1.1** Habitat types and attributes that support populations of *Euchloe ausonides insulanus* at American Camp, San Juan Island. Each habitat type contains specific host plants and pupation sites. Host plants in parentheses are secondary host plants found in these habitat types.

| Habitat Type | Attributes | Host plant(s) | Flowering phenology | Pupation site |
|---|---|---|---|---|
| Tidal lagoon and shoreline | tidal saturation and salinity; low nutrient rocky/sandy soils | *Lepidium virginicum var. menziesii* | late April - late June | *Salicornia virginica* |
| Grassland | strong summer winds; dense non-native grasses; moderately deep sandy soils over clay till | *Brassica rapa*<br>*(Sisymbrium altissimum)* | late March - mid June | *Holcus lanatus*<br>*Teesdalia nudicaulis* |
| Sand dune | open sand; microclimate conditions caused by varied topography | *Sisymbrium altissimum* | late April - late June | *Elymus mollis* |

**Table 1.2** Mean number of days in each stage of development for *Euchloe ausonides insulanus*. Data was collected in the field at American Camp, 2005 and 2006.

| | 2005 | | | 2006 | | | Combined Data | | |
|---|---|---|---|---|---|---|---|---|---|
| Stage | Mean | S.D. | N | Mean | S.D. | N | Mean | S.D. | N |
| Egg | $10.06^{a}$ | 2.06 | 64 | $11.54^{b}$ | 2.06 | 56 | 10.75 | 2.18 | 120 |
| 1st instar | $5.79^{a}$ | 1.97 | 150 | $5.50^{a}$ | 1.94 | 125 | 5.66 | 1.96 | 275 |
| 2nd instar | $4.23^{a}$ | 2.26 | 110 | $5.30^{b}$ | 1.49 | 76 | 4.67 | 2.05 | 186 |
| 3rd instar | $5.04^{a}$ | 2.33 | 66 | $4.78^{a}$ | 1.43 | 55 | 4.92 | 1.97 | 121 |
| 4th instar | $6.88^{a}$ | 3.39 | 12 | $5.51^{a}$ | 2.31 | 37 | 5.85 | 2.64 | 49 |
| 5th instar | $6.00^{a}$ | | 2 | $7.00^{a}$ | — | 1 | 6.33 | 0.58 | 3 |

Differences in development time between years were compared using *t*-tests. Mean values followed by a different letter are significantly different at $P < 0.01$

**Table 1.3** The mean length (mm) of larva at each instar at American Camp, 2005.

| Stage | Mean | S.D. | N |
|---|---|---|---|
| 1st instar | 1.97 | 0.80 | 238 |
| 2nd instar | 4.96 | 1.25 | 109 |
| 3rd instar | 8.76 | 2.38 | 105 |
| 4th instar | 15.96 | 4.00 | 77 |
| 5th instar | 25.62 | 5.25 | 17 |

**Table 1.4** Number of IV and V instar larvae that successfully located host plants in 5 minutes, 30 minutes, 120 minutes and 30 hours. Not all larvae located hosts in the first 120 minutes. The number of larvae locating hosts is out of a possible 15 larvae searching.

| Host Plants | Number of larvae that located hosts | | | |
|---|---|---|---|---|
| rock (control) | 2 | — | — | — |
| *S. altissimum* | 2 | 4 | 6 | 10 |
| *B. rapa* | 3 | 5 | 3 | 4 |
| *L. virginicum* var. *menzeisii* | 2 | — | — | 1 |
| Time | 5 min. | 30 min. | 120 min. | 30 hrs. |
| Chi-square test | $x^2 = 0.333$ $P = 0.954$ | NS | NS | $x^2 = 8.4$ $P < 0.015$ |

NS (non-significant) i.e., factors are independent

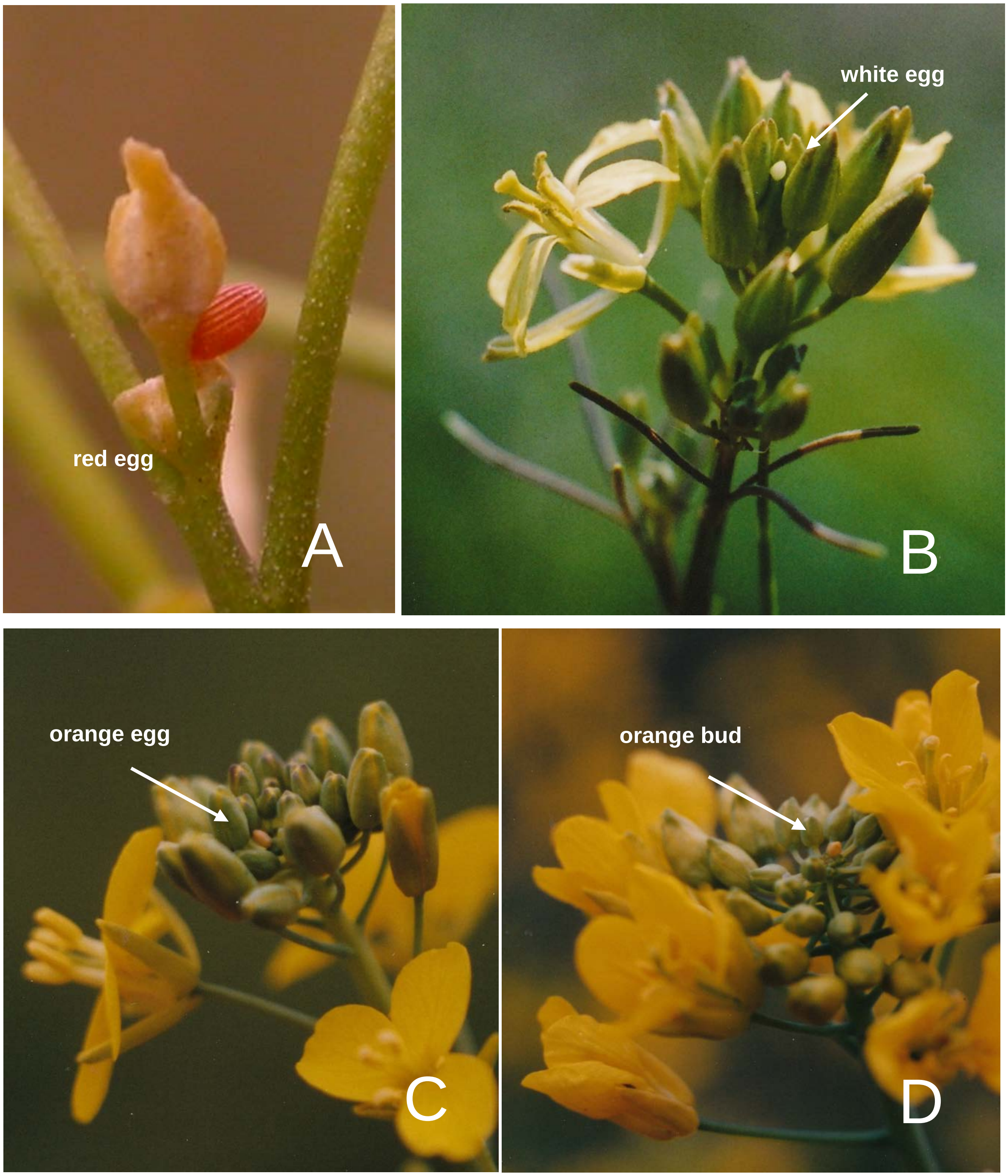

**Figure 1.1** Egg development on host plants, *Brassica rapa* and *Sisymbrium altissimum*, 2006. Eggs of *Euchloe ausonides insulanus* are typically found on the buds of host plants. As the egg matures over the course of approximately 10 days, the egg changes color from white to orange to red and then to brown. **A**) Eggs are the most conspicuous when they are the color red, 48-72 hours after female oviposition. Eggs are columnar in shape and have vertical ridges that adjoin at the top of the egg. **B**) Greenish-white egg among buds of *Sisymbrium altissimum*. **C**) Orange egg among cluster of buds of *B. rapa*. **D**) Single orange bud similar in size, shape and color to an orange egg. Host plants may mimic egg morphology to deter females from laying eggs.

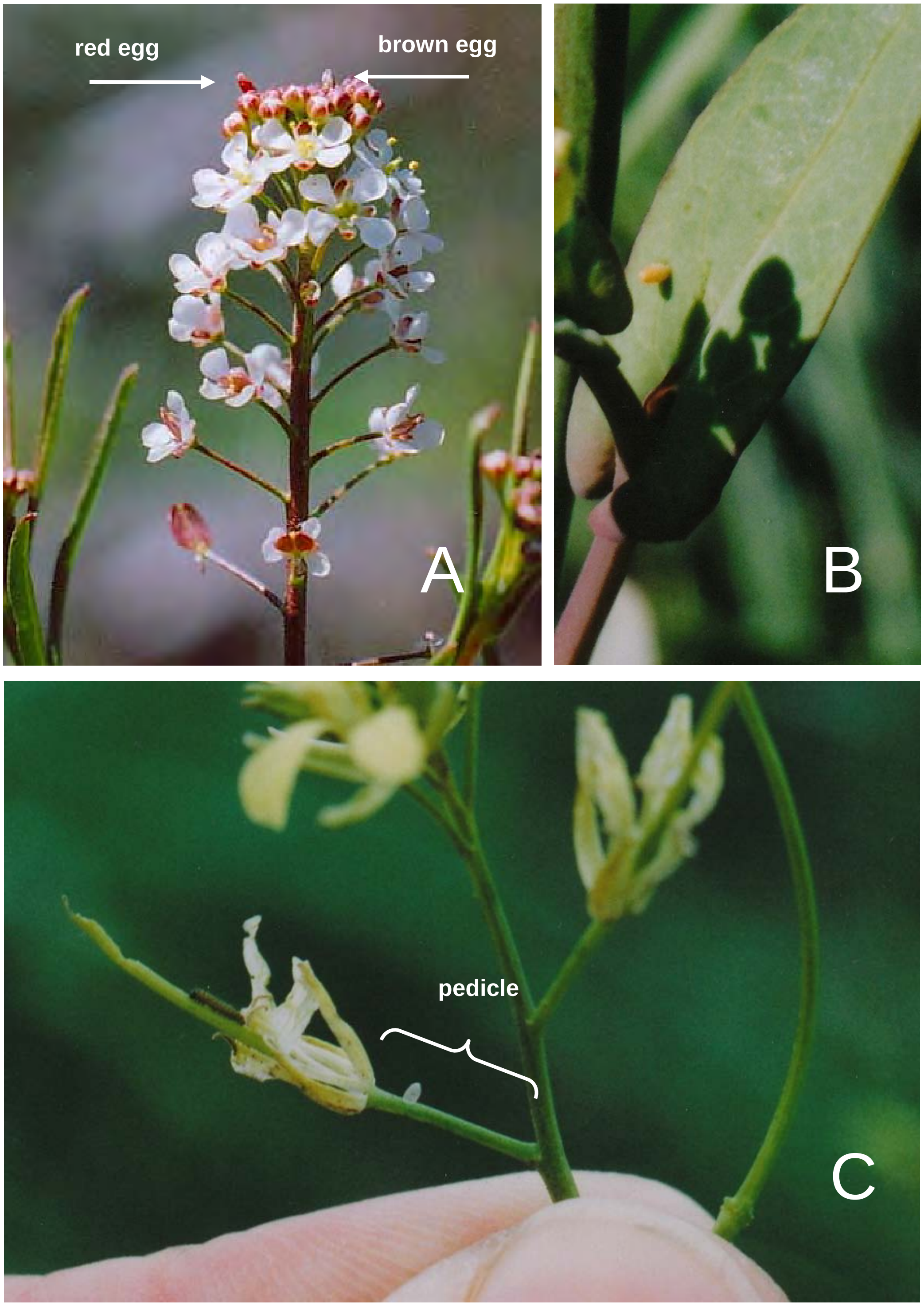

**Figure 1.2** Egg development on host plants, *Lepidium virginicum* var. *menziesii*, *Brassica rapa* and *Sisymbrium altissimum*, 2005. **A**) Buds of *L. virginicum var. menziesii* are similar to the morphology of *E. ausonides insulanus* eggs **B**) Orange egg on the leaf of *B. rapa*. **C**) The pedicle secures the flower/fruit of *S. altissimum* to the base of the inflorescence (or stem of the raceme). First-instar larvae recently emerged from egg case located on pedicle. Larvae that emerge from eggs deposited on pedicles of fruits are more likely to starve in the absence of buds.

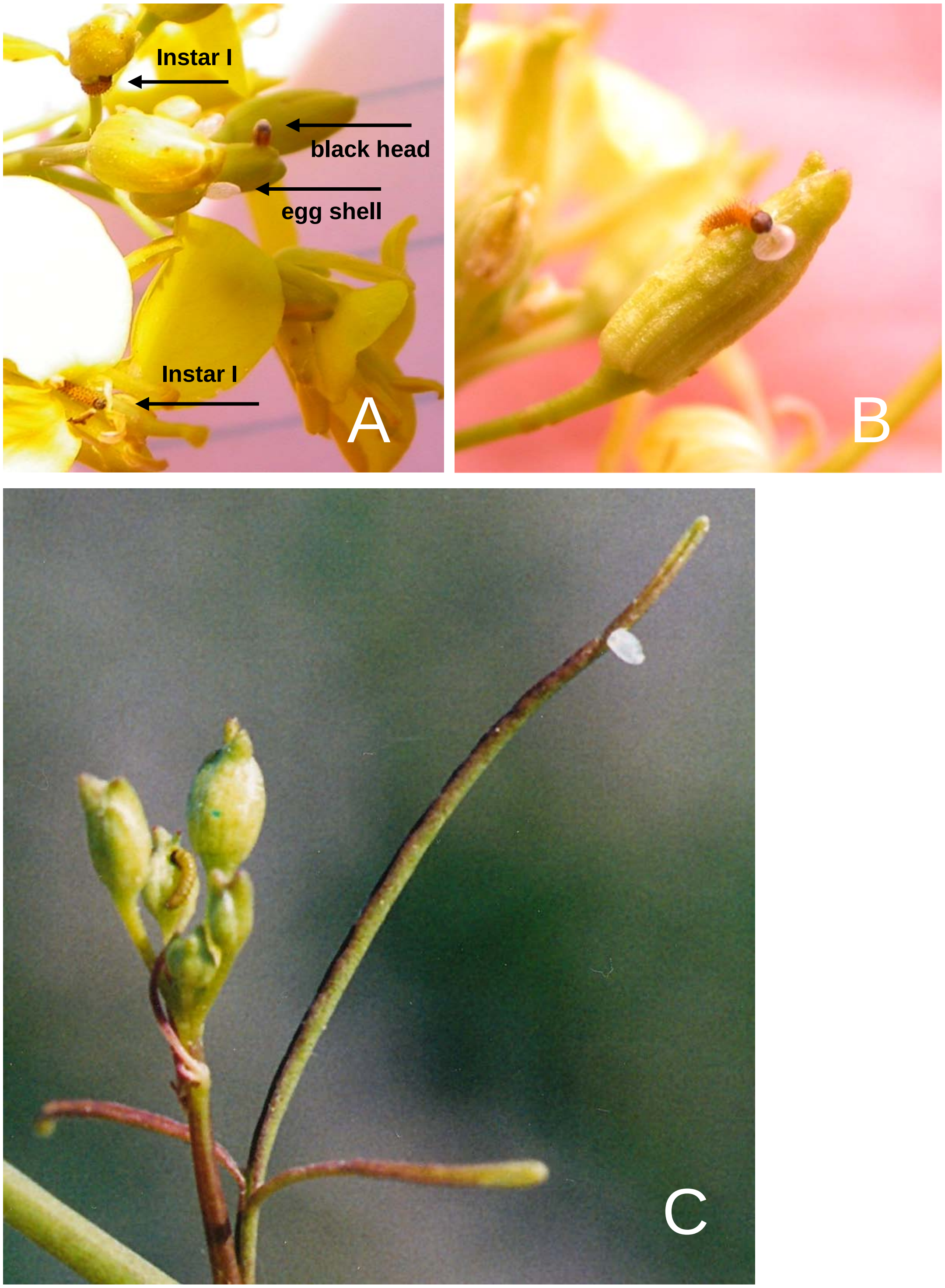

**Figure 1.3** The final stage of egg development and early first-instar larvae on *Sisymbrium altissimum*, 2006. **A**) The black head of the soon to be emerging larva is visible through the egg shell in the final stage of egg development. Two newly emerged larvae feed on flower buds. **B**) After emerging from the egg case, the larva feeds on the shell **C**) Larva hatched from an egg laid on tough vascular leaf tissue moved to edible flower buds. In this case, the larva did not consume the egg shell.

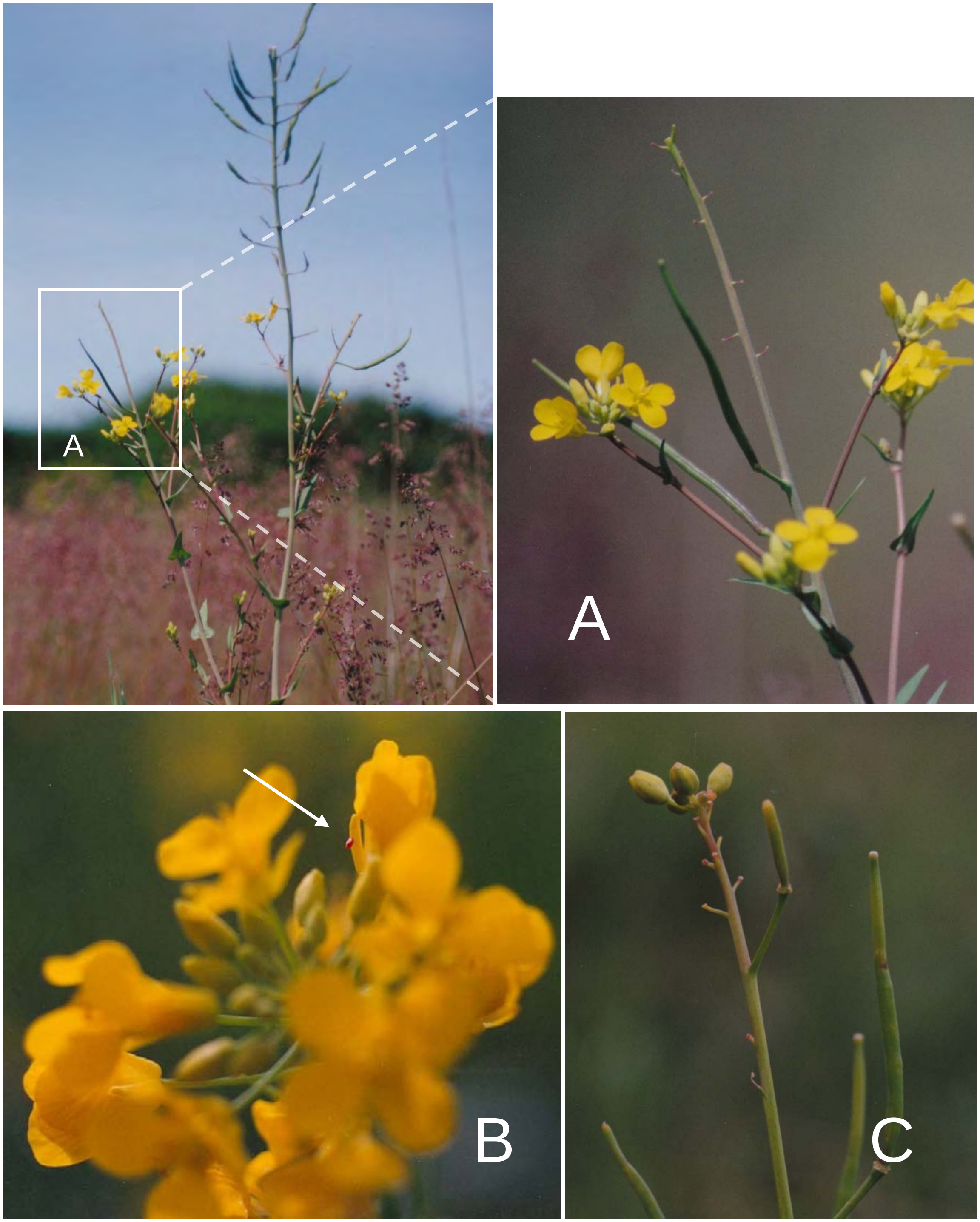

**Figure 1.4** New growth on axillary racemes of *Brassica rapa*, 2005-2007. **A**) Deer herbivory stimulates new growth of buds on axillary racemes. **B**) Red egg attached to flower petal of *B. rapa*. **C**) New flower buds emerge from axillary racemes after surge of precipitation late in the growing season.

**Figure 1.5** Cryptic first-instar larvae located beneath flower buds of *Sisymbrium altissimum*, 2005. Mean development time in the field from hatching to first molt is approximately 6 days. **A**) First-instar larva and uneaten egg shell. The coloration of newly emerged first-instar larvae is golden-yellow with a distinct black head. **B**) The mean length of first-instar is 2mm. The “pinhole” in the bud is evidence of larval feeding. **C**) Prior to molting first-instar larvae are dark grey-brown and hirsute.

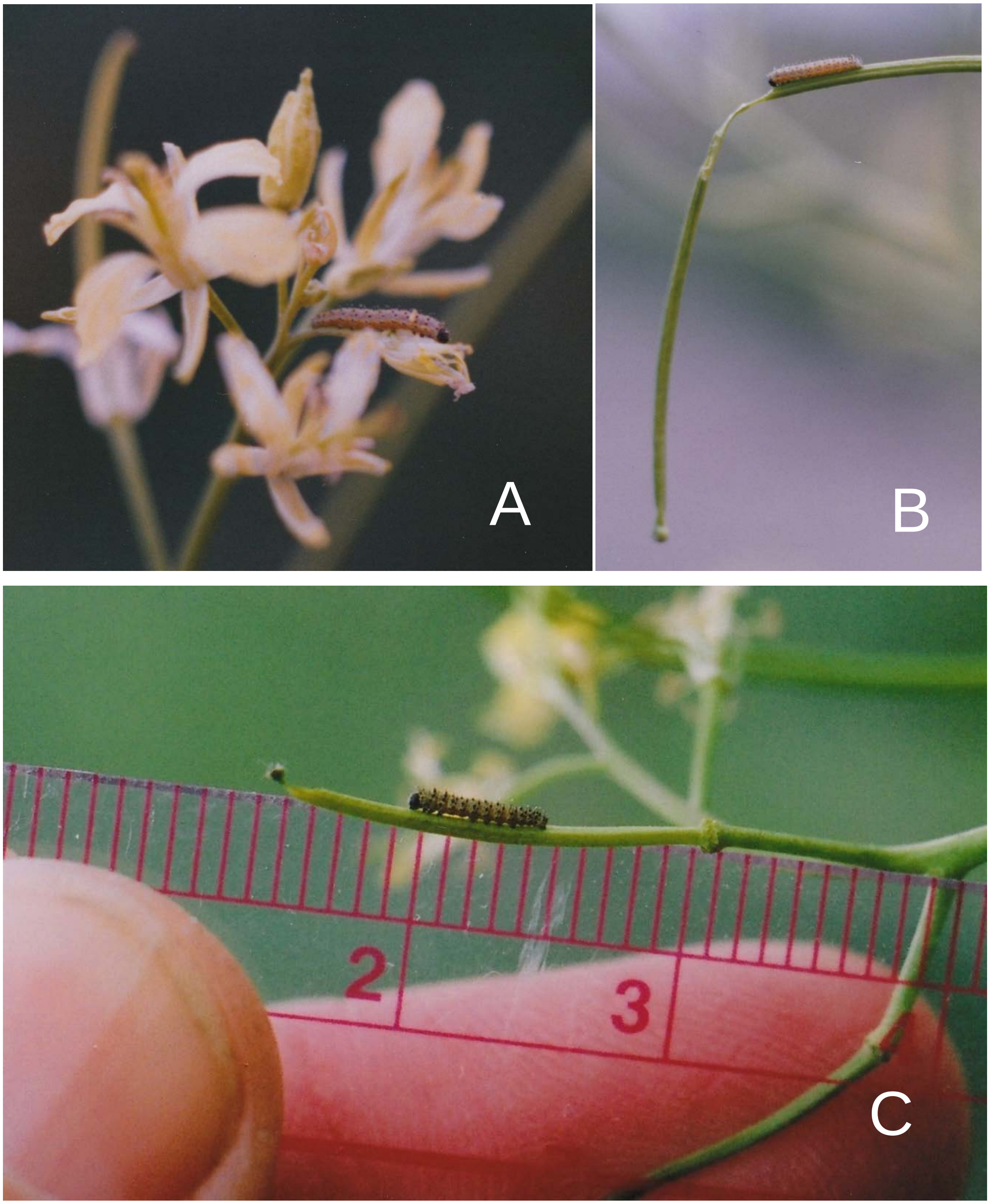

**Figure 1.6** Second-instar larvae feeding on flowers and fruit of *Sisymbrium altissimum*, 2005. Mean development time in the field is approximately 5 days. **A**) Pollen caught on setae of larva while feeding on flower. The color of second-instar larvae is golden-yellowish-green and the head capsule is black. Pinaculae are indistinguishably visible at this stage in development. **B**) Larva consumes fruit tissue but is unable to digest tough cellulose. **C**) Second-instar larvae become darker in color prior to molting. The mean length of second-instar is 5mm.

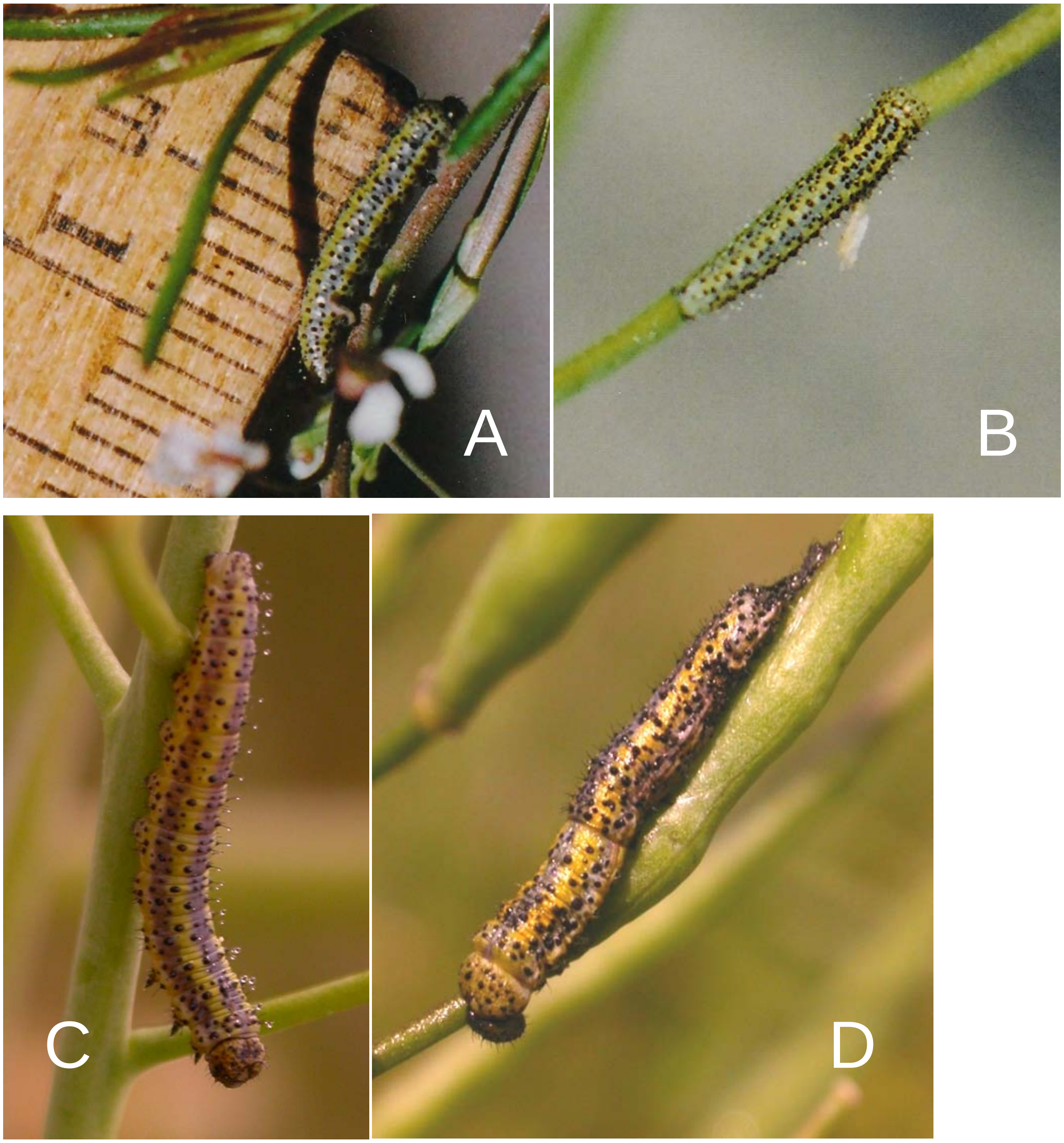

**Figure 1.7** Third-instar larvae feeding on flowers and fruit of *Sisymbrium altissimum*, *Brassica rapa*, and *Lepidium virginicum* var. *menziessii*, 2005. Mean development time in the field is approximately 5 days. **A**) Third-instar larva prior to shedding the head cuticle remaining from the second-instar. The mean length of third-instar larvae is 9mm. Grey-green and yellow-green stripes are visible on dorsal and subdorsal areas of the body. **B**) Early third-instar larvae have a greenish head that is larger or proportional to the width of the body. **C**) Late third-instar larvae have a brownish head that is smaller than the width of the body. The color white surrounds the spiracles. Yellow-green subspiracular stripes are visible on the lower sides of the body. Setae and pinaculae are easily recognizable in the field at this stage in development. **D**) Third-instar larva sheds old cuticle (ecdysis). The head capsule and body are shed separately during ecdysis. Once the cuticle of the body is shed the larva is identified as fourth-instar.

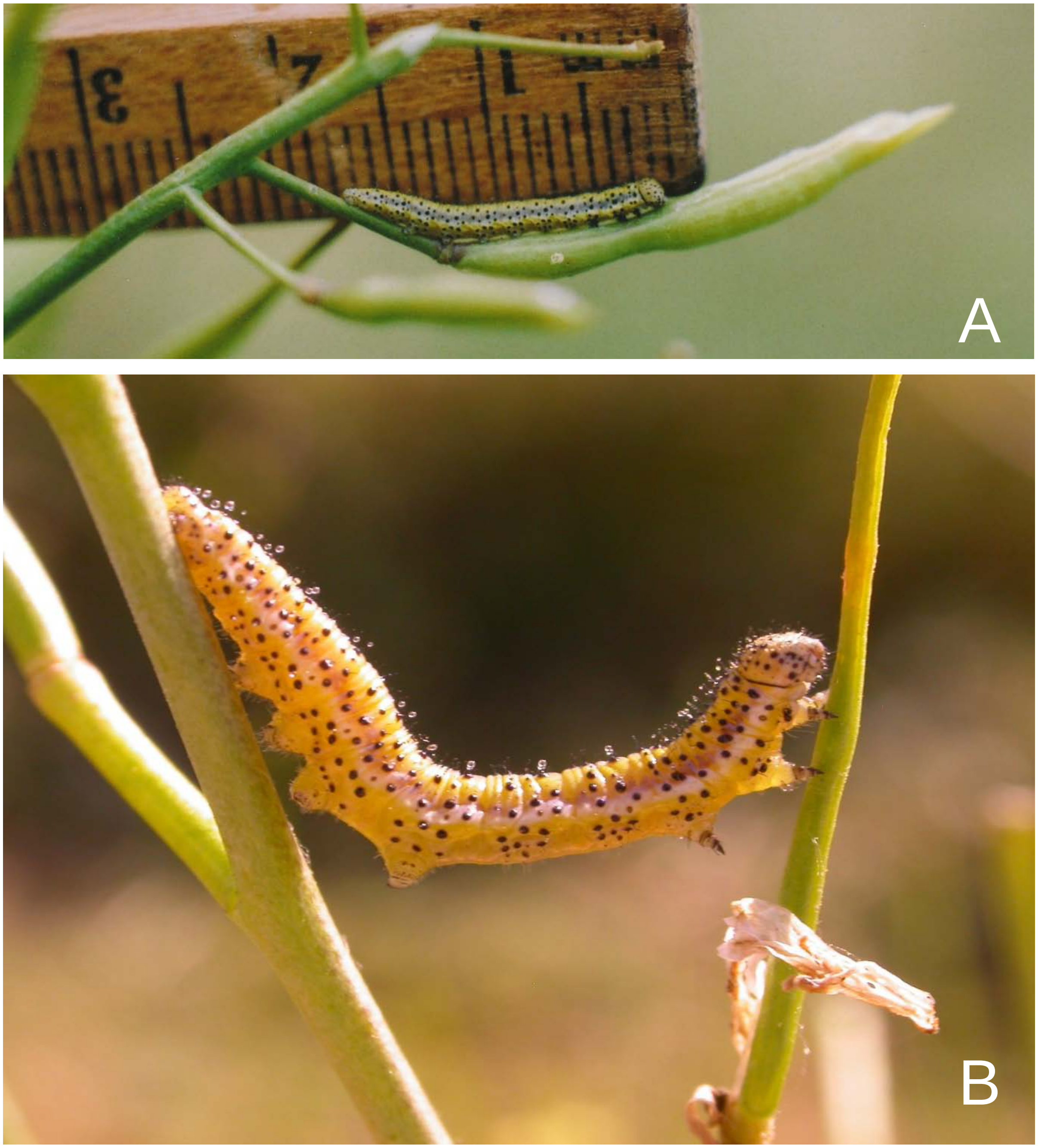

**Figure 1.8** Fourth-instar larvae on *Brassica rapa*, 2005. Mean development time in the field from fourth to fifth instar is approximately 6 days. **A**) Larva resting on the host plant pedicel after feeding on fruit. The color of fourth-instar larva is the same as third-instar larva although the white areas surrounding the spiracles is more developed and appears as a distinct white stripe along the lower side of the body. Pinaculae are also more prominent and easily recognizable in the field. The mean length of fourth-instar larva is 16 mm. **B**) Larva crawling from one raceme to another.

**Figure 1.9** Fourth-instar larva feeding on *Lepidium virginicum* var. *menziessii*, 2005. Larva consumed both fruit and pedicel in 19 minutes.

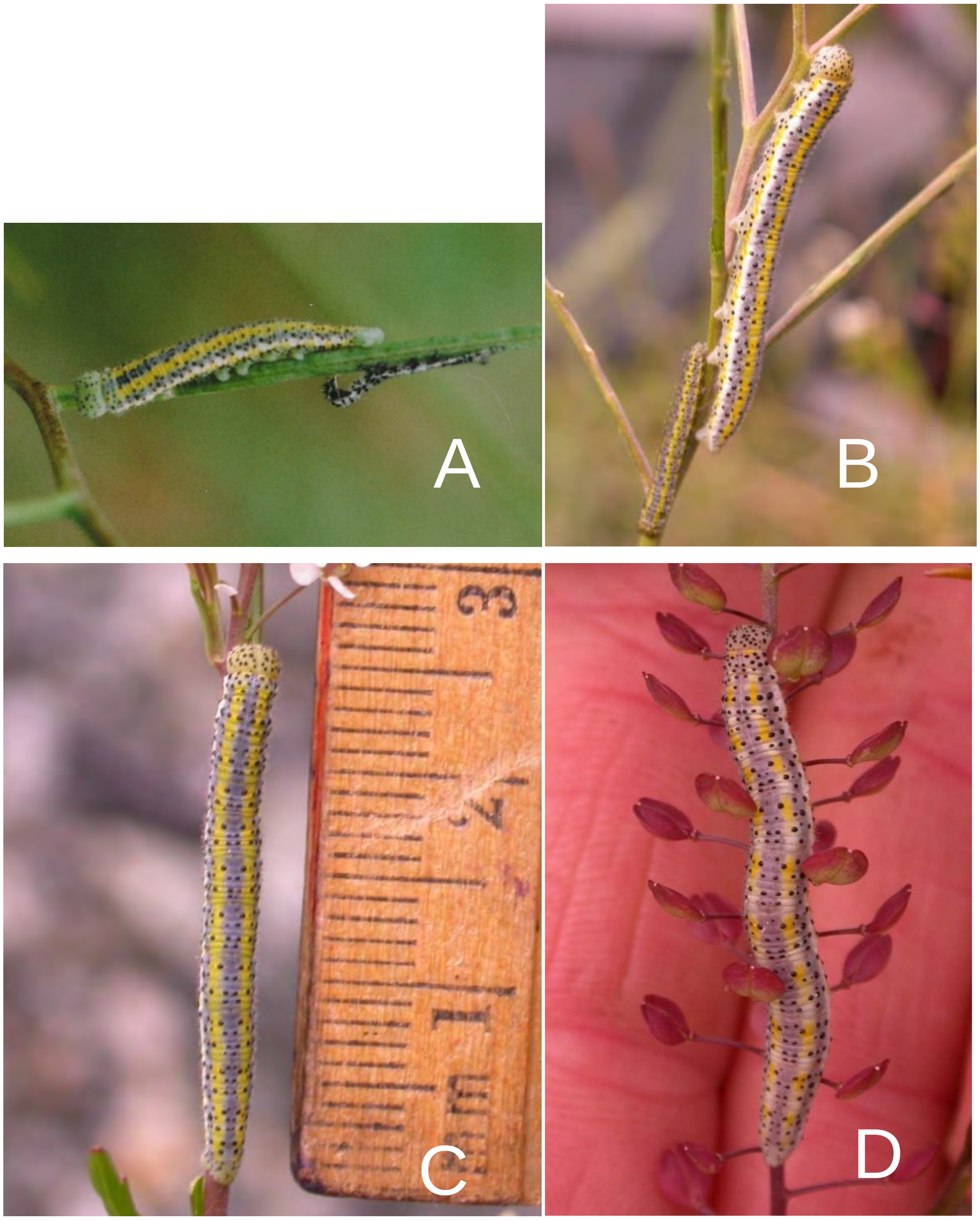

**Figure 1.10** Fifth-instar larvae on *Brassica rapa* and *Lepidium virginicum* var. *menziessii*, 2005. Mean development time in the field from fifth-instar to “wandering” phase (leaving the host plant in search of a pupation site) is approximately 6 days. **A**) Newly emerging fifth-instar with exoskeleton. Larvae molt from the front of the body and the head case remains intact. Early fifth-instar larvae have disproportionately large bulbous heads in comparison to the width of the body. **B**) Fifth-instar larvae are substantially larger in size and stripes are bold in color compared to fourth-instar larvae. Stripes are solid grey and yellow in the dorsal and subdorsal area, followed by a thin light-grey supraspiracular stripe and well-defined white spiracular stripe. **C**) The mean length of fifth-instar larvae is 26mm. D) Fifth-instar larva in late stages of development. The color of subdorsal and subspiracular stripes change from solid yellow to alternating yellow and white. At this stage the head is smaller relative to the size of the body.

**Figure 1.11** Behavior of fifth-instar larvae leading to “prepupal” stage. **A**) Larva “waits” on the stem of defoliated *Brassica rapa* prior to “wandering” in search of a suitable pupation site. At this stage, larvae cease feeding and position themselves downward on the stem of the food plant. They appear shriveled and lighter in color. **B**) Larva “wandering” across *Salicornia virginica*. “Wandering” occurred 50-60cm from the ground in lower to mid-canopy vegetation. **C**) Larva selects a dry stalk of *Holcus lanatus* positioned at a 60° angle in grassland habitat. Once larvae found a pupation site they spent approximately 70 minutes preparing for the formation of a pupa before entering the sedentary “prepupal” stage.

**Figure 1.12** Pupa and pupal case in restoration management area, June 25, 2006 - May 24, 2007. A) Pupa suspended from silk girdle attached to senescent stalk of *Teesdalia nudicaulis*. The color of the pupa is light paper-brown with linear black points parallel to the body and perpendicular to the wings. The wide dark-gray to black stripe extending the length of pupa is characteristic of the species. B) Pupal case after eclosion.

# Chapter 2

# Egg-Laying Patterns and Host Plant Biology

## INTRODUCTION

*Euchloe ausonides insulanus* Guppy and Shepard 2001 (Pieridae) is a rare pierid found on San Juan and Lopez islands in the San Juan Island archipelago, Washington (USA). *Euchloe ausonides insulanus* was presumed extinct until 1998 when several adults were rediscovered on San Juan Island (Fleckenstein and Potter 1999). *Euchloe ausonides insulanus* is currently a candidate for state listing  and is a conservation priority for US Fish and Wildlife and the National Park Service (Pyle 2006).

Since 1998, studies have been conducted to better understand adult abundance and distribution of *E. ausonides insulanus* (Lambert 2005, Miskelly and Potter 2005, Lambert 2007, Miskelly and Fleckenstein 2007, Hanson et al. 2009, Lambert 2009, Peterson 2009, Hanson et al. 2010, Peterson 2010). However, this is the first study to examine the relationship between egg-laying patterns and host plant biology (i.e., host plant traits, density and phenology). Host plant biology is thought to be the most common driver of population changes in butterfly species (Thomas 1984a, Thomas et al. 2010). For example, the presence of high quality host plant habitat (defined as a subset of host plants that are preferred by females over other less optimal host plants) is correlated with more persistent populations and an increase in carrying capacity within sites (Thomas et al. 2010). Furthermore, host plants preferred by females have similar plant traits and are closely correlated with larval survival (Thomas 1998, Thomas et al. 2010).

This study investigates the relationships between egg-laying patterns and host plants *Brassica rapa* L. var. *rapa*, *Sisymbrium altissimum* L., and *Lepidium virginicum var. menziesii* (DC) Hitchc. Larvae are metabolically and behaviorally adapted to specific host plant species, although host specificity is relatively broad within Brassicaceae as shown by the exploitation of introduced host plant species *B. rapa* and *S. altissimum*. *Lepidium virginicum var. menziesii* is the only known native host plant used by *E. ausonides insulanus*. Although several species of Brassicaceae occur on San Juan Island only *B. rapa, S.*

*altissimum* and *L. virginicum var. menziesii* support larval development under field conditions.

*Euchloe* species are known to select appropriate oviposition sites based on host plant chemistry (specifically mustard oil glucosides) (Renwick and Chew 1994, Stadler and Reifenrath 2009), structural features such as host plant size (Shapiro 1985, Karban and Courtney 1987, Dennis 1995), intrinsic factors such as egg load (i.e., the number of mature eggs found in ovaries and oviducts) or a combination of factors that influence oviposition behavior (see review Gibbs and Van Dyck 2009). This study focuses on oviposition preference for specific host plant traits (i.e., plant height, number of racemes and plant phenology) and host plant density.

Understanding the relationship of host plant traits and host plant density to oviposition site selection (i.e., egg-laying patterns) is important because if *E. ausonides insulanus* preferentially lays eggs on plants of particular size or arrangement (e.g., large plants on the edges of dense host plant patches) then larvae may be limited to such plants. Understanding what types of host plants are more likely to be occupied by eggs and larvae will improve managers' ability to: 1) monitor egg and larvae survival 2) predict the occurrence of eggs and larvae among host plants and host plant patches and 3) design host plant habitat that support oviposition site selection by *E. ausonides insulanus*.

To better understand how adult biology relates to egg-laying patterns, descriptive studies on adult phenology, mating behavior, egg phenology and egg dispersion were conducted. Finally, in an effort to synthesize and apply the knowledge gained in these areas of research a study was conducted on the relationship between host plant patch size and egg-laying patterns.

## METHODS

### Study species and sites

*Euchloe ausonides insulanus* is primarily white and yellow with a greenish marbled texture under the hind wing and wingspan of approximately 45 mm (Figure 2.1). The marbled pattern on the wing characterizes the species (Guppy and Shepard 2001). Pyle (2004) described *E. ausonides insulanus* as having dark markings expanding dorsally and wing bases strongly shadowed (Figure 2.2). The marbling texture on the ventral surface of the hind wing is composed of yellow and black scales combined with white patches between them and may reflect ultraviolet (Guppy and Shepard 2001). In flight, females appear yellowish white.

Research was conducted in open areas in American Camp, San Juan Island National Historical Park (SAJH) located on the southern end of San Juan Island, Washington, USA. Specific site locations are referenced in the methods section under individual studies (Figures 2.3 and 2.4). Grasslands, sand dunes and tidal lagoon plant communities support habitat unique to populations of *E. ausonides insulanus*. All three plant communities contain some type of topographic relief such as slopes, bluffs, sand banks or driftwood berms important for dispersal (Table 2.1) and each plant community contains specific host plants, nectar resources, mating sites and pupation sites (Table 2.2).

All *Euchloe* utilize plants in the family Brassicaceae (Opler 1974, Scott 1986, Braby and Trueman 2006). *Brassica rapa*, *S. altissimum* and *L. virginicum var. menziesii* are the sole known host plants for *E. ausonides insulanus*. *Brassica rapa* and *S. altissimum* are introduced host plant species planted at American Camp with other agricultural crops in the early 1850’s (Griffin 1852 as cited in Avery 2002). *Brassica rapa* is the most abundant of the three host plants and occurs in introduced grasslands with moderate levels of disturbance created by small mammals. *Sisymbrium altissimum* occurs in areas of high disturbance and is most often found in sand dunes. *Lepidium virginicum* var. *menziesii* is the only known native host plant and occupies intermediate beaches between tidal lagoons and shoreline. Among these, *B. rapa* is the most widely distributed throughout American Camp grasslands although not all stands of *B. rapa* are consistently occupied by *E. ausonides insulanus*.

### Adult phenology, behavior and egg phenology

Adult phenology and descriptive behavioral data were obtained while concurrently conducting population abundance surveys (Appendix B). Sixteen belt-transects (200m x 30m wide) located in grassland, sand dune and tidal lagoon habitat were established in 2004 based on methods outlined by Pollard and Yates (1993) (Figure 2.3). Transects were surveyed every 6 − 9 days from early April (prior to the emergence of adults) to late June (after adults were no longer observed). A total of 723 adults were observed and behaviors recorded from transect surveys from 2004 to 2008. Behaviors recorded included flying, resting, landing, searching, ‘foray searching’ (flying leeward and windward usually moving in the direction up a slope and returning down slope eventually returning to the original host plant habitat), patrolling, nectaring, interactions with conspecifcs and other butterfly species and mating behavior.

In addition to transect surveys, 36 adults (including 5 confirmed females) were observed for a mean time of 8.2 ± 7.6 minutes (range: 0.2−32.9 minutes) at nine sites (approximately 2 $km^2$ each) covering all habitats where butterflies were observed to fly in American Camp (Figure 2.3). Mating behavior (e.g., mating interactions, searching and “sweeping”), mate location and oviposition behavior were recorded. “Sweeping” refers to flying from one inflorescence to another in search of oviposition sites.

Egg phenology data were obtained from egg and larval survivorship studies (see Chapter 4). Host plants were searched for eggs at 7 study sites located in grassland, sand dune and tidal lagoon habitat at American Camp SAJH (Figure 2.3). One additional study site was located southeast of American Camp. Study sites contained only one host plant species and were spatially well defined; with the distance between any two sites ranging from 1800m – 500m. A total of 41 surveys were conducted from 2005 – 2008. The color, number and location of eggs on individual host plants were recorded.

### Egg dispersion and egg load

Patterns associated with egg dispersion and egg load (number of eggs a female lays at one time on one inflorescence) were studied from 2005 – 2008. An area 55m x 33m containing loosely distributed plants of *B. rapa* was selected for study (Figure 2.4).

The color and number of eggs per raceme were recorded. The co-occurrence of eggs laid by different females on the same inflorescence was distinguished by egg color. Eggs change color as they mature. Newly laid eggs were greenish-white and change to bright orange, to deep red and finally to brown in the final stages of development. Therefore it was assumed that different colors of eggs observed on the same inflorescence indicated that more than one female laid egg(s) on the same inflorescence at different times (Figure 2.5.A and B).

Host traits such as plant height, number of racemes and phenologic stage, that may contribute to oviposition site selection were examined. All axillary racemes (or inflorescences) on all *B. rapa* plants within the study area were counted and examined for eggs. In 2005, a total of 92 plants were surveyed for eggs. In 2006, 136 were surveyed; 2007, 227 and 2008, 349 respectively. Surveys were conducted on two occasions during the height of the flight season, approximately 10 days between surveys.

### Egg-laying patterns and host plant traits

To examine how plant traits (plant height, number of racemes and phenologic stage) influence egg-laying patterns a study was conducted using all three host plant species. A four year study of *B. rapa* (2005-2008) was conducted at the study site described above (see *Egg dispersion* and *egg load*). Two additional study sites containing *S. altissimum* and *L. virginicum* var. *menziesii*, were surveyed in 2004, one containing 111 plants of *L. virginicum* var. *menziesii* and another containing 205 plants of *S. altissimum* (Figure 2.4).

At all three study sites all host plants within the study boundaries were examined for eggs. Host plant racemes were counted and plant height was measured for all plants with and without eggs. Plant phenology was also recorded for all plants. Phenologic categories ranged from 1– 4. Phenologic stages run along a continuum, phenologic stage 1 represented young plants in bud and phenologic stage 4 represented senescent plants in fruit. Specifically, phenologic stages were determined by measuring the ratio of buds to flowers and flowers to fruits. Stage 1 plants contained >50% buds and <50% flowers; stage 2 plants <50% buds and >50% flowers; stage 3 no buds, <50% flowers and >50% developing fruit; and stage 4, no buds or flowers only developing fruit.

To test the difference in 1) mean plant height with and without eggs and 2) mean number of racemes with and without eggs within populations of *B. rapa* (2005-2008; n=802 plants), *S. altissimum* (2004; n=179 plants) and *L. virginicum* var. *menziesii* (2004; n=111 plants), a Mann-Whitney U test was used. To test for a difference in the mean height and number of racemes of *B. rapa* plants with eggs among four years (2005–2008), Analysis of Variance (ANOVA) was performed. To examine whether plants with eggs are independent of plant phenology (phenologic stages 1 – 4), Chi-square tests were conducted.

### Egg-laying patterns and host plant density

To investigation the relationship between host plant density and egg-laying patterns, two sites containing separate stands of host plants (*B. rapa* and *S. altissimum*) were selected for study in 2008 (Figure 2.4). Site selection was based on three criteria: 1) host plants were definitely grouped (resulting in a contagious distribution), 2) definite groups represented various densities (low, medium and high), and 3) females were observed using host plant habitat. The goals of the study were 1) to test if a correlation exists between numbers of eggs and host plant density and 2) evaluate the relative numbers of eggs in high and low density plots.

At both sites, host plant patches were contagiously distributed, although they differed in spatial scale due to differences in habitat structure. *Brassica rapa* occurred in areas of dense introduced grasses with moderate to low levels of disturbance by small mammals. Plants of *B. rapa* were distributed in loose groups (patches) and occurred predominantly in areas where the soil had been raised by small mammals. Conversely, *S. altissimum* occurred in sand dunes where there were high levels of wind disturbance and areas of open sand. Open areas are frequently colonized by dense stands of *S. altissimum*. Additionally, the *S. altissimum* study site included both dunes and swales containing moderately dense stands of native and non-native vegetation unlike the *B. rapa* site, which was generally flat and dominated by introduced grasses.

The *B. rapa* study site was 200m x 10m (2 square kilometers), rectangular in shape and comprised of approximately 571 *B. rapa* plants. The *S. altissimum* study site was 100 m x 100 m (10 square kilometers) and comprised of 7,987 host plants. Due to differences in habitat structure, the size (number of racemes and plant height) and number of host plants varied between *B. rapa* and *S. altissimum* sites. For example, most plants of *B. rapa* produced multiple axillary racemes (8.99 racemes per plant, n = 802 plants) compared to *S. altissimum* plants that produced 1.3 racemes per plant (n = 205 plants).

A 5m x 5m quadrat was used to measure number of plants, eggs and percent cover at the *B. rapa* and *S. altissimum* study sites. The size of the quadrat was used to capture variation in host plant density in addition to female dispersal (the distance a female moves between ovipositing eggs). Based on field observations, females were estimated to oviposit egg(s) every 1 − 6 meters in areas of moderately dense *B. rapa* (Lambert unpublished data).

All racemes on all plants within the *B. rapa* study site (2 km$^2$) were counted and examined for eggs. A total of 33, 5m x 5m quadrats were established along the 200 meter transect. At the *S. altissimum* site, percent cover was measured and the numbers of racemes were counted in 80, 5m x 5m plots (2 square kilometers). A subset of 12 plots were then randomly selected from plots with high host plant cover (>15 racemes/m$^2$) and low host plant cover (<2 racemes/m$^2$) and subsequently searched for eggs.

Spearman's rank correlation tests were used to examine the relationship between number of racemes and number of eggs for *Sisymbrium altissimum* plots (n=12) and *B. rapa* plots (n=33). Mann-Whitney U tests were used to test for differences in mean number of eggs between high and low density plots at both sites. High and low densities of *B. rapa* were categorized as > 8 racemes/m$^2$ (n=9) and < 2 racemes/m$^2$ (n=9), respectively. High and low densities of *S. altissimum* were categorized as >15 racemes/m$^2$ (n=6) and <2 racemes/m$^2$ (n=6) respectively.

In order to interpret the density of host plant per m$^2$ described in the *Host plant patch study*, the number of racemes/m$^2$ was adjusted to plants/m$^2$. Based on measurements of *B. rapa* plants with and without eggs, the mean number of racemes per plant of *B. rapa* is 8.99 (SD±12.18; range: 1−150, n=802) (Lambert unpublished data). To convert the number of racemes per plant for *B. rapa*, the number of racemes was divided by 8.99. The mean number of racemes per plant of *S. altissimum* is 1.3 (SD±0.69; range: 1 – 4, n=205) (Lambert unpublished data). To convert the number of racemes per plant for *S. altissimum*, the number of racemes was divided by 1.3.

### Host plant patch study

To examine how host plant patch size might influence egg-laying patterns a detailed study of *S. altissimum* host plant habitat was conducted in 2006. The site was located in an area below the redoubt at American Camp and was approximately 65km$^2$ (Figure 2.4) Host plant patches were mapped (Figure 2.6) and patch size, plant density and egg density were recorded (Tables 2.3 and 2.4). The number of eggs was recorded per plant. Results were tabulated and used to make predictions about the density and size of host plant patches and how patch size relates to egg densities.

## RESULTS AND DISCUSSION

### Adult phenology, behavior and egg phenology

*Euchloe ausonides insulanus* is a spring butterfly of coastal lowlands. Adults fly from early-April to mid-June. The univoltine (one brood per season) life cycle of *E. ausonides insulanus* is closely associated with host plant phenology (Figure 2.7). Host plants and butterflies emerge in synchrony at different times in the spring. The timing of host plant emergence is influenced by microclimatic conditions. For example, plants of *B. rapa* located at a exposed, dry site 6.5 km north of American Camp (i.e., Pear Point gravel pit) were observed flowering several weeks earlier than *B. rapa* plants located in grasslands at American Camp (SAJH). Following the early flowering period, adults were also observed a week earlier (April 19th) at the Pear Point gravel pit (Peterson 2009) than at American Camp (SAJH) (April 25th) (Hanson et al. 2009). This suggests that *E. ausonides insulanus* may have a flexible terminating cue for diapause.

In general males were observed to eclose from pupae in early April (depending on weather), shortly after *B. rapa* leaves and flower stalks emerge. Males emerged before females (protandrous males) and patrolled hillsides in search of females. Females emerged several days after males (4-7 days) and mating began. Males in search of females sought out white (ultraviolet-reflecting) objects that resembled females. Males approached objects that were the color white (Figure 2.1). Scott (1986) also observed that males of *E. ausonides* were attracted to the color white. Males of *E. ausonides insulanus* were observed to investigate white flowers on plants such as *Cerastium arvense* L. and *Achillea millefolium* L., white picket fences surrounding historical buildings and grounds and painted white lines along surface roads. Males also advanced toward cabbage white butterflies, *Pieris rapae* L. Similarly, *Euchloe ausonides* approached white paper models in the field and flew toward white butterflies such as *Coenonympha tullia californica* Westwood and *Pieris rapae* (Scott 1975a). *Euchloe ausonides insulanus* males flew approximately 1.5 meters above the ground at a rate of 4.9 meters per second along ridgelines, bluffs, road-cuts, trail edges, fence-lines and shrub/forest edges to reach mate locations. Similarly, Scott (1975a) found that *E. ausonides* can fly up to 5 meters per second.

Mating behavior may play an important role in movement and dispersal (Shields 1967, Scott 1975b). *Euchloe ausonides insulanus* flew to mating sites outside of host plant habitat increasing the area of occupancy (i.e., range of habitat). Adults were observed in areas several hundred meters outside of host plant habitat. Figure 2.1 illustrates a mating pair observed in native prairie 284 meters from the nearest host plant. In this case, both sexes had to move substantial distances to reach the white flowers of *Cerastium arvense*.

Mating behavior of *E. ausonides insulanus* is similar to the mating behavior of *E. ausonides* described by Scott (1973a, 1975a, 1986). *Euchloe ausonides insulanus* mating was observed most often in the afternoon during warm, calm conditions. When male and female encountered each other during flight they fluttered around each other in tight circles, the male hovering beside the female. Scott (1986) suggested that males cuff the female with beating wings to transfer pheromones during the courting ritual. Courting pairs of *E. ausonides insulanus* flew vertically into the air, over 10 meters high above coastal bluffs. Males courted sedentary females resting on vegetation. Males hovered above females for 6 − 12 minutes. Scott (1975a) found that males of *E. ausonides* hovered up to 20 minutes while courting unmated females, but males discontinued courtship in less than 30 seconds if they encountered mated females.

Shortly after *E. ausonides insulanus* courtship, receptive females joined the male’s abdomen. Mating pairs faced opposite directions usually resting without movement in grasses or other vegetation (Figure 2.1). Mating lasted approximately 4 hours. Unreceptive females rejected males by spreading their wings and lifting their abdomen between the wings in the direction of the pursuing male (Figure 2.2). This posture is commonly associated with the behavior of unreceptive females in Pieridae (Scott 1973a, 1986).

After mating, *E. ausonides insulanus* females flew to host plant habitat to oviposit eggs. Females were observed to fly both short distances (< 0.25 m) within host plant patches and, on occasion, moderately long distances between host plant patches (greater than 1km) (Lambert unpub. data). During dispersal events, females flew >1meter from the ground in a linear direction over open ground or along ridgelines, bluffs, road-cuts, trail edges, fences and/or shrub or forest edges. Upon finding appropriate host plant habitat, females searched for suitable host plants to oviposit eggs. Females flew < 1 m from the ground in small circular movements to investigate potential host plants (i.e., “searching”). “Searching” behavior was accompanied by bouts of short flights described as “sweeping”. “Sweeping” females flew short distances (< 0.25 m) between inflorescences and landed for 1 – 4 seconds. “Searching” females fluttered above host plants (touching host plants while in flight). “Searching” and “sweeping” behaviors likely contribute to host plant selection and presumably lead to acceptance or rejection of host plants based on host plant chemistry. After *E. ausonides insulanus* females selected appropriate host plants, they “walked” around on host plant buds and/or flowers to find suitable sites for ovipositing eggs. Other researchers have observed similar behaviors associated with oviposition site selection in Pieridae (Courtney 1986).

Over the course of this study females were not observed to lay eggs on more than one species of host plant, however, females were observed to move between patches of different host plants located within relatively close proximity. For example, females were observed to fly between *L. virginicum* var. *menziesii* habitat along the shoreline and adjacent upland grassland habitat occupied by *B. rapa* (> 300 meters). In addition, females flew between patches of *B. rapa* and *S. altissimum* within 200 meters in open grassland habitat. In both cases, different host plant species were located on the same slope but at different elevations. For this reason, movement between host plant patches is likely associated with dispersal aided by topography and off-shore wind (as opposed to host plant preference). In fact, the majority of adults that flew between patches of different host plant species returned to the original host plant patch. For example, in open grassland habitat females were observed to fly < 1 meter from the ground in wide circles (100 - 500 meters or more), moving in the direction up a slope and returning down slope eventually returning to the original host plant patch (i.e., foray searching) (Lambert unpub. data).

Females were not observed to lay eggs on more than one host plant species while conducting adult observational studies, however, eggs were observed on both *B. rapa* and *S. altissimum* in areas where both species co-occurred (e.g., overlapping host plant stands located in introduced grasslands in 2004) (Lambert unpub. data). It is likely that females are able to use more than one host plant species if both species are present and suitable for oviposition (e.g., relatively tall plants with multiple flower buds) (see Chapter 4; *B. rapa* and *Turritus glabra*).

Eggs were observed over a period of 48 ± 9 days (range: 37 – 59 days) from late April to early June (n=1617; 2005-2008) (Figure 2.7). The earliest record of eggs at American Camp was April 25, 2005. However, eggs were observed on *B. rapa* one day earlier on April 24, 2008 at Pear Point gravel pit (approximately 1500m north of American Camp). The latest record of eggs was observed on *L. virginicum* var. *menziesii* on June 22, 2005. Overall, females laid the greatest number of eggs between May 21st and May 24th (350) when all three host plant species overlapped in phenology (Figure 2.7).

In 2005, two *E. ausonides insulanus* adults were tracked during behavioral studies and were observed to live 6 – 9 days. Detailed observations of unique markings on the ventral hind wings were made in order to track adults. Individuals were easy to relocate over time because the population was relatively closed to immigration and emigration due to natural barriers (i.e., forest, open water). The estimated lifespan of *E. ausonides insulanus* based on a mark-recapture study conducted at the Pear Point Gravel pit in 2008 was 4.8 days (SE: 0.5; N=62; 68% recapture) (Peterson 2009). The recapture rate was biased towards males (2.9:1) and accounted for the relatively low persistence time (i.e., 4.8 days). In another study on *E. ausonides*, Scott (1973b) estimated the persistence time to be 5.5 days based on a mark-recapture study for which males were most often recovered. The discrepancy in estimated lifespan between observational and mark-recapture studies may be due to the potentially longer lifespan of females. Females of *E. ausonides* move at a greater distance and speed than males (Scott 1975b). Females may increase emigration rates in open populations and lower survival rates calculated for mark-recapture studies. In fact, the lifespan of females of *E. ausonides insulanus* may be double that of males. Under controlled lab conditions, one adult survived 14 days (S. Vernon pers. comm.). The increased longevity of females may be due to several factors. It may be that the life-span of females are longer because it takes more time for egg maturation, dispersal and selection of suitable host plants. Additionally, males may have a shorter life-span than females because conspicuous mate-locating behavior may expose them to higher levels of predation.

## Egg dispersion and egg load

In general, females oviposited a single egg on one inflorescence and dispersed to other unoccupied inflorescences to lay eggs, but in some cases females were documented to lay egg(s) on already occupied inflorescences. Eggs in different stages of development (i.e., greenish-white and red eggs) were rarely observed on the same *B. rapa* inflorescence (10%, N=328). Co-occurrence of different color eggs on the same inflorescence was rarely observed in three out of the four years of study. However in 2005, many greenish-white and red eggs were observed on the same inflorescences (25%, N=136; Table 2.5, Figure 2.5.A and B). This study discusses the possible mechanisms for both patterns of egg dispersion and egg loads observed in *E. ausonides insulanus*.

In general *E. ausonides insulanus* followed the same egg dispersion patterns described for *E. ausonides* (Shapiro 1980, Shapiro 1981a). For example, Shapiro (1980, 1981a) also observed that females of *E. ausonides* avoid laying eggs on already occupied inflorescences. He postulated that the presence of “red eggs” on host plants deter females from ovipositing more eggs on the same host plant and encourage them to disperse in search of host plants without red eggs (Shapiro 1980). This hypothesis is also known as “egg-load assessment”. Thomas (1984b) experimentally tested the “egg-load assessment” theory using a related pierid species, *Anthocharis cardamines* L. and found that females were more likely to oviposit on inflorescences without eggs. A further study by Dempster (1992) showed that *Anthocharis cardamines* can detect the presence of con-specific eggs on plants and will avoid them when laying eggs.

The patterns behind the “egg load assessment” hypothesis have been observed in other pierid species but since Shapiro (1981a), researchers have determined that one of the mechanisms by which females disperse eggs is driven by ovipositing-deterring-pheromones (ODP) (Rothschild and Schoonhoven 1977, Schoonhoven et al. 1990, Dempster 1992, reviewed by Renwick and Chew 1994) not necessarily the color of the “red egg” (Courtney 1986). For example, Dempster (1992) determined that *Anthocharis cardamines* deposits a water soluble pheromone at the time of egg-laying and that ODP deters the same female from laying additional eggs on the same inflorescences.

Ovipositing-deterring-pheromones could explain why most eggs of *E. ausonides insulanus* were observed to be the same color (i.e., laid by a single female) on single inflorescences, however, it does not explain the relatively high co-occurrence of greenish-white and red eggs (i.e., eggs laid on a single inflorescence by more than one female) in 2005.

It's unclear why egg-laying patterns are inconsistent in *E. ausonides insulanus*, although it is possible that the presence of ODP plays a role. Some research supports the idea that ODP are important in regulating intraspecific competition (e.g., Dempster 1992) whereas other studies have shown that ODP do not always regulate intraspecific competition. For example, in field experiments, Ives (1978) and Root and Kareiva (1984) showed that the presence of *P. rapae* eggs on host plants was not a deterrent to ovipositing females, and Shapiro (1981a) demonstrated that newly laid green eggs also of *P. rapae* (presumably having ODP) were not a deterrent to ovipositing females. Still, other studies show that ODP is a key factor reducing interspecific competition between *P. brassicae* and *P. rapae* (Schoonhoven et al. 1990). Perhaps, ODP has evolved to reduce interspecific competition (as opposed to intraspecific competition) in species such as *P. rapae* and *E. ausonides insulanus*. In the absence of interspecific competition ODP may act as a mechanism reducing intraspecific competition especially in butterfly populations where there are low levels of interspecific competition. In the case of *E. ausonides insulanus* at American Camp low levels of competition between other pierids (i.e., *P. rapae*) may increase the effects of ODP on intraspecific competition, thus resulting in single eggs on single inflorescences. This egg-laying pattern (i.e., single egg on a single inflorescence) is the most commonly observed pattern in *E. ausonides insulanus* but on occasion (e.g., 2005) multiple eggs on a single inflorescence were observed (Figure 2.5).

Observations of multiple eggs on the same inflorescence were mainly attributed to data collected in 2005 when multiple eggs of the same color were frequently observed (84%, n=136; Table 2.5). There was a sharp increase in the number of multiple eggs between survey dates in 2005 (May 14th and May 24th). Although there was no difference in the distribution of eggs on plants between surveys (half of the plants were bearing eggs on both survey dates), there was a difference in the number of eggs per raceme (i.e., inflorescence). For example, for the first survey, 33 eggs were observed on 20 racemes compared to the second survey when 128 eggs were observed on 54 racemes. More than 80% of plant racemes had multiple eggs on the second survey. The largest number of eggs recorded consisted of 10 green eggs on a single inflorescence (Figure 2.5.C). Thus if ODP are normally the mechanism by which *E. ausonides insulanus* regulates egg dispersion, this mechanism did not deter females selecting oviposition sites in 2005 and does not explain observations of multiple eggs on single inflorescences or the co-occurrence of greenish-white and red eggs.

The failure of ODP to regulate egg dispersion has been observed in *E. ausonides*. Shapiro (1984) suggests that “egg-load assessment” behavior (or ODP) of *E. ausonides* may on occasion fail when population density is unusually high. Factors such as population density (i.e., adult abundance) may have also contributed to unusual patterns of egg dispersion in *E. ausonides insulanus* in 2005. Other factors may have included 1) host plant density, 2) availability of robust host plant resources, and 3) an abundance of young females searching for oviposition sites simultaneously.

Multiple egg loads and unusual patterns of egg dispersion in the 2005 may have been attributed to relatively high levels of adult abundance (34 adults) in areas of low plant density (0.051 plants/m$^2$). If host plant resources are nominal (i.e., low density) and females are numerous, host plants are likely to receive more than one egg. Shapiro (1984) also found that when host density is reduced while population density is normal that an entire stand may receive disproportionate numbers of eggs.

The four year study further demonstrates that plants at low density (usually found at the edges of host plant patches) are more likely to receive proportionally more eggs. For example, the study site contained contagiously distributed host plants at low density in 2005. As *B. rapa* became more established at the study site over time, the number (and density) of host plants increased 279% (from 92 plants in 2005 to 349 plants in 2008) and egg abundance decreased 65% (from 136 eggs in 2005 to 48 in 2008). These results suggest that egg dispersion (multiple egg loads) may be related to host plant density. Multiple egg loads on the periphery of host plant patches (i.e., low density) have also been observed in *E. ausonides* (Shapiro 1981a) and other related pierid species (see review Courtney 1986). Based on models presented by Parker and Courtney (1984) egg loads are likely to increase where females are not encountering host plants as often (low density hosts or on edges of patches). For example, Courtney (1986) found that *Colias vauthieri* may oviposit up to 4 eggs per plant on plants at very low densities.

In addition to host plant density, multiple egg loads and unusual patterns of egg dispersion may have been attributed to robust host plant resources (i.e., large number of racemes on host plants in 2005). *Euchloe ausonides insulanus* females may lay multiple eggs on robust host plants in response to variation in resource availability for offspring. *Brassica rapa* plants surveyed in 2005 were more robust than plants surveyed in 2006-2008. For example, the mean number of racemes on plants with eggs in 2005 was 24.06 racemes/plant compared to 14.13 racemes/plant in 2006, 16.29 racemes/plant in 2007 and 8.44 racemes/plant in 2008. The difference in size was likely attributed to high levels of cumulative precipitation leading up to the growing season (November thru March). Cumulative precipitation was highest in 2005 (33.2 cm) compared all other years that surveys were conducted (2006, 14.66 cm; 2007, 24.31cm; 2008, 20.04cm). Additionally, there were several small bouts of precipitation over the course of the growing season (March-May) that may have contributed the increase in the number of racemes per plant. Modification of egg-laying behavior in response to host plant resources has been found in other pierid species (Shapiro 1979, 1980, 1981, Parker and Courtney 1984, Courtney 1984). Parker and Courtney (1984) found that plants that were more robust provided more resources to offspring of *P. brassicae* and as a result females increased the number of eggs laid at one time (i.e., “clutch size determination”). Clutch size has been correlated with leaf size of host plant species for other Lepidoptera (Pilson and Rausher 1988, Vasconcellos-Neto and Monteiro 1993, Kagata and Ohgushi 2001). Similar to leaf size, robust plants of *B. rapa* are likely to influence egg-laying patterns in *E. ausonides insulanus*.

Several factors relating to adult abundance and behavior may also help to explain the unusual egg-laying patterns observed 2005. An abundance of young females laying eggs simultaneously may have contributed to the co-occurrence of greenish-white and red eggs on single inflorescences. Data collected from relative abundance surveys bordering the study site suggest that several young females were laying eggs in host plant habitat simultaneously during the height of flight season in 2005. Adult surveys were conducted on May 13th, 21st and 25th spanning the period between egg surveys during which time the numbers of adults remained relatively constant over the course of the egg survey period (May 13 – 5 adults, May 21 – 8 adults and May 25 – 4 adults) (Appendix B). Because time for oviposition is limited and average lifespan of adults is relatively short (6-9 days), the high number of adults (17) observed within a relatively short period of time (May 13-25) suggests that young females were laying eggs on host plant over the course of the study. Most females die before laying their entire egg complement, so it is thought to be advantageous for young females to lay as many eggs as possible to increase their lifetime fitness (Parker and Courtney 1984). Nomakuchi et al. 2001 found younger females of *Anthocharis scolymus* to be less discriminate than older females. Younger females were more likely to lay eggs on plants with existing eggs compared to older females. For example, 59% of females of *Anthocharis scolymus* died before reaching the average longevity which suggests that the time and resources normally used for dispersal may be used to lay as many eggs as possible in the first few days. Egg loads (the number of eggs laid by a single female at one time) may also be higher when the female is young (Parker and Courtney 1984). Finally, poor weather conditions may also contribute to punctuated bouts of high eggs loads. Adults emerge over a relatively short period of time (mid-Apr- late May) when weather conditions are unpredictable (e.g., high winds, precipitation or cold temperatures) which may prevent females from dispersing and laying eggs. The inability of many pierid species to lay eggs due to poor weather conditions is well documented and closely linked to reproductive success (Shapiro 1979, Hayes 1981, Cappuccino and Kareiva 1985, Doak et al. 2006). For this reason, when weather is favorable it may be advantageous for females to lay as many eggs as possible to increase their lifetime fitness. Thus observations of high numbers of eggs and multiple eggs of the same color on the same host plant may also be related to factors associated with weather.

## Egg-laying patterns and host plant traits

Host plant traits such as plant height, number of racemes and phenologic stage influenced egg-laying patterns on all three host plant species (*B. rapa*, *S. altissimum* and *L. virginicum var. menziesii*). Plants with eggs were significantly taller (mean height) than plants without eggs for *B. rapa*, *S. altissimum* and *L. virginicum* var. *menziesii* (U=23132.5, *P*<0.001;

U=887.5, *P*=<0.001 and U=228.0, *P*=<0.001 respectively) (Figure 2.8). Similarly, plants with eggs had a greater number of racemes than plants without eggs in *B. rapa*, *S. altissimum* and *L. virginicum* var. *menziesii* (U=26965.5, *P*=<0.001; U=1346, *P*=<0.001 and U=228, *P*=<0.001 respectively) (Figure 2.8). These results suggest that females prefer to oviposit on plants that are taller and have a greater number of racemes.

Interestingly, the height of *B. rapa* plants supporting eggs was consistently similar across the four year study (mean height ranged from 84–86cm); there was no significant difference in mean height of *B. rapa* plants with eggs among populations between years (F=0.041, d=3,130, *P*=0.99). The combined mean height of *B. rapa* plants with eggs over the four year period was 84.43 ± 18.30 cm SD (n=134; range: 37 − 130) (Figure 2.8). This suggests that females may select plants within a very narrow height range, and that this host plant trait may be a good indicator of the presence of eggs. Measuring host plant height may be particularly important when sampling eggs at large scales when a complete census of all host plants is impractical.

Similar to height, the mean number of racemes on *B. rapa* with eggs was significantly greater than plants without eggs. The mean number of racemes on plants with eggs was 15.10 ± 15.88 SD (n=134; range: 1− 82) close to doubled the number of racemes on plants without eggs 7.76 ± 10.90 SD (n=668; range: 0 - 150) (Figure 2.8). However, unlike plant height, there was a significant difference in mean number of racemes for plants with eggs between years (F=3.868, d=3,130, *P*=0.01). The difference between years was attributed to the mean number of racemes for plants with eggs in 2005 (24.06 racemes per plant, n=18) compared to the mean number of racemes in 2008 (8.44 racemes per plant, n=27). The low number of racemes in 2008 may be due to factors such as increased herbivory, low mean temperature and low precipitation. For example, deer herbivory was not observed in 2005, however, in 2008, 48% of plants with eggs were observed to be eaten by deer. Deer were observed to eat multiple racemes of host plants. Additionally, mean temperature and precipitation was substantially lower in 2008 than in 2005. The mean temperature in April 2008 was 6.67° C and 9.44° C in 2005. Cumulative precipitation leading up to host plant establishment (November thru March) was 20.1 cm in 2008 and 33.2 cm in 2005. The combined effects of low temperature and low precipitation were likely to have slowed the growth rate of host plants.

Similar egg-laying patterns were observed for *S. altissimum* and *L. virginicum* var. *menziesii*. For example, plants with eggs were taller and more robust compared to plants without eggs (Figure 2.8). Managers interested in tracking eggs and larvae on host plants may use plant height and number of racemes to identify potential host plants with eggs. The mean height of plants with eggs of *S. altissimum* and *L. virginicum* var. *menziesii* was 45.62 ± 9.60 cm SD (n=26; range: 30 − 66) and 24.11 ± 5.82cm SD (n=19; range: 12 − 37) respectively. The mean number of racemes of plants with eggs of *S. altissimum* and *L. virginicum* var. *menziesii* was 1.96 ± 1.00cm SD (n=26; range: 1 − 4) and 3.16 ± 1.89cm SD (n=19; range: 1 − 7) respectively.

It may be that females use visual cues such as taller and more robust host plants to locate oviposition sites. In other words, females may perceive large plants more easily than small plants. Larger plants have a greater number of oviposition sites and are more conspicuous in the landscape. Many researchers have focused on understanding the relationship between plant size and oviposition rates in pierid species. For example, several studies of *P. rapae* found a positive correlation between plant size and oviposition rates (Ives 1978, Jones and Ives 1979, Latheef and Irwin 1979, Langan et al. 2001). Courtney (1982) found that host plant species with the highest floral surface area (*Cardamine pratensis* and *Barbarea vulgaris*) were typically used more often by *Anthocharis cardamines* L. compared to host plants species with smaller inflorescences (*Cardamine hirsute* L. and *Thlaspi alpestre* L.). A positive relationship between plants size and oviposition choice has also been found in several *Euchloe* species (Shapiro 1985, Karban and Courtney 1987, Dennis 1995). Karban and Courtney (1987) found *Euchloe hyantis* females to oviposit on significantly taller plants of *Streptanthus tortuosus* occurring on rocky exposed slopes, which suggest that taller plants were more apparent to searching females. Oviposition selection in *E. ausonides insulanus* is also likely to be influenced by visual cues (plant height) that aid females in host plant location.

## Plant phenology

The presence of eggs was dependent on host plant phenology (i.e., phenologic stage) for some host plants but not others. The presence of eggs was marginally dependent on host plant phenology (phenologic stages 1 – 3) for *L. virginicum var. menziesii* ($x^2$ =5.42; df=2; P=0.066) while the presence of eggs were not dependent on host plant phenology (phenologic stages 1 – 3) for *S. altissimum* ($x^2$ =1.87; df=2; P=0.39). However, the presence of eggs was highly dependent on host plant phenology (phenologic stages 1– 4) for *B. rapa* ($x^2$ =10.50; df=3; P=0.015). It is likely that the marginally significant and non-significant result in *L. virginicum var. menziesii* and *S. altissimum* were due to the combination of shorter growing season (Figure 2.7) and lack of variability in size classes within host plant stands. For example, out of 111 *L. virginicum var. menziesii* sampled 15% were classified as phenologic stage 1, 83% of plants as phenologic stage 2 and 2% as phenologic 3.

In contrast, stands of *B. rapa* showed more variability in phenology and each phenologic stage had higher sample sizes and therefore differences may have been easier to detect. Young host plants of *B. rapa* with buds and flowers (phenologic stages 1 and 2) were selected over host plants that were more developed (i.e., phenologic stages 3 and 4, plants with no buds). Young plants in phenologic stage 2 received 76% of all eggs (n= 134) compared to plants in phenologic stage 3 (plants with no buds and less than 50% flowers) which received 16% of all eggs. Older plants in phenologic stage 4 (no buds or flowers only developing fruit) received only 1% of all eggs. Surprisingly, the youngest plants with more buds than flowers (phenologic stage 1) received only 7% of all eggs. One of the reasons for this outcome may be that plants in early stages of development are shorter in height than surrounding vegetation, which may make host plants physically difficult to access (Lambert personal observation).

Overall, the data indicates that eggs will most likely be encountered on host plants that have flowers compared host plants without flowers (phenologic stages 1–3). Ultimately, host plants that possess both flowers and buds (i.e., phenologic stage 2 plants <50 percent buds and >50 percent flowers) were selected by ovipositing females over the course of the four year study. The presence of buds more than doubles the likelihood that a female will select a particular plant to oviposit eggs.

The 2005 host plant phenology study of *B. rapa* serves as one example of egg-laying patterns over one season. The study site consisted of both *B. rapa* plants that emerged early in the season and *B. rapa* plants that emerged later in the flowering season (i.e. over-lapping stands in different stages of phenology). The early stand of *B. rapa* germinated and bloomed in early April and subsequently developed fruit by the time of the first survey (May 14th), as a result there were low numbers of eggs observed on older plants at the study site (i.e., females likely dispersed to other areas to lay eggs). In contrast, plants surveyed on the second survey (May 24th) consisted primarily of buds and flowers. The later stand of *B. rapa* received 4 times the amount of eggs compared to plants surveyed during the first survey. This suggests that host plants in earlier stages of development were selected over plants in later stages. Similarly, Shapiro (1984) found that *E. ausonides* preferred to oviposit on plants in earlier stages of development and concluded that plant phenology is one of the most important criteria for oviposition.

Not only is host plant phenology an important factor influencing oviposition selection but it is also a key determinant of egg and larval survivorship. *Euchloe ausonides insulanus* larvae were observed to survive on young plant tissues (buds and flowers) compared to older seedpods that are difficult for first instars to penetrate. For example, senescent plant material led to the desiccation of 104 larvae (n=1617) (Lambert unpub data). It is thought that females may select younger plants over older plants as suitable oviposition sites because developing larvae are more likely to survive on young nutritious plant tissue than on older and tougher plant material (Opler 1974, Ives 1978, Wiklund and Ahrberg 1978, Courtney and Duggan 1983).

In another host plant, *S. altissimum*, it was also found that larval growth and survival was dependent on the timing of oviposition. Data from 2006 showed that plants that grew earlier in the season supported larval growth and survival for a longer period of time than plants that emerged later in the season. In other words, eggs laid earlier in the season (May 7 – June 2), and larvae emerging from these eggs, had higher survival than eggs that were laid later in the season (June 3 – June 19). Thirteen percent of eggs laid between May 7th and June 2nd survived to 5th instar compared to 4% of eggs laid between June 3rd and June 19th (n=341) (Lambert unpublished data). Plants that grew later in the season were more mature and most likely offered fewer resources to developing larvae. Constraints of host plant phenology on survival and growth of larvae has been observed in numerous Lepidoptera (e.g., Ehrlich et al. 1975, Shapiro 1979, Weiss et al. 1988, Cushman et al. 1994, Boughton 1999, Doak et al. 2006). For this reason, the relationship between oviposition preference and larval survival is important to understanding many aspects of host plant use.

Variation in host plant phenology among different host plant species can contribute to diet breadth (Shapiro 1975, 1976, Chew 1977, Wiklund 1982, Courtney and Forsberg 1988). Courtney and Forsberg (1988) showed pierid species, *Anthocaris belia* and *Pontia daplidice* broadened their diets to include other host plants when primary host plant species were rare. In the current study, differences in plant phenology likely contributed to the use of non-native host plant species, such as *B. rapa* and *S. altissimum*. For example, even though the flowering phenology of *S. altissimum* and *L. virginicum* var. *menziesii* closely overlap (Figure 2.7), *L. virginicum* var. *menziesii* grows more sporadically and matures at a faster rate than *S. altissimum*.  In addition, *B. rapa* flowers much earlier than *L. virginicum* var. *menziesii* providing oviposition sites for females that emerge from diapause early.

### Egg-laying patterns and host plant density

Many researchers have documented higher egg loads on host plants that are located on the edges of host plant patches or host plants that are highly dispersed. This has been demonstrated for *Euchole* spp. (Shapiro 1975, 1984, Shapiro 1985), *Colias vauthieri* (Courtney 1986), *Anthocharis cardamines* (Wiklund and Ahrberg 1978, Courtney and Courtney 1982) and *Pieris rapae* (Harcourt 1961, Kobayashi 1965, Jones 1977a). Consistent with other studies, results showed that areas of low density host plants received higher egg loads at both study sites (Figure 2.4) containing separate populations of *B. rapa* and *S. altissimum*.

At the *S. altissimum* study site there was a significant negative correlation between numbers of eggs and host plant density ($\rho = -0.71$, $P$=0.01). In other words, more eggs occurred in low density host plant study plots. In addition, a significant difference was detected between the mean numbers of eggs in high and low densities plots (U=3, $P$=0.005). Both tests confirm that areas of low density *S. altissimum* received higher egg loads compared to areas of high density host plants.

The findings were similar for the *B. rapa* site. Overall, the majority of eggs (88%) were found on host plants growing in relatively low densities (< 6.5 racemes/m$^2$ or approximately 1 plant/m$^2$) compared to plants growing at high densities (>8.4 racemes/m$^2$ or approximately >1 plant/m$^2$) (Figure 2.9). The Spearman's rank correlation test detected a marginally negative significant relationship between numbers of eggs and density of host plants ($\rho = -0.32$, $P=0.07$). In other words, more eggs occurred in low density host plant plots.

The lack of a stronger correlation was likely a result of sampling effects related to an over representation of low density plots. If the study site had equal areas of high and low density *B. rapa* plots or included a larger sample of high density plots (>8.4 racemes/m$^2$) the results may have shown a stronger inverse-density relationship. In fact, when a subset of high and low density plots (n=18; >8 racemes/m$^2$ and <2 racemes/m$^2$ respectively) were analyzed separately, mean number of eggs laid was found to be statistically higher in low than in high densities of *B. rapa* (U=17, $P=0.03$).

The distribution of high egg loads on host plants at low densities is thought to be related to the flight patterns of many pierids (Jones 1977a, 1977b, Courtney and Courtney 1982, Yamamoto 1982, Root and Kareiva 1984). Isolated plants located at the edges of host plant patches receive proportionally more eggs than plants located in dense patches (see *Host plant patch study*). This pattern may be due to the distance females disperse beyond the edges of host plant patches and deposit eggs on isolated plants at the boundaries of the patch. In spatially heterogeneous habitat, *E. ausonides insulanus* females fly moderately long distances (i.e., > 4m) before locating and landing on host plants. The distance *E. ausonides insulanus* females fly between oviposition events likely influences the distribution of eggs on host plants. For example, females were observed to fly shorter distances between laying eggs when they were observed in patches that were relatively large and moderately dense (Lambert unpub. data).

Research by Root and Kareiva (1984) have shown similar patterns in *P. rapae* egg densities among low and high dense patches of host plants. In an experimental garden study, low densities of host plants received more *P. rapae* eggs compared to host plants in high density areas. Interestingly, they found that females flew shorter distances between oviposition events in high density host plant patches but shorter movement patterns did not increase the proportion of eggs per plant in comparison to low density patches. In addition they found that *P. rapae* does not alter it's turning behavior according to changes in host plant densities and that females of *P. rapae* disperse by linear flight paths (<45º). These findings support the theory that *P. rapae* selects host plants in a relatively random manner (Root and Kareiva, 1984).

The flight paths of female *P. rapae* described by Root and Kareiva (1984) likely depict the distribution and landscape-scale movement of *E. ausonides insulanus*. Root and Kareiva (1984) suggested that females disperse randomly and that this behavior may insure that females "spread the risk" of mortality among their offspring in environments that are unpredictable. Other researchers suggest linear flight paths are a result of females avoiding areas of high parasitoid attack. Shapiro (1975) and Ohsaki and Sato (1994) found that parasitoid attack was greater in dense host plant patches. However, it should be noted Karban and Courtney (1987) did not find a relationship between host plant density and parasitism of *Euchloe hyantis*.

Throughout its range, *E. ausonides insulanus* utilizes hosts that are ephemeral, ruderal and occur predominantly in areas of high disturbance (i.e., unpredictable environments) and flight patterns may have evolved to reduce offspring mortality as Root and Kareiva (1984) suggested. However, it is also likely that flight patterns of *E. ausonides insulanus* have co-evolved with host plant patterns of succession and are driven by patchy host plant dynamics. Open population structure (i.e., high rate of emigration and persistent local colonization of new host plant patches) of *E. ausonides insulanus* may be a result of the species dependence on *R*- strategist host plant species. Other research supports this idea. For example, Dennis et al. (2004) found a clear link between butterfly mobility and host plant strategies using Grime's plant strategy model. They showed that *R*-strategist (i.e., ruderal host plant strategy) was highly correlated with butterfly mobility, geographic range and patchy host plant distribution. In an early study of *Pieris rapae* movement, Jones (1977b) argued that host plant ecology contributed to differences in movement patterns in two separate populations of

*P. rapae* located in Canada and Australia and that these differences were likely a result of adaptations to unpredictable host plant resources (Jones and Ives 1979, Jones et al. 1987) . Finally, Chew and Courtney (1991) found that ovipositing females of *Euchloe belemia* Esper, a butterfly that occurs in open dry grasslands in the Mediterranean neglected to lay eggs on populations of hosts in mesic montane areas where host plants were more abundant and stable. These studies illustrate the importance of how flight patterns of butterflies may have co-evolved with host plant patterns of succession.

The idea that females select host plants in a relatively random manner (Root and Kareiva 1984) aptly describes the behavior of *E. ausonides insulanus* at large spatial scales (i.e., patches on the landscape). However, host selection involves different behavioral mechanisms at different spatial scales. For example, in the study conducted by Root and Kareiva (1984) females flew shorter distances in areas of high density host plants suggesting that female dispersal is not completely random but that behavior may also be influenced by cues in the environment (i.e., patch size and color). More recent research examining flight behaviors of *P. rapae* suggest that flight behavior is a combination of random movement and attraction toward a particular object in the environment (Turchin 1998, Bukovinszky et al. 2005). Bukovinszky et al. (2005) showed an inverse-density relationship between *P. rapae* and patch size based on visual cues (smaller patches with greater perimeter area ratios were more likely to be encountered). Other visual cues such as color (i.e., hue and tint) have been shown to play a role in host plant location of *P. rapae* (Hovanitz and Chang 1964, Traynier 1979, Myers 1985, Renwick and Radke 1988). Flight behavior in *E. ausonides insulanus* is also likely to be influenced by visual cues such as color. For example, it may be that females are attracted to the bright yellow color of *B. rapa* flowers especially when clusters of flowers appear in high contrast to the opaque green landscape comprised of thick grasses. Future research is needed to determine the mechanisms behind egg-laying patterns in *E. ausonides insulanu,s* but data in this study indicates that low density host plants received higher egg loads.

### Host plant patch study - background

To further investigate the relationship of egg-laying patterns and host plant structure (i.e., host plant density and patch size) a host plant patch study was conducted in 2006 (Figure 2.4

and 6). The goal of this study was to provide recommendations for the design of host plant habitat. Historical components of the site may be important to understanding *E. ausonides insulanus* occupation of host plant habitat at this location. At the time of study, the study site was being restored to native grassland habitat. Prior to restoration activities, the site was part of contiguous introduced grassland and very few individuals of *S. altissimum* were observed at the study site (34 plants restricted to two locations within the study site). Between 2004 and 2006 the study site was repeatedly burned, herbicide was applied and several hundred native grass plugs were planted. Above-ground biomass was reduced which effectively eliminated the dense stand of introduced grasses between host plants. This resulted in an open matrix between host plant patches. Due to significant increases in soil disturbance and lack of competition from introduced grasses, *S. altissimum* established in small numbers on the edges of experimental restoration plots. Shortly thereafter, *E. ausonides insulanus* began to occupy *S. altissimum* habitat. On June 15, 2005, 40 late-instar larvae were removed from 36 plants of *S. altissimum* and relocated to alternate *S. altissimum* habitat in an effort to protect *E. ausonides insulanus* from a scheduled controlled burn. A year later, the number of *S. altissimum* plants in the restoration area increased more than 15 times for a total of 570 plants. Of the total host plants present at the site in 2006, 210 plants were occupied by 341 eggs (116 late-instar larvae). Large-scale restoration activities such as burning were complete in 2005. Poast© herbicide was applied to a portion of the study site during the spring of 2006. By spring 2007, stands of native and non-native vegetation established and as a result, the number of host plants at the restoration site decreased in 2007 to 61 plants. No eggs were observed.

*Sisymbrium altissimum* plant phenology, structure and spatial distribution differed dramatically in the study site (i.e., disturbed grasslands) compared to sand dune habitat. The timing and frequency of disturbance likely caused the sudden increase in *S. altissimum* and may be compared to other habitats where *S. altissimum* quickly establishes in open sandy soils that are frequently disturbed by wind or erosion. However, the establishment of *S. altissimum* at the restoration site differed from other areas of *S. altissimum* habitat in three respects. First, plants in the restoration site were definitely clustered as compared to dense continuous stands of *S. altissimum* growing in open sand. Second, many *S. altissimum* plants occurring in isolation or on the edges of a patches were taller and more robust compared to plants associated with large stands of *S. altissimum* in sand dune habitat. Third, the maturation of *S. altissimum* plants located in the restoration site progressed at a slower rate and as a result supported greater numbers of eggs and developing larvae later in the season compared to *S. altissimum* plants growing in dry sandy soils that senescence quickly as seasonal temperatures increase. For example, at the restoration site females oviposited 84 eggs on plants of *S. altissimum* from June 12th – June 18th (27% of all eggs at the restoration site) compared to 2 eggs on plants located in sandy soils (3% of all eggs surveyed in sites with sandy soils). Furthermore, late-instar larvae were observed feeding on *S. altissimum* as late as July 12th at the restoration site. Late-instar larvae were not observed in sandy sites after June 17th due to plant senescence.

The population structure of *S. altissimum* in highly disturbed grasslands is similar to the population structure of *B. rapa*. Some of the factors that contributed to *S. altissimum* establishment at the restoration site in 2006 were the implementation of different disturbance regimes (e.g., burning and herbicide), variation in soil moisture, location and viability of the seed bank and competition with other early successional species. Many of these environmental factors are also associated with the establishment of *B. rapa* in introduced grasslands. For example, clusters (or patches) of *S. altissimum* located in the restoration site resembled the patchy distribution of *B. rapa* in adjacent grassland habitat. For these reasons, recommendations and conclusions from the host plant patch study may be applied to populations of *S. altissimum* in highly disturbed grassland sites or minimally disturbed introduced grasslands where *B. rapa* occurs.

### Host plant patch study - results and discussion

The results from the host plant patch study provide managers with several ways to think about the spatial structure of host plants (i.e., patch size, connectivity and host plant density) and how that relates to egg densities. The results of the patch study may be useful for understanding how to maximize the number of eggs at different scales. Number of eggs may be measured per square meter or per host plant. The percent of plants occupied by eggs may also be useful to patch design depending on the goals of restoration.

Medium size (100 – 300m$^2$), moderately dense (0.25 – 0.75 plant/m$^2$) patches received the highest number of eggs per square meter (0.25 – 0.35 eggs/m$^2$). Host plant patches 3 and 4 received 30% of all eggs at the study site (Table 2.3; Figure 2.6). Small size (75 – 80m$^2$), moderately dense (0.20 – 0.40 plant/m$^2$) patches also received relatively high numbers of eggs (e.g., patch 7; Table 2.3). These results suggest that medium, moderately dense patches maximize the number of eggs per square meter. Medium-moderately dense patches support high numbers of eggs because dense patches are likely to have plants that vary in plant phenology. Individual plants mature at different rates within the patch and collectively provide females with suitable oviposition sites over the course of the flight season. For example, females were reported to lay eggs in medium-moderately dense patches from early May to mid June. The longevity of host plant patches (and subsequent variation in host plant phenology over the course of the flight season) may also be influenced by site-specific factors such as soil moisture. For example, host plant patch 3 was located in an area known to have relatively high levels of soil moisture (Lambert 2006). Other site related factors such as the presence of a seed bank may have also contributed to the abundance of host plants in patch 3. For example, plants of *S. altissimum* were observed in patch 3 in 2004 and 2005. Lastly, the edge of moderately dense patches may be perceived by females more readily than isolated host plants which may contribute to repeated oviposition by multiple females.

Large size (>800m$^2$), low density (< 0.1 plants/m$^2$) patches (patch 1; Table 2.3, Figure 2.6) maximize the number of eggs per host plant (as opposed to moderately dense patches that maximize the number of eggs per square meter). It is likely that host plants received multiple eggs per plant because of the distance a female moves between host plants (i.e., >4m). In this case, large, low density patches may be comparable to large groups of isolated plants. Isolated plants were more likely to receive multiple egg loads from more than one female and females dispersing distances greater than 4 meters were more likely to lay multiple eggs on a single plant. For example, patch 1 (854.04m$^2$ at 0.09 plant/m$^2$) received one of the highest egg loads averaging 1.95 eggs per plant.

Patch 1 also contained the highest number of eggs (72 eggs) of all patches at the study site. It is likely that numbers of eggs increase with patch size because with an increase in patch size there is also an increase in host plant abundance that offsets variation in host plant availability (i.e., plant growth form, phenology). Conversely, patch 1 had one of the lowest numbers of eggs per $m^2$ (0.08; Table 2.3). This is not surprising considering that large patch size reduces the number of eggs per square meter.

In addition to receiving multiple eggs per plant, almost half of all plants growing in large, low density patches were occupied by eggs. This phenomenon is similar to plants that grow in isolation or at the edges of host plant patches. For example, 45% of the 85 plants growing in isolation were occupied by eggs. These data are comparable to egg data collected from plants growing in low to medium dense patches (<0.1 – 0.25 plants/$m^2$). In low to medium dense patches 46 – 68% of plants received eggs irrespective of patch size. These studies support the idea that females disperse 4 meters on average before laying eggs and that host plants growing in isolation (separated by 4 or more meters) are more likely to be occupied by eggs. Large, low density patches may provide the optimal distance between host plants and act like “stepping stones”. For this reason, large, low density patches may be useful for planning the matrix between patches of medium, moderately dense host plant habitat.

Outcomes of the *Host plant patch study* corroborate results from the *Egg-laying patterns and host plant density study*. Dense host plant patches consistently received fewer eggs per square meter in both *S. altissimum* and *B. rapa* host plant studies. In the *B. rapa* density study, plots that had > 1 plant/$m^2$ received significantly fewer eggs than plots with < 1 plant/$m^2$ (Figure 2.9). In the *S. altissimum* patch design study, host plant patch 5 (86.94 $m^2$ at 0.89 plant/$m^2$) received the second lowest number of eggs per square meter (0.05 eggs/$m^2$; Table 2.3). Furthermore, in the *S. altissimum* density study, areas of low density *S. altissimum* received higher egg loads compared to areas of high density host plants. Number of plants averaged 0.42 plants/$m^2$ (SD±0.43; range: 0.09 − 1.14, n=5) in plots with eggs compared to plots without eggs that averaged 18.32 plants/$m^2$ (SD±11.14; range: 0.22 − 36.71) (Figure 2.10). Importantly, these results indicate that high density host plant patches (>1 plant/$m^2$) should be avoided when planning the design of host plant habitat.

To better understand the role of host plant density at small scales, isolated plants (Figure 2.6) were grouped according to the number of plants growing in 1$m^2$. Most eggs on plants growing in isolation were found on single plants (70%) compared to plants growing in groups of 2 (10%) or 3 (20%) (Table 2.4). Even though distance between plants is likely to play an important role in determining the presence of eggs, data suggests that even on a small scale ($1m^2$) host plant density contributes to egg-laying patterns.

Understanding the relationship between patch size and density may be useful for locating the maximum number of eggs in areas where host plant habitat is abundant and widespread and resources to track eggs are limited. Patch size may also be useful in the design of host plant habitat if the goal is to maximize the number of eggs per square meter or per host plant. In general, larval resources (i.e., host plants) were observed to be abundant and survivorship was found to be independent of patch size and density. Late instar larvae were observed in 9 of the 13 patches and 2 of the 3 isolated plants groups (Tables 2.3 and 2.4). The mean percent survival of eggs to instar V was relatively consistence across patches (0.12 ± 0.159 SD; n=13; Table 2.3) which suggests that larvae are not limited by the abundance of larval food resources. However, the synchrony (or asynchrony) of egg and larval development and host plant development is likely to influence survivorship. For example, more than 50% of the eggs that survived to instar V were laid early in the season (May 15 – 20). This suggests that eggs laid on young plants are more likely to survive because larvae and host plants mature at the same rate.

Medium-moderately patches support high numbers of eggs because dense patches are likely to have plants that vary in plant phenology. Individual plants mature at different rates within the patch and collectively provide females with suitable oviposition sites over the course of the flight season.  The longevity of host plant patches (and subsequent variation in host plant phenology over the course of the flight season) may also be influenced by site-specific factors such as soil moisture. For example, host plant patch 3 was located in an area known to have relatively high levels of soil moisture (Lambert 2006).

## CONCLUSION

The results of this research suggest that females prefer to oviposit on plants that are taller and have a greater number of racemes among all three host plant species. In *B. rapa,* the presence of eggs was also highly dependent on plant phenology. In the host plant density study, egg loads were highest in areas where host plants (*B. rapa* and *S. altissimum*) occurred at low densities. The host plant patch study showed that medium size, moderately dense patches received the highest number of eggs per square meter and that dense (>1 plant/$m^2$) host plant patches received the lowest numbers of eggs per square meter. The fundamental relationship between adult biology, egg-laying patterns and host plant ecology is critical to understanding population changes in *E. ausonides insulanus*. This study aims to help researchers predict the occurrence of eggs and larvae among host plants and host plant patches. The results of the patch study may also be useful for understanding how to design host plant habitat that maximizes oviposition site selection by *E. ausonides insulanus*.

**Table 2.1** Plant communities and habitat attributes that support *Euchloe ausonides insulanus* at American Camp, San Juan Island National Historical Park, San Juan Island, Washington.

| **Plant community** | **Attributes** | **Topography** | **Disturbance** |
|---|---|---|---|
| Tidal lagoon and shoreline | tidal saturation and salinity; low nutrient rocky/sandy soils | north-facing shoreline, driftwood berms | active, tidal action, seasonal flooding |
| Grassland | strong summer winds; dense non-native grasses; moderately deep sandy soils over clay till | north and south-facing slopes, shrub and tree edges, banks, bluffs | intermittent, small mammals |
| Sand dune | open sand; microclimate conditions caused by varied topography | north and slope-facing sand banks, swales, bluffs | active, wind action |

**Table 2.2** Plant communities and associated host plants, nectar resources, mate location and pupation sites that support *Euchloe ausonides insulanus* at American Camp, San Juan Island National Historical Park, San Juan Island, Washington. Host plant species in parenthesis are host plants also used for nectar and mate location.

| Plant community | Host plant(s) | Nectar resources | Mate location | Pupation site(s) |
|---|---|---|---|---|
| Tidal lagoon and shoreline | *Lepidium virginicum var. menziesii* | *Cakile maritima*<br>*(Lepidium virginicum var. menziesii)* | *Achillea millefolium*<br>*(Lepidium virginicum var. menziesii)* | *Salicornia virginica* |
| Grassland | *Brassica rapa*<br>*(Sisymbrium altissimum)* | *Brassica rapa*<br>*(Sisymbrium altissimum)* | *Brassica rapa*<br>*(Sisymbrium altissimum)* | *Holcus lanatus, Teesdalia nudicaulis* |
| Sand dune | *Sisymbrium altissimum* | *Abronia latifolia*<br>*Amsinkia intermedia*<br>*Cerastium arvense*<br>*(Sisymbrium altissimum)* | *Cerastium avense*<br>*(Sisymbrium altissimum)* | *Elymus mollis* |

**Table 2.3** Host plant patch study, *Sisymbrium altissimum*, 2006.

| **map number** | **patch area ($m^2$)** | **number of plants** | **patch density ($plant/m^2$)** | **number of eggs** | **number of plants with eggs** | **number of eggs per plant** | **number of eggs per $m^2$** | **number of instar V larvae** | **% survival to instar V** |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 854.04 | 80 | 0.09 | 72 | 37 | 1.95 | 0.08 | 7 | 0.10 |
| 2 | 370.48 | 40 | 0.11 | 45 | 27 | 1.67 | 0.12 | 5 | 0.11 |
| 3 | 230.02 | 145 | 0.63 | 56 | 42 | 1.33 | 0.24 | 5 | 0.09 |
| 4 | 103.64 | 25 | 0.24 | 36 | 15 | 2.40 | 0.35 | 4 | 0.11 |
| 5 | 86.94 | 77 | 0.89 | 4 | 4 | 1.00 | 0.05 | 2 | 0.50 |
| 6 | 81.33 | 15 | 0.18 | 9 | 7 | 1.29 | 0.11 | 1 | 0.11 |
| 7 | 74.17 | 27 | 0.36 | 21 | 9 | 2.33 | 0.28 | 2 | 0.10 |
| 8 | 68.59 | 20 | 0.29 | 2 | 2 | 1.00 | 0.03 | 0 | 0.00 |
| 9 | 60.74 | 8 | 0.13 | 7 | 4 | 1.75 | 0.12 | 0 | 0.00 |
| 10 | 55.83 | 10 | 0.18 | 14 | 8 | 1.75 | 0.25 | 1 | 0.07 |
| 11 | 55.56 | 8 | 0.14 | 7 | 5 | 1.40 | 0.13 | 0 | 0.00 |
| 12 | 45.80 | 19 | 0.41 | 7 | 6 | 1.17 | 0.15 | 3 | 0.43 |
| 13 | 41.91 | 11 | 0.26 | 5 | 3 | 1.67 | 0.12 | 0 | 0.00 |

**Table 2.4** Isolated plants of *Sisymbrium altissimum* growing in groups of one, two and three. Host plant patch study, 2006.

| number of plants/m$^2$ | number of eggs/m$^2$ | total plants | total eggs | number of instar V larvae | % survival to instar V |
|---|---|---|---|---|---|
| 1 | 0 | 2 | 0 | 0 | 0 |
| 1 | 1 | 22 | 22 | 0 | 0 |
| 1 | 2 | 3 | 6 | 1 | 0.5 |
| 1 | 11 | 1 | 11 | 0 | 0 |
| 2 | 0 | 8 | 0 | 0 | 0 |
| 2 | 1 | 8 | 4 | 0 | 0 |
| 2 | 2 | 2 | 2 | 1 | 0.5 |
| 3 | 0 | 18 | 0 | 0 | 0 |
| 3 | 1 | 9 | 3 | 0 | 0 |
| 3 | 2 | 12 | 8 | 0 | 0 |

**Table 2.5** Numbers of white and red eggs on host plants, May 14 and May 24, 2005.

| 14-May | | |
|---|---|---|
| **number of racemes** | **greenish-white eggs** | **red eggs** |
| 12 | 0 | 1 |
| 5 | 0 | 2 |
| 2 | 0 | 5 |
| 1 | 2 | 0 |

| 24-May | | |
|---|---|---|
| **number of racemes** | **greenish-white eggs** | **red eggs** |
| 18 | 0 | 1 |
| 7 | 0 | 2 |
| 4 | 0 | 3 |
| 2 | 0 | 4 |
| 3 | 0 | 5 |
| 8 | 1 | 0 |
| **3** | **1** | **1** |
| **1** | **1** | **2** |
| **1** | **1** | **3** |
| **1** | **1** | **7** |
| **1** | **2** | **1** |
| **1** | **2** | **2** |
| **1** | **2** | **4** |
| 1 | 4 | 0 |
| 1 | 5 | 0 |
| 1 | 10 | 0 |

**Figure 2.1** Pair of adults mating on *Cerastium arvense*, a native herbaceous perennial located in native prairie habitat at American Camp, 2005. *Euchloe ausonides insulanus* wings are white and yellow with a greenish marbled texture under the hind wing. *Cerastium arvense* has white flowers which attract males and serve as a mate location sites as well as a nectar resources.

**Figure 2.2** Mating behavior and wing coloration. Female resting on *Achillea millefolium* lifts abdomen in direction of pursuing male. This posture is commonly associated with the behavior of unreceptive females in Pieridae (Scott 1973, 1986). Wings of *E. ausonides insulanus* have dark markings that expand dorsally and wing bases are strongly shadowed (Pyle 2004).

**Figure 2.3** Adult phenology, behavior and egg phenology study sites at American Camp, San Juan Island National Historical Park, San Juan Island, Washington. Circles represent 16 belt-transects (200m x 30m wide), triangles represent 9 sites where adult behavioral studies were conducted (approximately 2 km2) and squares indicate 7 egg study sites. One egg study site is located southeast of American Camp and is not represented on the map. Overlapping shapes represent the same study location.

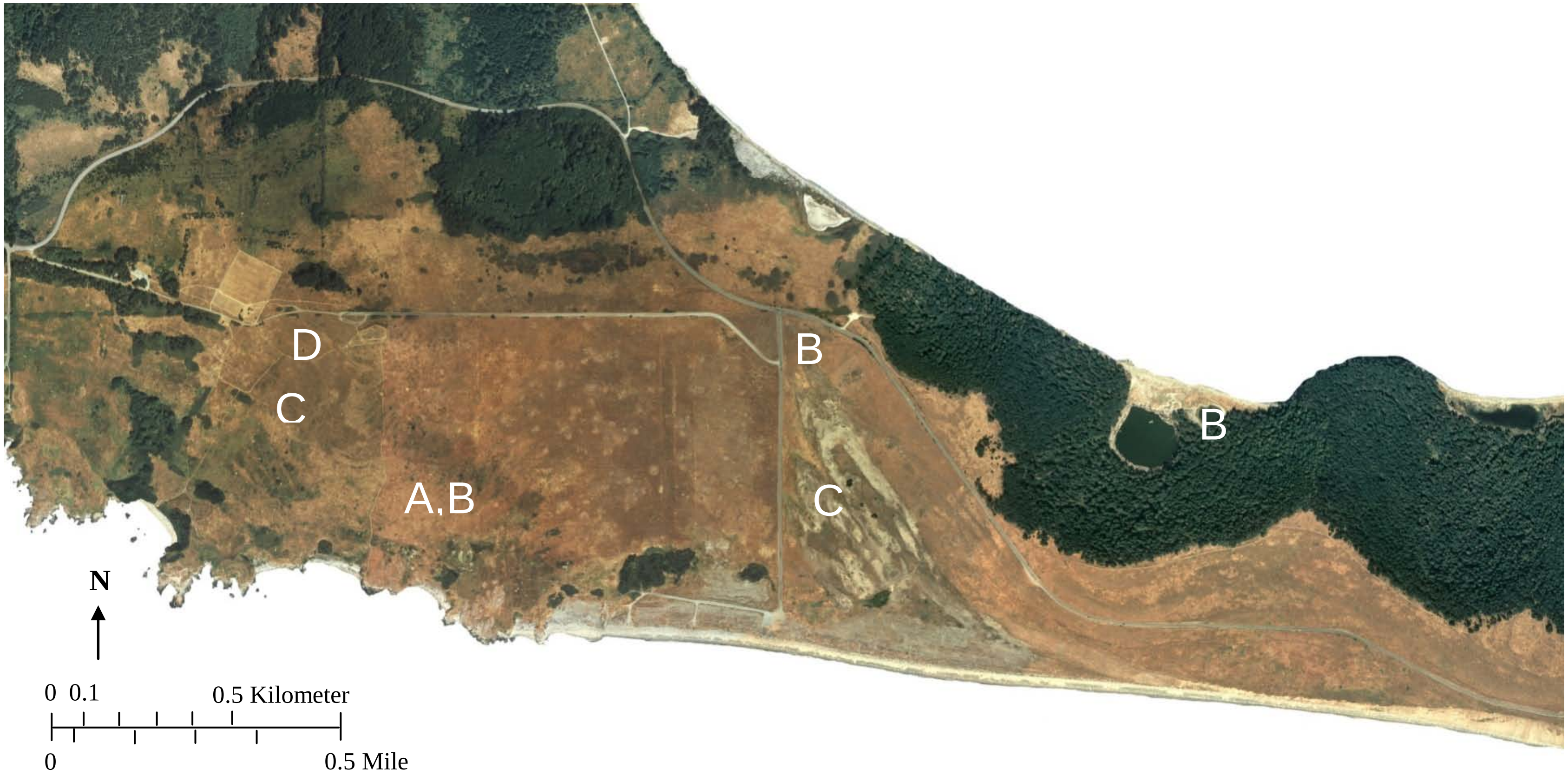

**Figure 2.4** Location of study sites at American Camp, San Juan Island National Historical Park, San Juan Island, Washington. A) Egg dispersion and egg load B) Egg-laying patterns and host plant traits C) Egg-laying patterns and host plant density and D) Host plant patch study

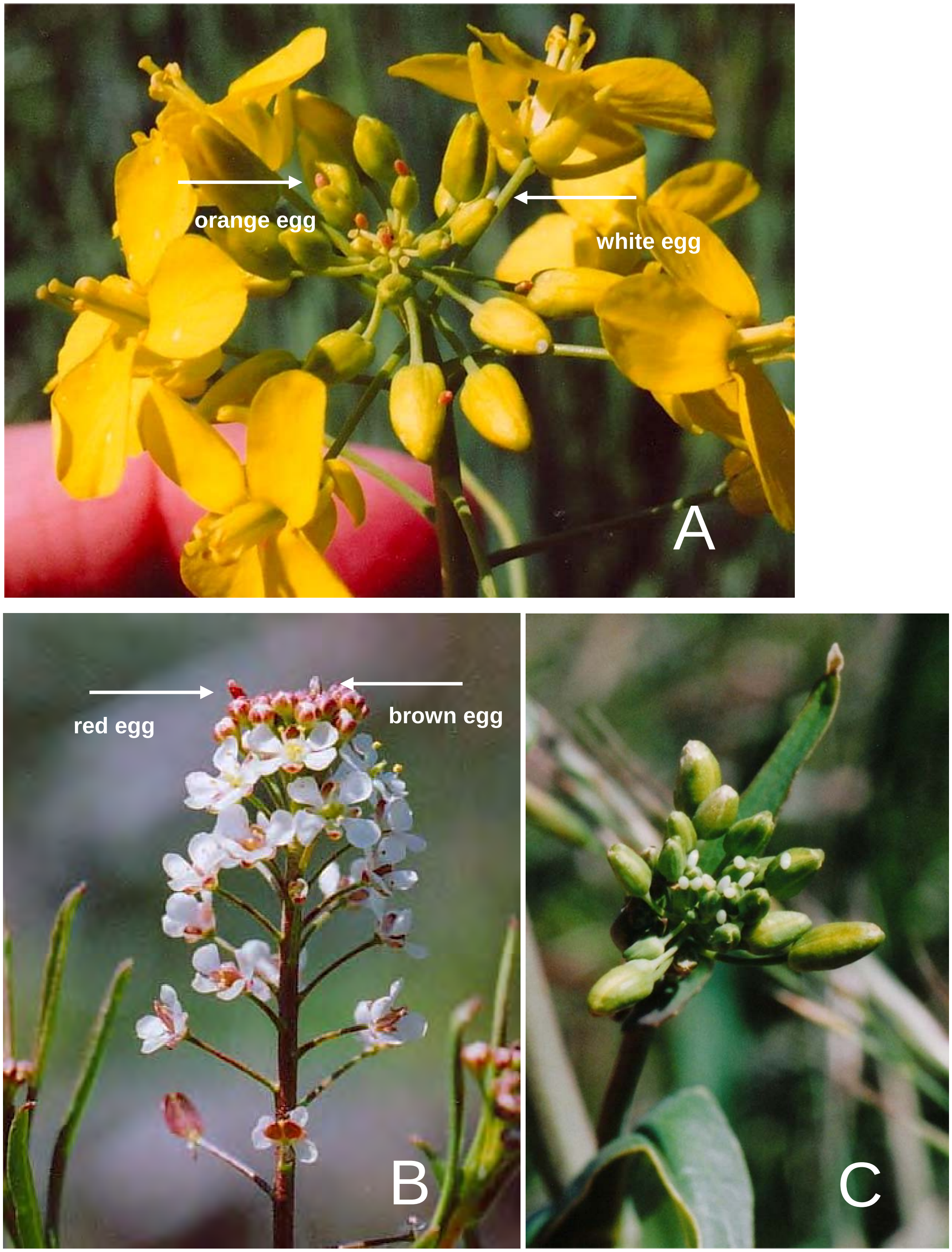

**Figure 2.5** Egg development on host plants, *Brassica rapa* and *Lepidium virginicum* var. *menziesii* in 2005. Different colors of eggs represent different stages of development indicative of multiple oviposition events on the same inflorescence. **A**) A total of 8 eggs (one greenish-white and 7 red eggs) and one first-instar larva on a single inflorescence of *B. rapa*. **B**) Red and brown eggs on a single inflorescence of *L. virginicum* var. *menziesii*. **C**) Multiple eggs (10 white eggs) laid during a single oviposition event on *B. rapa*.

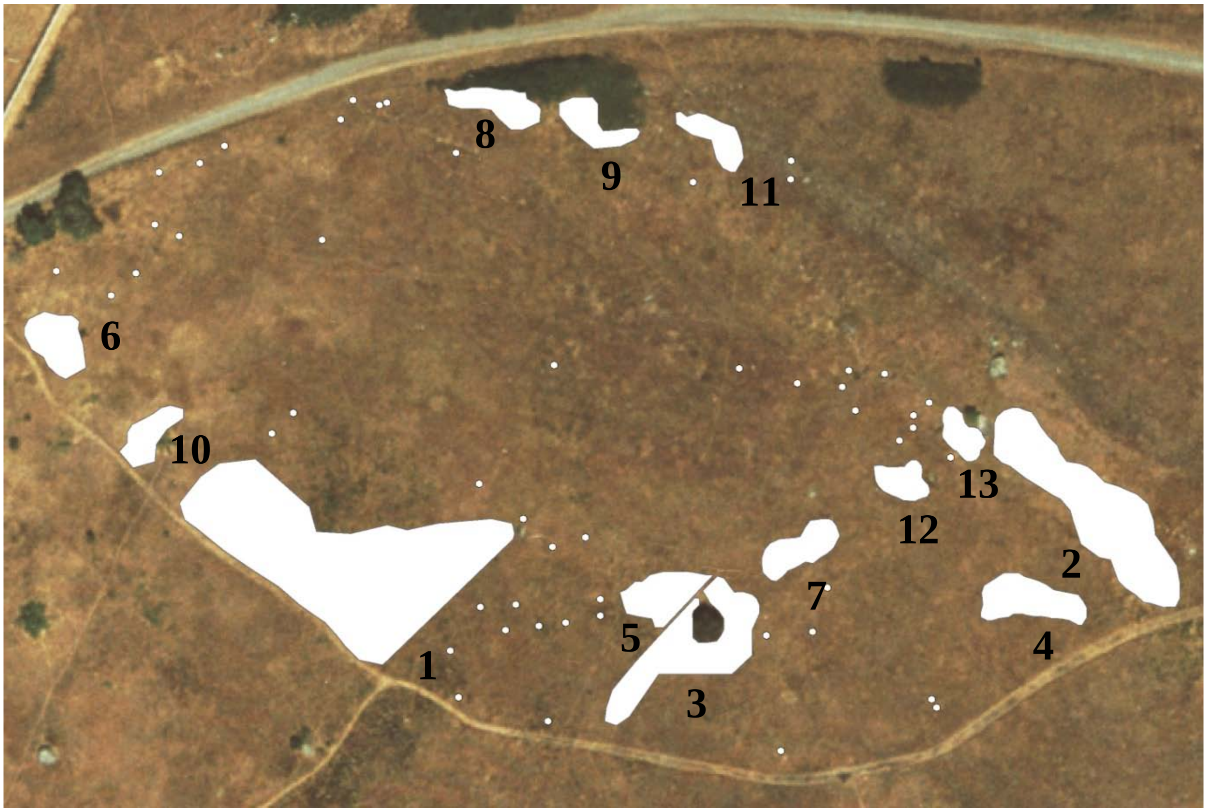

**Figure 2.6** Location of patches of *Sisymbrium altissimum* described in the host plant patch study, 2006. Numbers correspond to host plant patches (i.e., map number) described in Table 2.3.

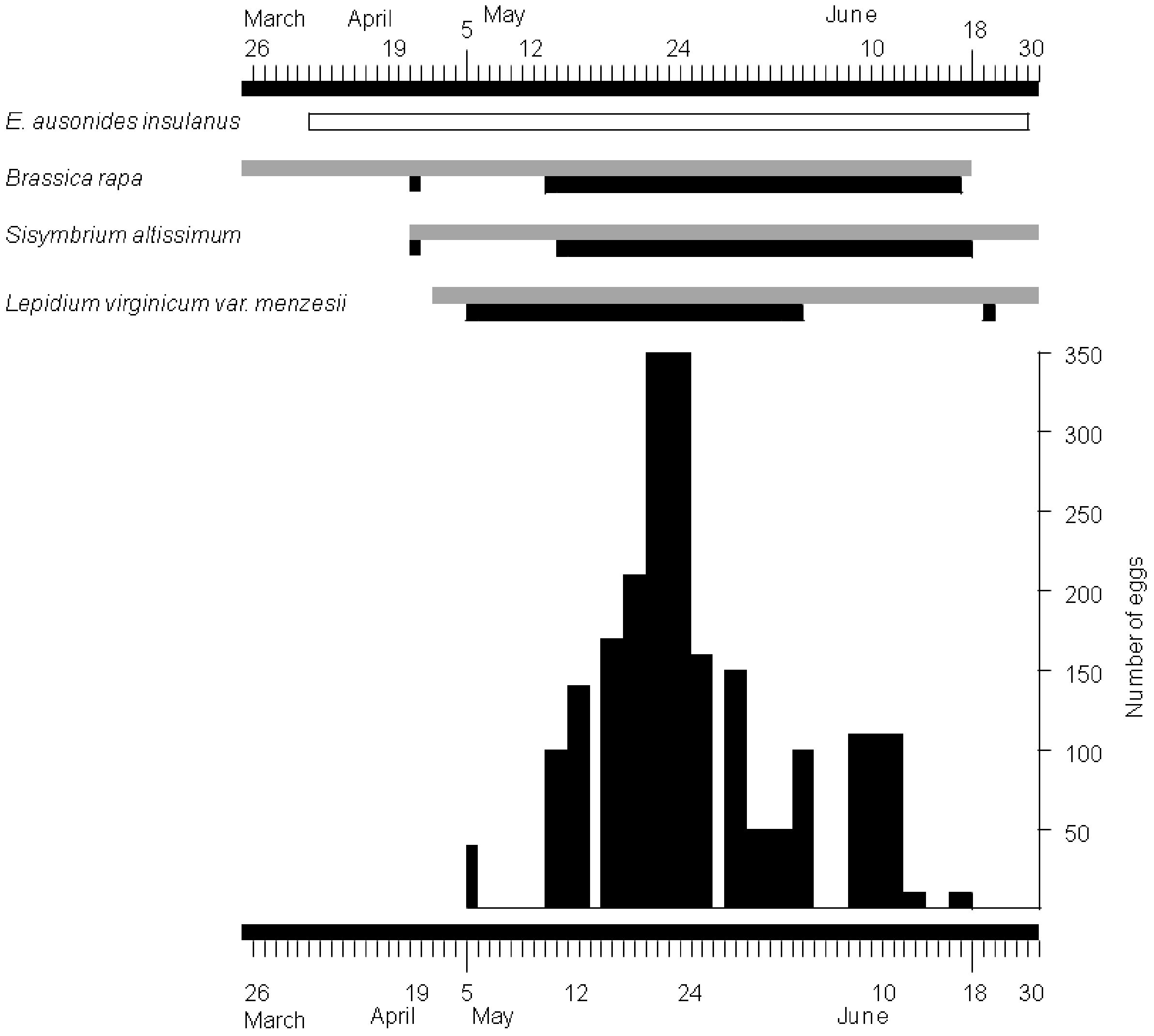

**Figure 2.7** Adult, egg and host plant phenology of *Euchloe ausonides insulanus* at American Camp (SAJH), San Juan Island, Washington, 2004 − 2008. Adult phenology (723 adults) indicated by the white horizontal bar, egg phenology (1617 eggs) indicated by black horizontal and vertical bars and host plant phenology indicated by gray horizontal bars. The distance between ticks from March 26 – May 5th and June 18 – June 30th represent two days. Adult flight period was from April 8–June 28th (white bar), host plants flowered from March 26 – June 30th (gray bars) and eggs were laid from May 5 – June 18th (black bars). Egg census periods were collapsed as indicated by the width of vertical bars. Black bars correspond with host plant flowering period and represent the phenology of eggs laid on each host plant species. The black boxes outside of the black bars indicate the dates when eggs were observed at extreme ends of the season. The earliest observation of eggs was April 25, 2005 and latest observation was June 22, 2005. The greatest number of eggs (350) was laid between May 21st and May 24th.

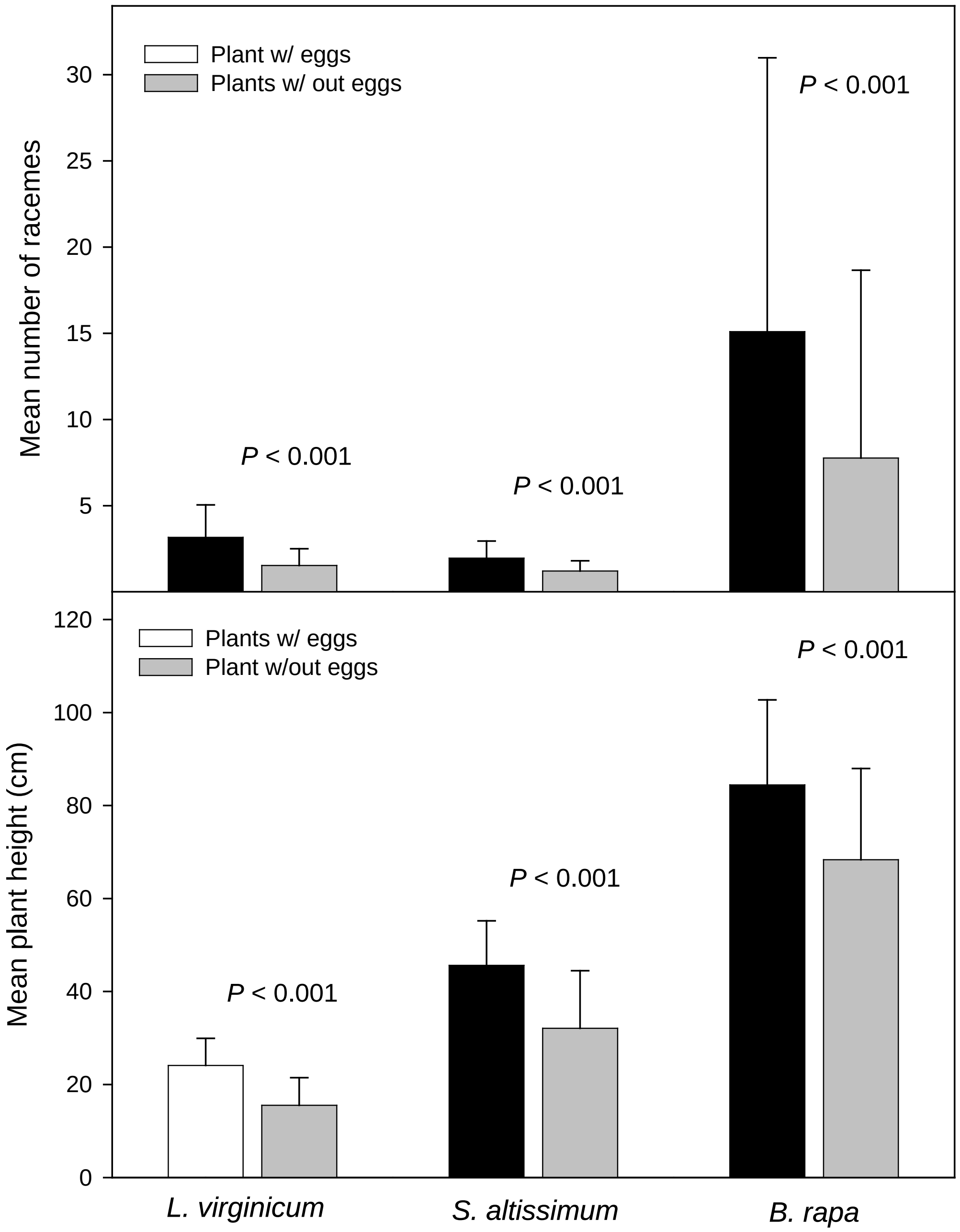

**Figure 2.8** Comparison of height and number of racemes for host plants *Brassica rapa*, *Sisymbrium altissimum* and *Lepidium virginicum* var. *menziesii*, with and without eggs Host plants, with eggs were significantly taller (mean height) and had a greater number of racemes than plants without eggs.

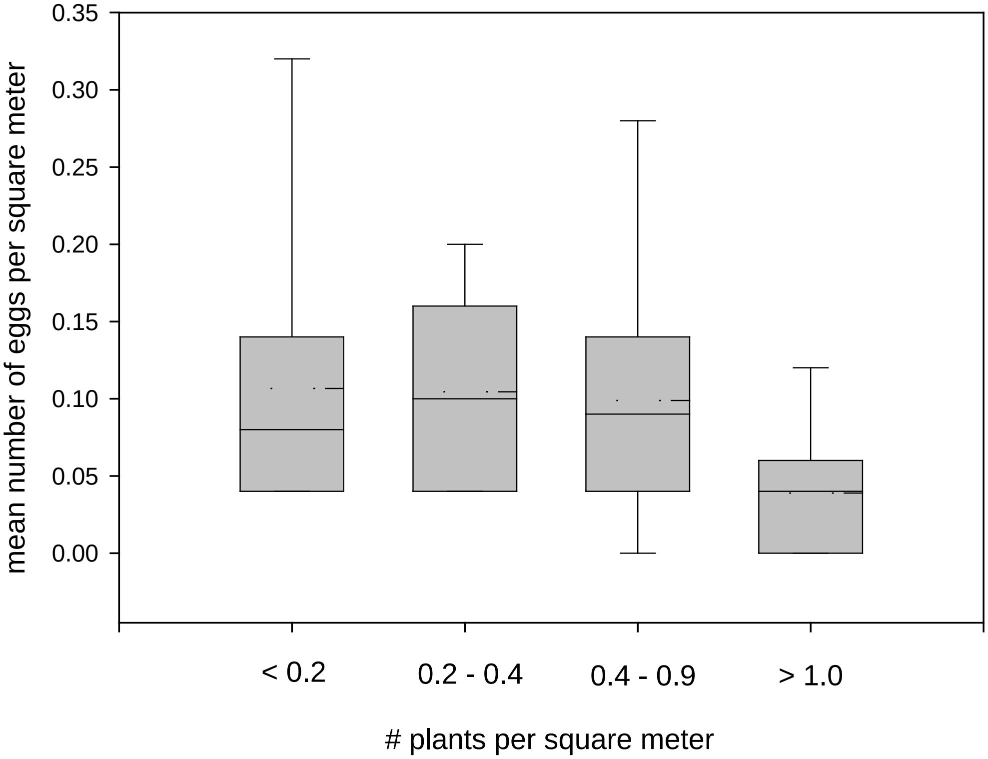

**Figure 2.9** Comparison of the mean number of eggs and number of plants of *Brassica rapa*, 2008. Plots that had > 1 plant/m$^2$ received significantly fewer eggs than plots with < 1 plant/m$^2$. Dashed line represents means (0.170, 0.150, 0.100, 0.040), solid line represents medians (0.08, 0.10, 0.08, 0.04).

**Figure 2.10** Number of plants per square meter with and without eggs. *Sisymbrium altissimum* host plant density study, 2008. Areas of low density *S. altissimum* received higher egg loads compared to areas of high density host plants. Dashed line represents means (18.32, 0.42) and solid line represents medians (17.5, 0.25) for plants with no eggs and plants with eggs, respectively.

# Chapter 3

# Mortality and Survivorship of Immature Stages

## INTRODUCTION

The island marble butterfly, *Euchloe ausonides insulanus*, is restricted to San Juan and Lopez Island in the San Juan archipelago and known populations are limited to a few to no more than one hundred individuals (Miskelly and Potter 2009). Host plant resources are abundant and widely distributed across both islands but the butterfly remains rare. This suggests *E. ausonides insulanus* is limited by factors other than the availability of host plant resources.

Theory predicts that females should avoid unsuitable host plants and select host plant species that support larval development and survival, in other words, host plant preference is heritable and responsive to selection and therefore host plant selection should optimize larval performance (Jaenike 1990, West and Cunningham 2002). Data derived mostly from manipulated field experiments have shown that multiple factors account for differences in egg and larval performance including host plant chemistry (e.g., Singer 1971, Tabashnik 1983, Thomas et al. 1987, Keeler & Chew 2008), exploitation of enemy-free space (Bernays and Graham 1988, see also Thompson 1988, Murphy 2004, Mulatu et al. 2004, Wiklund and Friberg 2008) and intra and interspecific competition (e.g., Rausher 1979, Dempster 1983, Chew and Robbins 1984). However, few studies have shown that larval growth and survival vary as a function of using different host plant species under naturally occurring field conditions (Raushner 1980, Feeny et al. 1985, Moore 1989, Agosta 2008, Wiklund and Friberg 2009). My research builds on field studies that focus on extrinsic sources of mortality and habitat variables (e.g., host plant size and density) that address local host plant use in *E. ausonides insulanus*. This study quantifies differences in host plant utilization, larval survival and the cause of mortality on three primary host plant species over a four year period.

Knowledge of factors causing mortality is essential to developing a better understanding of *E. ausonides insulanus* population dynamics. Studies on Pieridae suggest that eggs and larvae are susceptible to many species of parasitoids including species of the genus Apanteles (see review by Courtney 1986, Cole 1959, Warren 1984, Wiklund and Ahrberg 1978, Shapiro 1981 jewel flower, Karban and Courtney 1987, Kristensen 1994). Pieridae are also vulnerable to predation by invertebrates such as spiders, pentatomid bugs, Syrphid larvae, carabid beetles, mites and wasps (Parker 1970, Wiklund and Ahrberg 1978, Jones and Ives 1979, Hayes 1981, 1984, 1985, Dempster 1967, Jennings and Toliver 1976, Guppy and Shepard 2001, Schmaedick and Shelton 1999) and vertebrates such as birds (Dempster 1984, Kristensen 1994). Additionally, mammals have been found to have an impact on Lepidoptera populations by browsing host plants that reduce larval food plant resources (Miller et al. 1992, Littlewood 2008) or result in the incidental predation of eggs (Guppy and Shepard 2001, Gomez and Gonzalez-Megias 2007). Specifically, deer herbivory has been shown to have negative effects on insect herbivores (Bergström et al. 2000, Den Herder et al. 2004, Allombert et al. 2005). Black-tailed deer, *Odocoileus hemionus*, are endemic to the San Juan Island archipelago (Gonzales and Arcese 2008) and preliminary data suggest that deer may indirectly compete with *E. ausonides insulanus* for host plant resources. Deer herbivory is reported to impact populations of other threatened and endangered butterfly species. For example, the data collected by the New Hampshire Natural Heritage Inventory Program reported deer damage on wild lupine, *Lupinus perennis*, the primary host plant of the federally endangered karner blue butterfly, *Lycaeides Melissa samuelis* (Miller et al. 1992). In the current study, data suggests there may be differences in site-specific mortality caused by deer herbivory. Deer related mortality may be related to the distance of host plants from forest cover. Deer are more likely to browse on vegetation in open areas if vegetation is in close proximity to forest cover (Harlow 1984).

This study quantifies larval survival and mortality that may contribute to the rarity of *E. ausonides insulanus* and provides the first insights into the key role of immature stages in the demography of *E. ausonides insulanus*. The objectives of this research are to assess whether survivorship differs among three host plant species (one native and two non-native host plant species), assess which factors cause mortality (e.g., predation and deer herbivory) of immature stages and determine which immature stages (egg, instars I-V larval stages) are most vulnerable to different sources of mortality.

## METHODS

### Study sites and species

Demographic surveys were conducted at eight sites located on the southern end of San Juan Island, Washington, from 2005-2008. Seven sites were located within the boundaries of American Camp, SAJH (Figure 3.1) and an additional site was located in the Cattle Point Natural Resource Conservation Area (NRCA). The Cattle Point site (site 4) is located southeast of American Camp and not depicted on Figure 3.1. Sites were selected based on habitat type, patch occupancy and spatial distance between sites. The distance between any two sites ranged from 1800m–500m and were well defined (i.e., only one species of host plant located at the site).

*Euchloe ausonides insulanus* Guppy and Shepard 2001 (Pieridae) is a rare pierid found on San Juan and Lopez islands in the San Juan Island archipelago, Washington (USA). *Euchloe ausonides insulanus* was presumed extinct until 1998 when several adults were rediscovered on San Juan Island (Fleckenstein and Potter 1999). *Euchloe ausonides insulanus* is currently a candidate for state listing and is a conservation priority for US Fish and Wildlife and the National Park Service (Pyle 2006). *Euchloe ausonides insulanus* is supported by both introduced host plant species, *Brassica rapa* L. var. *rapa*, field mustard, and *Sisymbrium altissimum* L., tumble mustard and native host plant species, *Lepidium virginicum var. menziesii* (DC) Hitchc, tall peppergrass. *Lepidium virginicum* var. *menziesii* is the only native host plant species known to support *E. ausonides insulanus*. *Brassica rapa* is the most abundant of the three host plants and occurs in introduced grasslands with moderate levels of disturbance created by small mammals. *Sisymbrium altissimum* occurs in sand dunes but occasionally occupies disturbed areas in introduced grasslands. *Lepidium virginicum* var. *menziesii* occurs between tidal lagoons and shoreline.

### Sampling design

Host plants were searched for eggs and subsequently tracked through all immature stages of development until death or disappearance. A total of 1617 individuals were followed over the four year study (2005-2008). Not all sites were surveyed every year (Table 3.1). Censuses were conducted on 894 eggs occurring on Brassica rapa at four sites, 606 eggs on

Sisymbrium altissimum at three sites and 117 eggs on Lepidium virginicum var. menziesii at one site. For the purpose of analyzing death due to deer herbivory, an additional 63 eggs occurring on B. rapa (site number 1) were tracked in 2007. I conducted a complete census (surveyed every host plant within the site boundaries for eggs and subsequently tracked development of all eggs found) over four years (2005-2008) at site 2 and two years (2006-2007) at site 3 and 4 (Table 3.1). This type of exhaustive survey could only be conducted at a small number of sites (2-4) because few sites had less than 200 host plants that could be monitored repeatedly within the short seasonal time frame. All other sites (1, 5-8) contained abundant widely distributed host plants. I used a stratified sampling design to sample host plants. A random sample design was not used because of low probability of finding eggs. The sampling design was based on the results of research that showed that adults select host plants based on plant height, age class and density (Lambert 2011, Chapter 2). Eggs were surveyed at each site within the seasonal time frame. I surveyed as many eggs as possible to avoid low sample sizes due to attrition.

### Census procedure

Newly deposited eggs (less than 48 hours old) were tracked. The age of eggs was determined by the color of the egg. Eggs in early development ranged in color from white to orange. Color coded wire and ink markings on buds, flowers and leaf petioles were used to locate eggs and larvae as they developed. At each site an initial survey was conducted (Table 3.1). Cohorts of eggs and larvae were sampled at each site from the egg stage to late instar larvae, or until all larvae died. Individuals were recorded as egg, larval instars I-V or pupa. Eggs were inspected at five-day intervals, early instar larvae at three-day intervals and late instar larvae were inspected several times a day. Coloration of eggs and larvae size and morphology were used to identify larval instars (Lambert 2011, Chapter 1). Diagnostic drawings of setae and pinaculae (or chalazae) for all larval stages (instars I-V) of *E. ausonides* (Opler 1974) were used as a baseline for field observations. Larvae that were observed in the final instar (instar V) were recorded as having survived. This is the accepted procedure for species that wander from the host plant in search of a pupation site (Doak et al. 2006, Wiklund and Friberg 2009). However, this method assumes that there is no predation on instar V larvae during the time they are feeding, moving between host plants in search of food or wandering to pupation sites. To get the best estimate of survival to pupation, only those larvae observed in the last stage of instar V (i.e., inactive, positioned downward on the host plant stem and with marked changes in color) were recorded as survived to pupation. Predation was not observed in observational studies of larvae wandering to pupations sites (Lambert 2011, Chapter 1). However, observations of wandering larvae were limited to 3 individuals and therefore predation could have occurred in other instar V larvae without detection.

Methods for reporting larval survivorship of Pieridae vary. Several studies do not report on survivorship beyond instar IV because of larval movement in late stages of development (Cappuccino and Kareiva 1985, Doak et. al. 2006), other studies report observations of larvae at instar V as having survived to pupation (Wiklund and Friberg 2008, Wiklund and Friberg 2009). For this reason I have incorporated both of these methods. Survivorship of *E. ausonides insulanus* is reported for larvae that survive to instar IV, instar V and estimate survival to pupation (based on observations of larvae in the final stage of instar V).

If eggs and larvae were not observed between two consecutive surveys individuals were recorded missing from the host plant. Eggs and larval instars I-III missing from the host plant were recorded as predated because of the inability of early instar larvae to move to alternative host plants. Larvae that disappeared between instars IV- early instar V could not be confirmed to be dead because of the tendency of larvae to walk off the host plant in search of another food plant. When a disappearance occurred, neighboring host plants and the surrounding vegetation were thoroughly examined for larvae. Plants were inspected for damage contributing to the death of eggs and larvae including senescent or wilting plant material and shredded or torn stems. Sign of mortality including desiccated eggs and larvae, presence of spiders, webs, presence of competing Lepidoptera (unidentified moth species), Lepidoptera cocoons, torn and broken plant racemes and wilted and senescent (dry and tough) host plant material. Deer do not have upper incisors and therefore when feeding tear the stem from the plant. Tearing the stem leaves ragged ends of the primary stem, but is not easily recognizable on new growth. Therefore, evidence of deer herbivory included both the absence of buds, flowers and stems between two consecutive surveys as well as the absence of color coded wire demarcating the location of eggs and larvae. Direct observations of deer feeding on plant racemes as well as predation by spiders, wasps and birds were also recorded.

Mortality factors were grouped into five categories, predation, deer herbivory, starvation, plant damage and disappearance (Appendix C). Starvation included mortality caused by senescent host plant material and egg placement on petals of host plants (causing eggs to fall from the host plant). Plant damage included mortality caused by physical factors such as high wind (causing plant racemes to tear and break from contact with adjacent vegetation), wilted host plant racemes caused by insect herbivory or below-ground mammalian herbivory and inflorescences damaged by competing Lepidoptera. Disappearance included instar IV and early instar IV larvae that likely walked from the host plant. Unknown causes of mortality included observations of desiccated eggs and larvae.

### Statistical analysis-survivorship and mortality

Logistic regression models of survival were used to examine how different host plant species relate to the number of eggs likely to survive to pupation (beyond larval instar IV). Additionally, logistic hazard models were used to provide a focused look at how host plant species may affect mortality at intermediary stages of egg and larval development (instars I-IV). Hazard models assumed site and year effects were the same across egg and larval stages and offered a parsimonious way to examine how host plant species related to survival and mortality. Site was modeled as a random effect rather than fixed effects and assumed that the site effects were normally distributed with mean 0 and some constant variance. For this reason, models applied to eggs and larvae at a “typical” site. Models were fit with a complementary log-log link providing the exponentiated coefficients with hazard ratios for interpretation.

### Statistical analysis-deer herbivory

The impacts of deer herbivory on egg and instar I larvae on host plants *B. rapa* and *S. altissimum* were investigated from 2006–2008 using logistic regression models. Models were fit with Generalized Estimating Equations (GEE) to account for correlated observations due to multiple eggs on single host plants. The outcome of multiple eggs on the same host plant species was assumed to be similar but survival may vary from host plant to host plant. In other words, if there were multiple eggs, they were more likely to all be eaten at once, but whether or not eggs were eaten at all varied among host plants. The data were too sparse to reliably estimate coefficients for *L. virginicum* var. *menziesii* and life stages II-V and therefore was not included in the model. Variables included in the model were host plants *B. rapa* and *S. altissimum,* egg or instar I larva at death, and their interaction (all categorical variables). Individuals that were reported missing from the host plant were not included in the model. Other variables affecting mode of death were year, site and the number of eggs per host plant. Site was nested within host because only one host plant species occurred at each site; in other words, the host effect was an average of the site effects. Number of eggs was log-transformed to decrease the influence of a few very large numbers of eggs on single host plants. A significance level of 0.05 was used for inferential analyses.

The model is expressed as:

$$logit(p_{ijkm}) = \mu^* + y_i^* + h_1^* + s_{k(j)} + t_1^* + \rho * log(\# \, individuals)$$

where $\mu^*$ is the log-odds of deer death for the referent group (e.g., host plant *B. rapa*, year 2006, average site effect, egg stage, and 1 individual on a plant), $y_i^*$ is the log-odds for year i (i=2, 3), $h_1^*$ is the log-odds of deer death for individuals on *S. altissimum* plants, $s_{k(j)}$ represents site effects, and $t_1^*$ is the log-odds for individuals in instar I. An interaction term between stage and host plant species was added to the model to test for effect modification, however, the stage-host interaction was not statistically significant (p=0.615) and therefore not included in the final model results. Kendall’s coefficient of rank correlation was used to test for association between death due to deer herbivory (as percentage mortality) and the distance of research sites from nearest forest cover.

## RESULTS

### Survivorship

For all host plant species, years and sites combined, the average survivorship from egg to instar IV was 12%; survivorship to instar V was 5% and an estimated 3% of eggs survived to pupa (n=1617; Figure 3.2). Survival of eggs beyond instar IV (i.e., instar V) differed slightly depending on host plant species, 8.5% on *L. virginicum* var.*menziesii* and 7.1% on *S.*

*altissimum* to 3.8% on *B. rapa*, respectively (Figure 3.3). Mixed effects logistic models did not detect a significant difference in survivorship between host plant species that included year and site. However, the odds of survival beyond instar IV were estimated to be 2.45 times higher in *L. virginicum* var.*menziesii* (95% CI [0.89, 6.72]) and 1.79 times higher in *S. altissimum* (95% CI [0.91,3.5]) compared to *B. rapa* (logistic regression, Wald test of host plant effect, p=0.11) (Table 3.2). In terms of percentage, survivorship was highest on *L. virginicum* var.*menziesii* in 2007 (14.2%; Figure 3.3). Year was not a significant predictor of survivorship (p=0.19).

Although the models did not show differences in overall survivorship among host plants, chances of survival in early life stages (egg to instar IV) did vary between host plant species *L. virginicum* var.*menziesii* and *B. rapa,* as well as between *S. altissimum* and *B. rapa*. For example, the odds of survival to instar II (i.e., beyond instar I) on *L. virginicum* var.*menziesii* were 5.6 times that of survival on *B. rapa*, holding year and site constant (95% CI [1.6, 19.4], p=0.007) (Table 3.2). In short, chances of survival in early life stages were highest in *L. virginicum* var.*menziesii* and *S. altissimum* and lowest in *B. rapa* (Figure 3.4). Tests for differences in survival between *L. virginicum* and *S. altissimum* were not significant at any stage (Table 3.2).

### Mortality

There are noteworthy similarities and differences in mortality across stages between the three host plant species (Figure 3.5). In all three host plants, mortality was the highest from the egg stage to instar I (especially on *B. rapa*). Mortality then declined from instar II to instar III. After instar III, mortality increased again to instar IV, exception in *B. rapa* where larvae continued to decline. There were differences in mortality at each stage across the three host plant species. Model-fitted hazard profiles comparing host plant effects at each stage suggest that mortality differed significantly by life stage (egg to instar IV) (Table 3.3). Eggs and instar I larvae on *B. rapa* had a significantly higher estimated odds of mortality than both *L. virginicum* var.*menziesii* (p<0.001, p=0.001) and *S. altissimum* (p<0.001, p<0.001). Egg mortality was also significantly higher in *S. altissimum* compared to *L. virginicum* var.*menziesii* (OR=0.50, 95% CI [0.28, 0.88], p=0.016) (Table 3.3).

As larvae developed from instar II to instar III differences in mortality rates between host plants became more similar and no significant differences were detected. Instar IV mortality was somewhat higher in *L. virginicum* var.*menziesii* and *S. altissimum* compared to *B. rapa* (Figure 3.5). However, models were unable to detect a statistical difference due to declining numbers in the last stage of larval development.

A likelihood ratio test for an overall effect of year was significant (p=<0.001) when year was treated as a categorical variable. However, there was only a marginally significant linear change (p=0.06) in overall hazard rate between years. Hazard rates were lowest in 2007, followed by 2005, 2006 and 2008.

### Deer herbivory

The odds of mortality on B. rapa due to deer herbivory were estimated to be 3.57 times higher than the odds of mortality on S. altissimum (95% CI [0.081-0.989], p=0.048). The risk of mortality due to deer herbivory at the egg stage was estimated to be 4 times higher than the odds of mortality of larvae in instar I stage (95% CI [0.152-0.423], p<0.001). The odds of mortality due to deer was higher in the egg stage than instar I for both B. rapa and S. altissimum but the interaction between stage and host plant species was not significant (p=0.615); in other words, the effect of stage did not differ for B. rapa and S. altissimum. Finally, multiple eggs on host plants was significantly associated with higher risk of mortality due to deer (p=<0.001). There was no significant correlation between forest cover and mortality caused by deer herbivory among Brassica rapa sites (sites 1–4) (Kendall's tau –0.183, n=4, p=0.718).

## DISCUSSION

### Survivorship

*Euchloe ausonides insulanus* survivorship of eggs to instar IV was 12% (n=1617 eggs tracked), comparable to both common and rare *Pieris* spp. but considerably less than *Euchloe hyantis* (26.9 % [n=52; Karban and Courtney 1987]). No data on larval survivorship for other *Euchloe* species have been published. The survival rate of the generalist butterfly, *Pieris rapae* was found to be 15.5% (n=43,464; Parker 1970). Similarly, survival of the rare species, *Pieris virginiensis* was 16% in one study (n=189; Cappuccino and Kareiva 1985) and 19.6% in another (n=143; Doak et al. 2006). Relatively low survivorship of *E. ausonides insulanus* is likely not attributed to one single factor, but rather to multiple factors including the high rates of mortality in egg and instar I stages (Appendix C; Figure 3.9).

*Euchloe ausonides insulanus* survivorship was lowest in early stages of development (egg and instar I) (Figure 3.4). Low survivorship in early stages of development is common in many species of butterflies (Scott 1986) and in general, the trend in low survivorship in early stages (egg and instars I) in *E. ausonides insulanus* was similar to trends found in other pierid species (Courtney 1986). However, egg and instar I survivorship was exceptionally low in *E. ausonides insulanus* (54% and 26%, respectively) compared to a close relative *E. hyantis*. For example, in studies on *E. hyantis* 62% of eggs survived to instar I (n=53) and 77% of instar I survived to instar II (n=52) (Karban and Courtney 1987).

Low egg survivorship (54%) was particularly low in *E. ausonides insulanus* compared to other pierids. In review of life table data for several Pieridae (including *Pieris sp*., *Colias sp*. and *Anthocharis cardamines*), Courtney (1986) found that survivorship was greater in the egg stage than early instars I and II. For example, in studies on *Anthocharis cardamines* approximately 90% of eggs survived to instar I (n=3416) and 30-50% of instar I survived to instar II (Wiklund and Friberg 2009). In general, low egg and instar I survivorship may be attributed to high rates of mortality in egg and instar I stages. For individuals that survived beyond instar I, the rate of mortality declined substantially in the instar II stage (Figure 3.5). However, the high rates of mortality in egg and instar I stages outweighed the relatively low levels of mortality in instars II and III.

### Stage-specific mortality among host plant species

Overall, egg and instar I mortality was most severe on *B. rapa* (Figure 3.5). Even though mortality on *B. rapa* was reduced in later stages (instars II and III), the difference was not large enough to compensate for the heavy losses in egg and instar I stages. In other words, the low survivorship of eggs to instar V (beyond instar IV) on *B. rapa* (3.8%; Figure 3.4) is attributed to the high rate of mortality at the egg and instar I stages.

In contrast, mortality was greater in *L. virginicum* var.*menziesii* and *S. altissimum* at instar IV stage compared to *B. rapa* (Figure 3.5). Mortality of late instar larvae on *L. virginicum* var.*menziesii* and *S. altissimum* is likely attributed to starvation and disappearance (i.e., crawling off the original host plant in search of another food plant). Starvation and premature disappearance of larvae occurred most often as a result of early host plant senescence. Observations of larval death due to starvation and disappearance were largely confined to individual plants of *L. virginicum* var.*menziesii* and *S. altissimum* growing in exceptionally dry sandy or rocky nearshore soils. Sites of open sand and rocky nearshore soils were composed of little sediment, highly porous, held little moisture and increased in temperature at a faster rate than other sites. Late stage larval mortality was particularly high on *L. virginicum* var.*menziesii* and *S. altissimum* in 2006. For example, there was 81% mortality of instar III larvae on *S. altissimum* in 2006. Host plant senescence and disappearance accounted for 92% of instar III deaths. Furthermore, there was 75% mortality of instar III larvae on *L. virginicum* var.*menziesii* in 2006; similarly, all deaths were attributed to disappearance. In both cases disappearance and early host plant senescence may be related to low levels of precipitation during the months of April and May. These results indicate that host plants with limited access to resources may not support the growth and development of *E. ausonides insulanus*.

### Sources of mortality among sites

Some differences between sites are noteworthy, specifically, differences in sources of mortality (e.g., deer herbivory, starvation, disappearance). For example, percent mortality due to deer herbivory was substantially lower at site 1 (5%) compared to all other *B. rapa* research sites (site 2–4, 69%, 56% and 47% respectively) (Figure 3.6). The difference in deer related mortality may be related to the distance of host plants from forest cover. Site 1 was located the furthest from the nearest forest cover (470 m) and surrounded by extensive open grassland, while the mean distance of sites 2–4 from forest cover was 133±40.4 m. However, there was no significant relationship between percent mortality due to deer and the distance from forest cover. This result may be because a larger sample size is needed to detect a relationship between deer herbivory and distance from forest cover.

Site differences also influenced trends in mortality on *S. altissimum* (sites 6–8). For example, percent mortality of eggs and instar I larvae on *S. altissimum* located in introduced grasslands (site 8) was 52% and 62% respectively, compared to 21% and 41% mortality observed on *S.*

*altissimum* located in sandy soils (sites 6 and 7) (Figure 3.7). The difference in mortality between *S. altissimum* sites may be attributed to differences in the presence of deer. Mortality due to deer herbivory was twice as high on *S. altissimum* located in introduced grasslands (site 8, 11%) than on *S. altissimum* located in sandy soils (site 6, 5% and site 7, 4%; Figure 3.8). Deer were rarely observed in sand dune habitat (Lambert pers. obs.) and likely avoid crossing areas of open sand to get to *S. altissimum.* Additionally, the nearest forest cover to *S. altissmum* in sand dune habitat was ~ 400 meters. At site 7, low deer herbivory could be attributed to inaccessibility, as the site was bordered by a surface road and steep bluff that may prevent deer from actively using the site. In contrast, herbivory was higher at site 8 on *S. altissimum* located where deer had access to cover (i.e., tall grasses) and connectivity to forest vegetation.

### Stage-specific mortality among habitat types

It is suspected that many site related factors influence trends in mortality including factors related to differences in habitat (e.g., sand dune and introduced grasslands). Similarities and differences in stage specific mortality may be attributed to factors associated with habitat type as opposed to factors strictly associated with host plant species. For example, trends in egg and instar I larval mortality on *S. altissimum* located in areas of introduced grassland habitat were similar to trends in egg and instar I larval mortality on *B. rapa* also found in grassland habitat (Figure 3.7). In other words, there was a high percentage of egg and instar I larval mortality on both *S. altissimum* and *B. rapa* that occurred in introduced grasslands. In contrast, trends in mortality on *S. altissimum* located in areas of introduced grassland habitat were substantially different than trends in mortality on *S. altissimum* located in sand dunes (Figure 3.7).

Understanding the relationship between trends in stage specific mortality and the factors that cause mortality within and among habitat types may be useful for managing host plant habitat. For example, percent mortality due to starvation and disappearance was substantially lower on *S. altissimum* located in grassland habitat compared to *S. altissimum* located in sand dunes (Figure 3.8). Stressful environmental conditions (low precipitation and high temperatures) may have caused *S. altissimum* to develop at a faster rate in sand dune habitat.

Whereas *S. altissimum* located in grassland habitat may have access to higher levels of soil moisture which provides more reliable resources to developing larvae.

Understanding the difference in mortality factors between host plant species within the same habitat type may also be useful for designing host plant habitat with the goal of maximizing survival. For example, stage specific trends in mortality on *S. altissimum* and *B. rapa* located in introduced grassland habitat were similar (Figure 3.7), however, factors causing mortality on *S. altissimum* in grassland habitat were different from the factors causing mortality on *B. rapa* in grassland habitat (Figure 3.8). Deer herbivory caused high levels of egg and instar I larval mortality on *B. rapa*. However, predation and damage to inflorescences due to moth caterpillar competition (unidentified species) resulted in equally high levels of egg and instar I mortality on *S. altissimum*. Deer herbivory contributed to 51% mortality of egg and instar I larvae on *B. rapa*, while predation caused 51% mortality of egg and instar I larvae on *S. altissimum*. These results suggest that egg and instar I stages are the most vulnerable on host plants located in grassland habitat and that the reduction of mortality in early stages of development (e.g., deer enclosures or moth and spider removal) may increase survivorship. Efforts to increase survivorship by reducing mortality in early stages of development may be particularly effective in *S. altissimum* established in grassland habitat due to the low percent mortality in later stages of development (Figure 3.7). The low percent mortality of larvae in later stages of development on *S. altissimum* in grassland habitat (as opposed to sand dune habitat) may be important to consider in the design of host plant habitat. Over the course of this four year study, only once was a substantial number of plants of *S. altissimum* observed in introduced grassland habitat (2006). Isolated plants of *S. altissimum* were often observed on the edges of introduced grassland in areas of high soil disturbance but moderate to large patches of *S. altissimum* were uncommon in introduced grassland habitat. Thus, one management scenario to increase the survivorship of *E. ausonides insulanus* may be to increase the number of *S. altissimum* plants in grassland habitat using established restoration treatments (e.g., controlled burn plots) in conjunction with measures to reduce mortality in early stages of development (e.g., deer enclosures or moth and spider removal).

### Stage-specific survivorship and mortality among years

The two models examining survivorship and mortality among years showed mixed results. Logistic regression models of survival indicated that year was not a significant predictor of survivorship. Egg and larval survivorship did not vary dramatically among years. The model may have not detected year as a significant predictor because the number of eggs tracked was highest on *B. rapa*, the host plant species that was the most abundant and constant over the four year study.

In contrast, overall effect of year was significant when year was treated as a categorical variable in logistic hazard models (hazard rates were lowest in 2007, followed by 2005, 2006 and 2008) and may be partially explained by differences in stage-specific mortality among years on *S. altissimum* located in sand dunes (site 6) and *L. virginicum* var. *menziesii* located in nearshore habitat (site 5). Unlike, *B. rapa* that occupies relatively stable introduced grassland habitat, both *S. altissimum* and *L. virginicum* var. *menziesii* occur in highly disturbed habitats. Plants of *L. virginicum* var. *menziesii* and *S. altissimum* occupy sand dune and nearshore habitat both of which are impacted by large-scale disturbances such as off-shore wind, shifting dunes and seasonal storms that alter shoreline habitat.

Additionally, plants of *S. altissimum* and *L. virginicum* var. *menziesii* grow in dry sandy soils and are highly susceptible to fluctuations in annual precipitation and mean temperature. For example, low precipitation and high temperatures early in the 2006 growing season caused many host plants to develop fruits prematurely. Six larvae died from starvation on *S. altissimum* in 2006 (14% of the eggs tracked) because host plants developed at a faster rate than larvae and stranded early instar larvae on fruits that were too tough to consume. An additional eight instar III and instar IV larvae were forced to move from their original host plant in search of alternative food plants as a result of early host plant senescence. Percent mortality of larvae on *S. altissimum* located in sand dune habitat was substantially higher than larvae occupying *S. altissimum* and *B. rapa* in grassland habitat (Figure 3.7). Furthermore, the difference in host plant senescence among years, especially 2006 and 2007, may explain the sharp difference in percent mortality of instar III larvae on *S. altissimum* in sand dune habitat (77% in 2006 compared to 30% in 2007) and *L. virginicum* var. *menziesii* (75% in 2006 compared to 9% in 2007).

The number of plants fluctuated from year to year across all sites and all host plant species. For example, site 2 increased in number of *B. rapa* from 10 plants in 2005 to 511 in 2008. Likewise, the number of eggs occupying host plants also changed from year to year including several sites observed to be unoccupied. Some unoccupied sites are noteworthy (site 8, site 4 and site 5) in that all three sites had previously supported relatively large numbers of eggs. The absence of eggs at these sites may be related to a reduction in numbers of host plants (site 8) or reduction of available oviposition sites (site 4) or combination of both (site 5). For example, in 2008, no eggs were observed at the *L. virginicum* var. *menziesii* site. The absence of eggs may be partly attributed to the reduction in numbers of plants and oviposition sites. Mortality factors such as predation of eggs and flooding of overwintering pupae also likely contributed to low population numbers (and eventual absence of eggs). For example between 2005 and 2008, the number of adults declined from 11 to 0 at site 8 (Appendix B). The reduction of the number of eggs (and adults) was likely triggered by an unprecedented windstorm that coincided with a high tide in February 2006. The *L. virginicum* var. *menziesii* plant population was reduced by more than 50% (Table 3.1) due to the wave action that deposited large volumes of sand and gravel on extant populations of host plants (Lambert 2011, Chapter 5, Figure 3.1). Plants that recovered from the soil disturbance were short in stature and in very early stages of development (lacking robust flower buds) thus unavailable to ovipositing females. In addition, mortality of overwintering pupae most likely contributed to the eventual decline of adults (and eventual absence of eggs).

A combination of reduction in host plant resources and an increase in mortality due to predation and deer herbivory (consumption of eggs) is also likely to have contributed to the absence of eggs at other research sites (site 8 and site 4).

### Sources of mortality and host plant suitability

Predation was the greatest source of egg and larval mortality throughout the four year study period (n=752; 47% of all eggs tracked) (Figure 3.9). Predation by spiders was observed most often (n=109) although social paper wasps (Family Vespidae, *Polistes* spp.) were also observed to predate on larvae. Paper wasps are prominent generalist predators on lepidopteron (Raveret Richter 2000, Rayor 2004). Because wasps locate larvae on different species of host plants, host plant structure (e.g., height or number of racemes) and density has been shown to affect the ability of wasps to locate or capture larvae (Geitzenauer & Bernays 1996, Eigenbrode et. al. 2000). Geitzenauer & Bernays (1996) found predation rates of *Polistes arizonensis* to be higher on larvae that occupied plants with less branching and lower leaf surface area. Similarly, in this study, paper wasps were observed to predate on larvae occupying *S. altissimum* plants having very few racemes and growing in isolation (several meters from adjacent plants of *S. altissimum*) (site 8).

Arthropod predators observed to attack *E. ausonides insulanus* adults and larvae include crab (Family Thomisidae) and wolf spiders (Family Lycosidae). Wolf spiders *Pardosa distincta* and *Zelotes puritanus* were identified in nearshore habitat hunting from driftwood logs (Crawford unpub. data 2005) and may account for some of the high percent predation of eggs and larvae on *L. virginicum* var. *menziesii* (77%, Figure 3.9). Predation by ants and mites and other non-arthropod herbivores have been documented in other species of Pieridae (Dempster 1967, Hayes 1981, 1984) and also likely contributed to death of eggs and early instar larvae of *E. ausonides insulanus*.

The abundance of generalist predators is likely to be mediated by the structural diversity of vegetation within and between host plant patches and may explain the substantial difference in percent mortality caused by predation between host plants species. *Sisymbrium altissimum* in native sand dune vegetation and *L. virginicum* var. *menziesii* in nearshore habitat showed high rates of predation (site 6; 45%; site 5; 77%, respectively). These two host plants at these two sites are found in diverse habitat compared to introduce grassland where *B. rapa* occurs. Studies have shown that structurally diverse habitats provide cover for predators (Rypstra et al. 1999, Langellotto and Denno 2004, Janssen et al. 2007) and have also been shown to increase the predator richness (Root 1973). Thus, habitat structure may explain high rates of predation in *Sisymbrium altissimum* in native sand dune vegetation and *L. virginicum* var. *menziesii.*

Eggs and early instar larvae are especially vulnerable to host plant factors that are interrelated. For example, larvae unable to feed on senescent host plant material may leave the original host plant in search of an alternative food plant and therefore become more susceptible to predation. Competition for host plant resources may also indirectly contribute to egg and larval mortality. For example, competition for floral resources between early instar larvae and unidentified Lepidoptera (web building moth caterpillar) was observed in 2006 on *S. altissimum*. Sixteen percent of all eggs tracked at site 8 (n=341) died as a result of competition with an unidentified moth species. Moth competition for floral resources was only observed on *S. altissimum*.

Cannibalism was not been observed in *E. ausonides insulanus* over the four years of study. However, competition for resources via cannibalism of eggs and early instar larvae is thought to be a major factor that influences population dynamics of many species of Pieridae (Courtney 1981, Courtney and Courtney 1982) and therefore may be an adaptive behavior that has yet to be observed in *E. ausonides insulanus*.

Observations of egg cannibalism have been reported in studies of *Euchloe* and other related species (Ford 1945, Jones and Ives 1979, Yamamoto 1981, Courtney and Duggan 1983, Courtney 1986). Evidence for intraspecific competition for larval resources is generally observed only in Lepidoptera populations of high densities (Dempster 1983, Kivela & Valimaki 2008) and therefore may not be detected in *E. ausonides insulanus* because of the species low population density. Intraspecific competition is much more common in species of Pieridae that specialize on host plant resources such as buds and flowers (as opposed to leaves) (Courtney 1986, Shapiro 1975) because the quantity of food plant resources are limited and larval survival is dependent on ephemeral parts of the plant that may mature at a faster rate than larvae develop (Doak et. al. 2006). Thus it is surprising that cannibalism has not been observed in *E. ausonides insulanus* because larvae consume the majority of inflorescence on host plants and multiple eggs on a single plant are commonly observed (Lambert 2011, Chapter 2).

Similarly, parasitism of eggs or larvae was not observed in *E. ausonides insulanus* although parasitism is commonly attributed to mortality factors associated with Pieridae (see review by Courtney 1986, Wiklund and Friberg 2009). It may have been that the low population abundance of *E. ausonides insulanus* was unable to support parasitoid populations. For example, several studies on related pierid species noted few parasitoids in low-density host populations (Lees and Archer 1974, Warren 1984). It is also possible that some *E. ausonides insulanus* larvae may have been parasitized, but because parasitoids do not pupate until the last larval instar or pupal stage, parasitoids may have gone undetected.  Ichneumonid

(Hymenoptera) wasps were occasionally observed in *E. ausonsides insulanus* grassland habitat (Lambert pers. obs.) and have historically been known to parasitize pierids (Cole 1959). For example, Karban and Courtney (1987) found low levels of parasitism of *Euchloe hyantis* by *Cotesia* sp. (Hymenoptera: Braconidae) and an unidentified Ichneumonid.

It is also possible that some *E. ausonides insulanus* larvae may have been parasitized, but died of other causes. If, in fact, parasitism of *E. ausonides insulanus* larvae occurred, parasitoids could have contributed to the sudden disappearance of many instar IV larvae by altering their behavior. Parasitoids have been found to manipulate the behavior of their hosts (Godfray 1994, Moore 2002). For example, woolly bear caterpillars, *Platyprepia virginalis* (Lepidoptera: Arctiidae) have been shown to move to a different host plant in response to parasitism by the parasitoid *Thelaira americana* (Diptera: Tachinidae) (Karban and English-Loeb 1997). Thus it is possible that some of the instar IV larvae that 'disappeared' from healthy plants of *L. virginicum* var. *menziesii* wandered far from their last sighting and subsequently starved or were preyed upon by generalist predators.

### Impacts of deer

A significant portion of plants in this study were browsed by deer and evidence of deer herbivory was observed at almost every study period. Deer herbivory reduced *E. ausonides insulanus* abundance by indirectly reducing availability of oviposition sites (Lambert 2011, Chapters 3) and by direct consumption of eggs and larvae. Over the course of four years of study, death due to deer consumption of eggs and larvae accounted for a large percentage of total mortality; 415 individuals were eaten by deer, 26% of all eggs tracked.

Browsing pressure on *B. rapa* was especially high and accounted for the majority of deer deaths (Figure 3.10). For example, 58% of the total egg mortality on *B. rapa* was attributed to deer consumption of eggs (n=478 egg deaths on *B. rapa*). High egg mortality caused by deer (Figure 3.10) contributed substantially to overall low survivorship in *B. rapa* (3.8%; Figure 3.3). Even though mortality on *B. rapa* was reduced in later stages (instars II and III), the difference was not large enough to compensate for the heavy losses of eggs due to deer.

Multiple eggs on host plants, particularly *B. rapa*, was significantly associated with mortality caused by deer herbivory. This suggests that deer and *E. ausonides insulanus* may be selecting for similar plant traits. Plants selected by *E. ausonides insulanus* were relatively robust (tall with many plant racemes), in early stages of growth and growing in isolation or on the edges of host plant patches (see Lambert 2011, Chapter 2). Similarly, deer are known to select plants based on plant size, phenology and at the edges of cover (e.g., forest cover or sufficient cover by tall introduced grasses or shrubs) (Alverson et. al. 1988, see review by Cote et. al. 2004). For example, deer consume the early growth of forage crop plants (e.g., *B. rapa*) and select plant stems on the top or sides of plants in preference to lower stems of the plant (Baugher et al. 1985). Deer are also known to selectively browse flowering plants when available (Anderson 1994, Hanley 1997). In this study, deer were observed to select robust plants of *B. rapa* having several inflorescences containing compact flower buds, plant traits also preferred by ovipositing females. The removal of large compact inflorescences indirectly reduces the availability of potential oviposition sites (Lambert 2011, Chapter 4). Moreover, inflorescences that are already occupied by multiple eggs may be selectively consumed by deer effectively acting as a local population ‘sink’.

Additionally, deer browsing causes the regeneration of plant racemes and new flower buds, especially on robust host plants. The growth of new flower buds provides females with new sites for oviposition, particularly in the second half of the flight season when many host plants have already matured and the number of oviposition sites has decreased. Latter in the season, females may also be under pressure to lay their full complement of eggs, thus increasing the chances of multiple egg loads on a single plant inflorescence. In some circumstances, the regeneration of new flower buds caused by deer browsing provided oviposition sites that would have otherwise not been available to females as the season progressed and host plants matured. However, frequently plant racemes were browsed more than once and in many cases, multiple egg loads were also consumed. For example, in 2007 at site 3 (n=211 eggs), 43 plants of *B. rapa* were eaten by deer which stimulated new growth and oviposition sites that were later occupied by eggs. Plants with “new” oviposition sites accounted for 53% of the total number of plants occupied by eggs. However, 33 plants were browsed multiple times (77% of the initial 43 plants with new growth). Multiple browsing events contributed to 41% of the total egg and larvae mortality at site 3.  Thus, multiple browsing events had a major impact on egg mortality.

Browsing pressure on plants of *B. rapa* located within close distance of forest cover was particularly high and contributed greatly to overall deer related mortality. For example, scattered plants of *B. rapa* located in a small open grassland bordered by forest and shoreline (site 2) were browsed consistently over the four year study. Sixty nine percent of all eggs present at research site 2 (n=276) were consumed by deer.

Browsing pressure on host plants located in grassland habitat is expected to increase in the absence of active deer management on San Juan Island (Davison pers. comm.) Furthermore, increases in browsing pressure due to high deer densities is likely to reduce species richness of native plant communities in the San Juan and Gulf Island archipelagos (Gonzales and Arcese 2008, Martin et al. 2010) and prevent native host plants such as *Turritis glabra* from becoming established in grassland or restored native prairie habitat (Lambert 2011, Chapter 3). For example in an experimental study that tested restoration treatments and establishment of *T. glabra* in areas of *E. ausonides insulanus* habitat, deer damaged 64% of all *T. glabra* plants (n=44) (Lambert 2011, Chapter 4). Other research has shown deer herbivory to significantly affect island flora, fauna and ecosystems processes (Cote et al. 2004, Gaston et al. 2008, Martin et al. 2010) and thus it is not surprising that relatively high abundance of deer on San Juan Island (Davison pers. comm.) corresponds with the high rate of deer related mortality. Conservation and protection of *E. ausonides insulanus* may require the exclusion of deer from host plant patches that are susceptible to deer browsing (e.g., in short distance from forest cover), especially plants occupied by multiple eggs and larvae.

## CONCLUSION

Low egg survivorship in *E. ausonsides insulanus* may contribute to overall low population abundance and is likely related to high egg mortality in *B. rapa* and *S. altissimum*. Egg mortality was mainly attributed to predation and deer herbivory. Deer consumed the buds and flowers of host plants that led to direct consumption of eggs. Larval mortality due to starvation was also observed. Periods of low precipitation and high temperatures likely caused host plants to dry out faster than larvae were able to develop. Larval starvation was largely confined to plants of *S. altissimum* located in sand dune sites.

The only known native host plant, *L. virginicum* var. *menziesii* was found to have the highest percent survivorship to instar IV. However, *L. virginicum* var. *menziesii* habitat was impacted by a large-scale off-shore storm that likely caused mortality of overwintering pupae. In 2008, no eggs or adults were observed at the *L. virginicum* var. *menziesii* site (see Appendix B for relative adult abundance). The absence of eggs at research sites previously known to support eggs may be attributed to combined effects of reduced host plant resources (i.e., reduction in oviposition sites) and mortality factors such as predation and deer herbivory (consumption of eggs).

**Table 3.1** Number of eggs tracked by host plant species, site and year (n=1617 eggs tracked). Total number of host plants at each site, the number of host plants that were occupied by eggs and that were subsequently tracked over the course of the season and initial survey date that host plants were surveyed for eggs. Blank cells indicate year when survey was not conducted. 0 indicates the absence of eggs on site.

| | | 2005 | | | | 2006 | | | | 2007 | | | | 2008 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Host plant | Site | eggs | host plants on site | host plants with eggs | initial survey date | eggs | host plants on site | host plants with eggs | initial survey date | eggs | host plants on site | host plants with eggs | initial survey date | eggs | host plants on site | host plants with eggs | initial survey date |
| *Brassica* | 1 | 165 | 730 | 46 | 14-May | 47 | 1000 | 10 | 18-May | 56 | >1000 | 41 | 25-May | | | | |
| | 2* | 17 | 10 | 4 | 18-May | 145 | 32 | 18 | 20-May | 81 | 42 | 25 | 24-May | 33 | 511 | 26 | 19-May |
| | 3* | | | | | 55 | 81 | 17 | 17-May | 205 | 512 | 81 | 18-May | | | | |
| | 4* | | | | | 90 | 26 | 21 | 17-May | 0 | 15 | 0 | 25-May | | | | |
| *Lepidium* | 5 | 79 | 2000 | 55 | 5-May | 17 | 855 | 16 | 26-May | 21 | 1200 | 20 | 28-May | 0 | >1000 | 0 | 25-May |
| *Sysimbrium* | 6 | 130 | 1000 | 54 | 13-May | 43 | >1000 | 25 | 15-May | 67 | >3000 | 54 | 24-May | | | | |
| | 7 | | | | | 25 | 226 | 21 | 17-May | 0 | 155 | 0 | 25-May | | | | |
| | 8 | | | | | 341 | 570 | 210 | 15-May | 0 | 66 | 0 | 24-May | | | | |

*Indicates sites where a complete census of host plants was conducted. All host plants with eggs were subsequently tracked

**Table 3.2** Logistic regression analysis of survivorship on three host plant species, *Lepidium virginicum* var. *menziesii*, *Sisymbrium altissimum*, and *Brassica rapa*, 2005-2008. Exponentiation of the coefficients yields odds ratios of survival with respect to the reference host plant (*B. rapa*) and year (2005). Wald tests were used to test for any differences in survival by host plants and year.

| | *P*-value | Odds ratio (95% CI) |
|---|---|---|
| Egg Survival | | |
| Year | 0.012 | |
| 2006 | 0.100 | 1.40 (0.94-2.11) |
| 2007 | <.001 | 1.94 (1.28-2.93) |
| 2008 | 0.698 | 1.18 (0.52-2.67) |
| Host plant | <.001 | |
| *L. virginicum* | <.001 | 5.70 (2.15-15.10) |
| *S. altissimum* | <.001 | 2.59 (1.36-4.91) |
| Survival beyond Instar I | | |
| Year | 0.023 | |
| 2006 | 0.996 | 1.00 (0.65-1.54) |
| 2007 | 0.015 | 1.67 (1.11-2.53) |
| 2008 | 0.992 | 0.99 (0.27-3.67) |
| Host plant | 0.006 | |
| *L. virginicum* | 0.007 | 5.55 (1.59-19.38) |
| *S. altissimum* | 0.012 | 3.04 (1.27-7.24) |
| Survival beyond Instar II | | |
| Year | <.001 | |
| 2006 | 0.058 | 0.65 (0.41-1.02) |
| 2007 | 0.084 | 1.44 (0.95-2.19) |
| 2008 | 0.255 | 0.30 (0.04-2.38) |
| Host plant | 0.001 | |
| *L. virginicum* | <.001 | 4.40 (1.67-11.60) |
| *S. altissimum* | <.001 | 2.78 (1.39-5.56) |
| Survival beyond Instar III | | |
| Year | <.001 | |
| 2006 | <.001 | 0.40 (0.26-0.61) |
| 2007 | 0.054 | 1.56 (0.99-2.45) |
| 2008 | 0.984 | 0.00 (0.00-1.00) |
| Host plant | <.001 | |
| *L. virginicum* | <.001 | 3.59 (2.06-6.26) |
| *S. altissimum* | <.001 | 3.27 (2.21-4.83) |
| Survival beyond Instar IV | | |
| Year | 0.510 | |
| 2006 | 0.739 | 1.14 (0.53-2.44) |
| 2007 | 0.192 | 1.68 (0.77-3.64) |
| 2008 | 0.990 | 0.00 (0.00 inf) |
| Host plant | 0.112 | |
| *L. virginicum* | 0.082 | 2.45 (0.89-6.72) |
| *S. altissimum* | 0.092 | 1.79 (0.91-3.53) |

**Table 3.3** Host-stage interaction model of hazard comparing three host plant species, *Lepidium virginicum* var. *menziesii*, *Sisymbrium altissimum*, and *Brassica rapa* and *E. ausonides insulanus* life stages, egg to larval instar IV, 2005-2008.

| | *P*-value | Odds ratio (95% CI) | df | Wald test-statistic |
|---|---|---|---|---|
| Overall Host Plant Effect | | | | |
| Egg | <.001 | | 2 | 34.695 |
| Instar I | <.001 | | 2 | 22.285 |
| Instar II | 0.184 | | 2 | 3.387 |
| Instar III | 0.930 | | 2 | 0.145 |
| Instar IV | 0.301 | | 2 | 2.400 |
| *L. virginicum* vs. *B. rapa* | | | | |
| Egg | <.001 | 0.25 (0.14-0.44) | 1 | 23.912 |
| Instar I | 0.001 | 0.37 (0.21-0.66) | 1 | 11.444 |
| Instar II | 0.150 | 0.57 (0.27-1.22) | 1 | 2.071 |
| Instar III | 0.723 | 0.86 (0.39-1.94) | 1 | 0.125 |
| Instar IV | 0.127 | 2.11 (0.81-5.49) | 1 | 2.329 |
| *S. altissimum* vs. *B. rapa* | | | | |
| Egg | <.001 | 0.50 (0.37-0.68) | 1 | 19.010 |
| Instar I | <.001 | 0.45 (0.31-0.66) | 1 | 17.534 |
| Instar II | 0.112 | 0.66 (0.39-1.1) | 1 | 2.532 |
| Instar III | 0.983 | 0.99 (0.56-1.77) | 1 | <.001 |
| Instar IV | 0.383 | 1.35 (0.69-2.66) | 1 | 0.759 |
| *L. virginicum* vs. *S. altissimum* | | | | |
| Egg | 0.016 | 0.50 (0.28-0.88) | 1 | 5.827 |
| Instar I | 0.513 | 0.82 (0.46-1.46) | 1 | 0.429 |
| Instar II | 0.715 | 0.87 (0.41-1.83) | 1 | 0.133 |
| Instar III | 0.725 | 0.87 (0.4-1.88) | 1 | 0.123 |
| Instar IV | 0.342 | 1.56 (0.62-3.9) | 1 | 0.903 |

**Figure 3.1** Location of egg study sites at American Camp, San Juan Island National Historical Park, San Juan Island, Washington, 2005-2008. Numbers 1−4 represent study sites where *Brassica rapa* occurs in introduced grassland habitat. Study site 4 is located southeast of American Camp in the Cattle Point Natural Resource Conservation Area (NRCA) and is not represented on the map. Number 5 represents the study site where host plant species *L. virginicum var. menziesii* occurs in nearshore habitat. Numbers 6−8 represent *Sisymbrium altissimum* sites; site 6 is located in sand dune habitat, 7 on bluffs with sandy soils and 8 in a restoration management area (formerly introduced grassland).

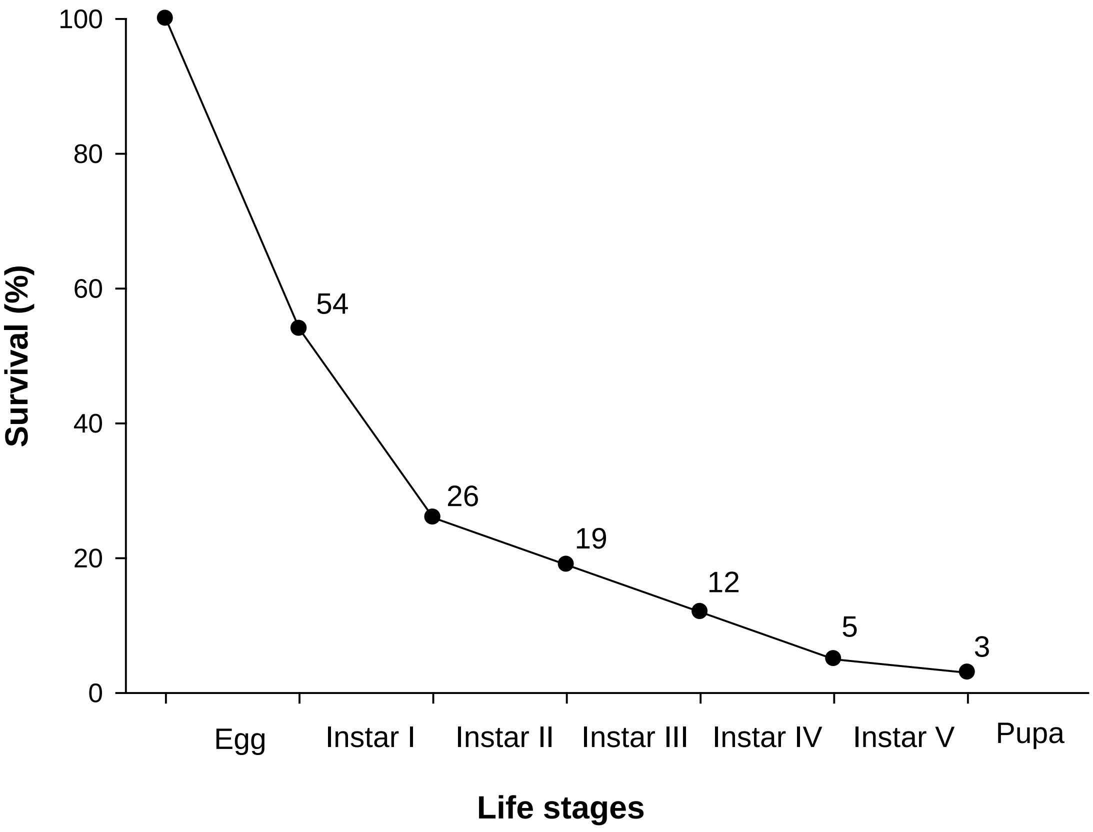

**Figure 3.2** Survival of 1617 eggs among all sites, years and host plant species, *Lepidium virginicum* var. *menziesii*, *Sisymbrium altissimum*, and *Brassica rapa*, 2005-2008.

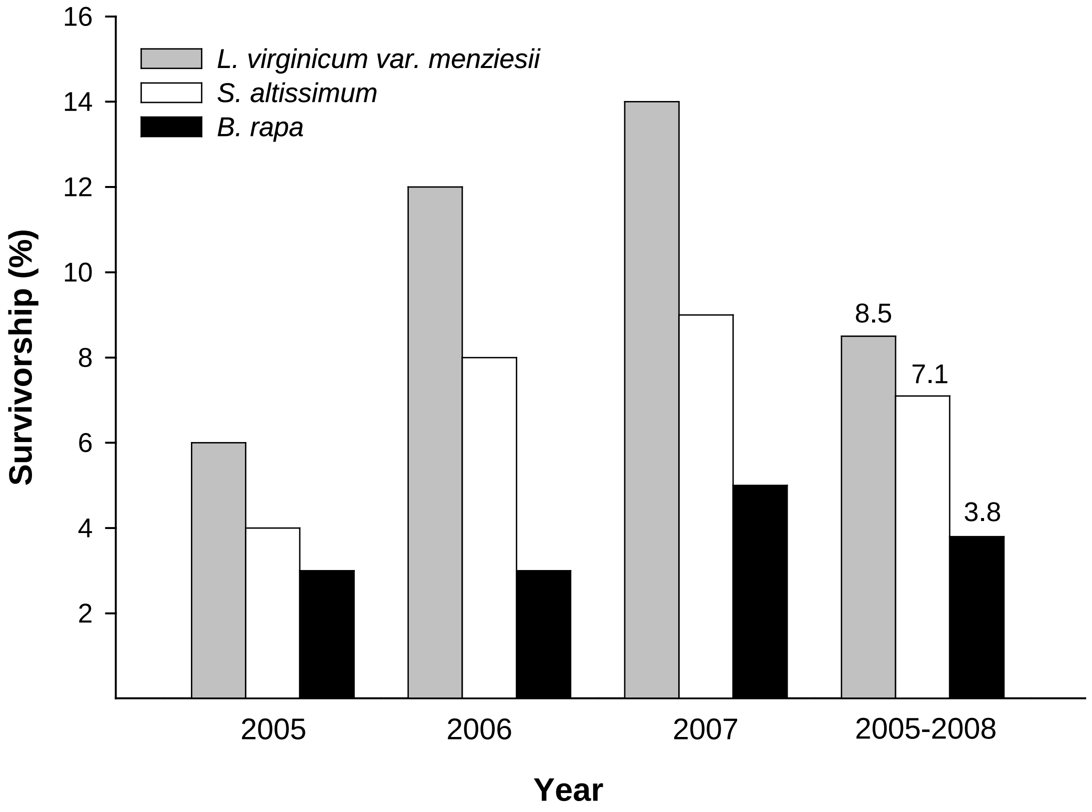

**Figure 3.3** Yearly survivorship of larvae (beyond instar IV) on three host plants species, *Lepidium virginicum* var. menziesii, *Sisymbrium altissimum*, and *Brassica rapa*, 2005-2008 (n=1617). Separate data for year 2008 was not included in the graph because only one population (*B. rapa*) was measured and larvae did not survive beyond instar III. However, 2008 data were included in summary data from 2005-2008. No significant difference in survival between host plant species was detected (logistic regression, Wald test of host plant effect, p=0.11). In terms of percentage, survivorship was highest on *L. virginicum var. menziesii* in 2007 (14.2%).

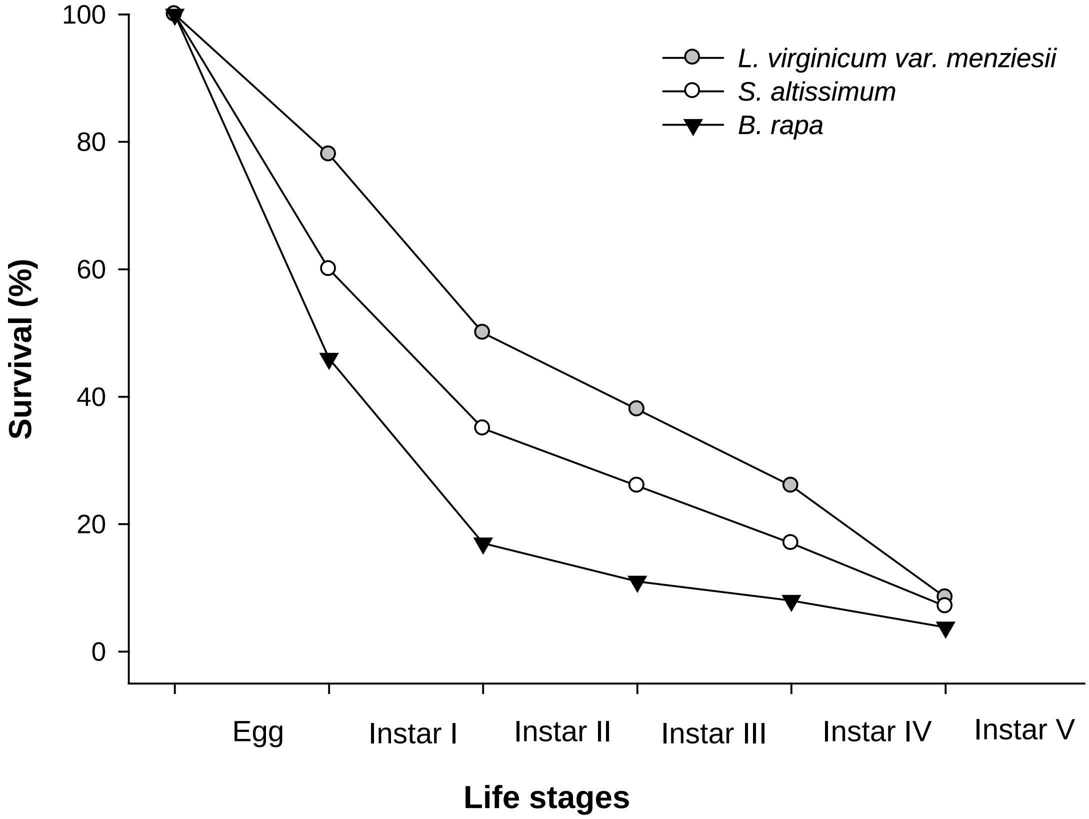

**Figure 3.4** Stage-specific survivorship on three host plant species *Lepidium virginicum* var. *menziesii*, *Sisymbrium altissimum* and *Brassica rapa*, 2005-2008. Chances of survival from egg to larval instar IV were highest in *L. virginicum* var. *menziesii* and *S. altissimum* and lowest in *B. rapa*. Survivorship varied significantly between host plant species *L. virginicum var.menziesii* and *B. rapa* and *S. altissimum* and *B. rapa* in early life stages (egg to instar IV) (logistic regression, Wald test of host plant effect, see Table 2). Tests for differences in survival between *L. virginicum* var. *menziesii* and *S. altissimum* were not significant at any stage.

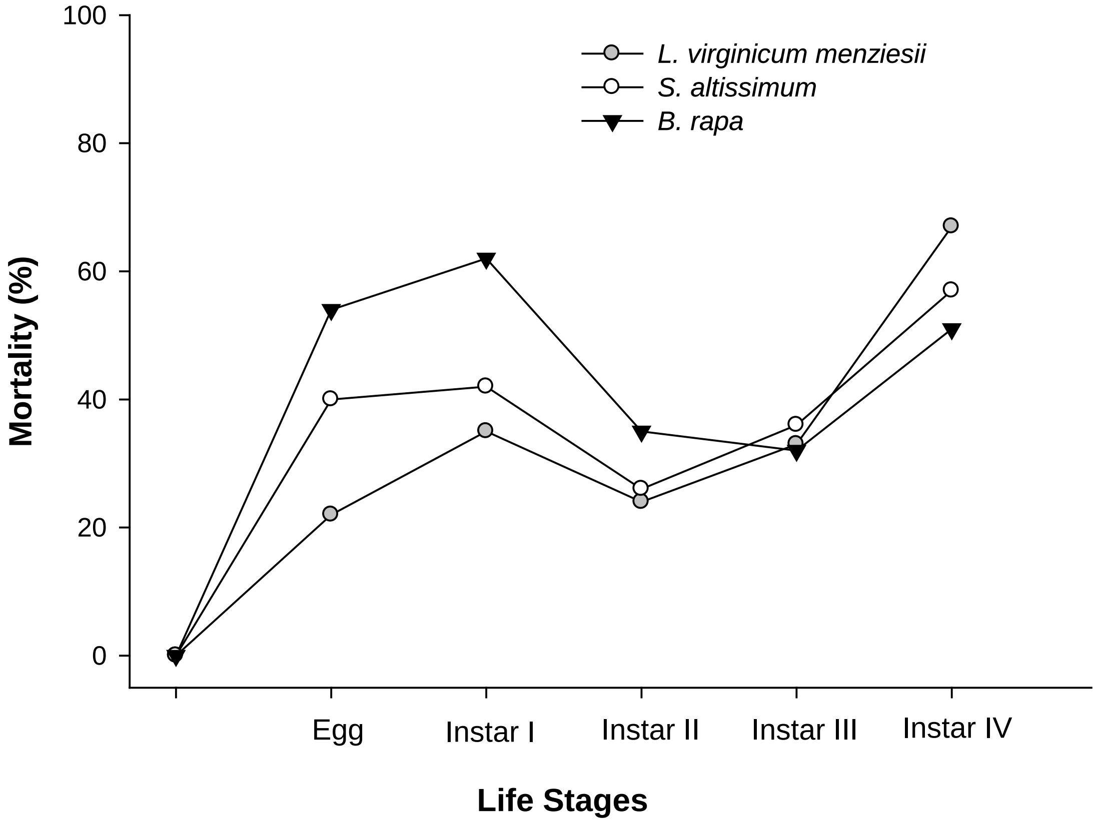

**Figure 3.5** Stage-specific mortality on three host plant species *Lepidium virginicum* var. *menziesii*, *Sisymbrium altissimum* and *Brassica rapa*, 2005-2008. Mortality differed significantly between host plants at egg and instar I stages. Eggs and instar I larvae on *B. rapa* had a significantly higher estimated odds of mortality than both *L. virginicum* var.*menziesii* ($p<0.001$, $p=0.001$) and *S. altissimum* ($p<0.001$, $p<0.001$). Egg mortality was also significantly higher in *S. altissimum* compared to *L. virginicum* var.*menziesii* (OR=0.50, 95% CI [0.28, 0.88], $p=0.016$).

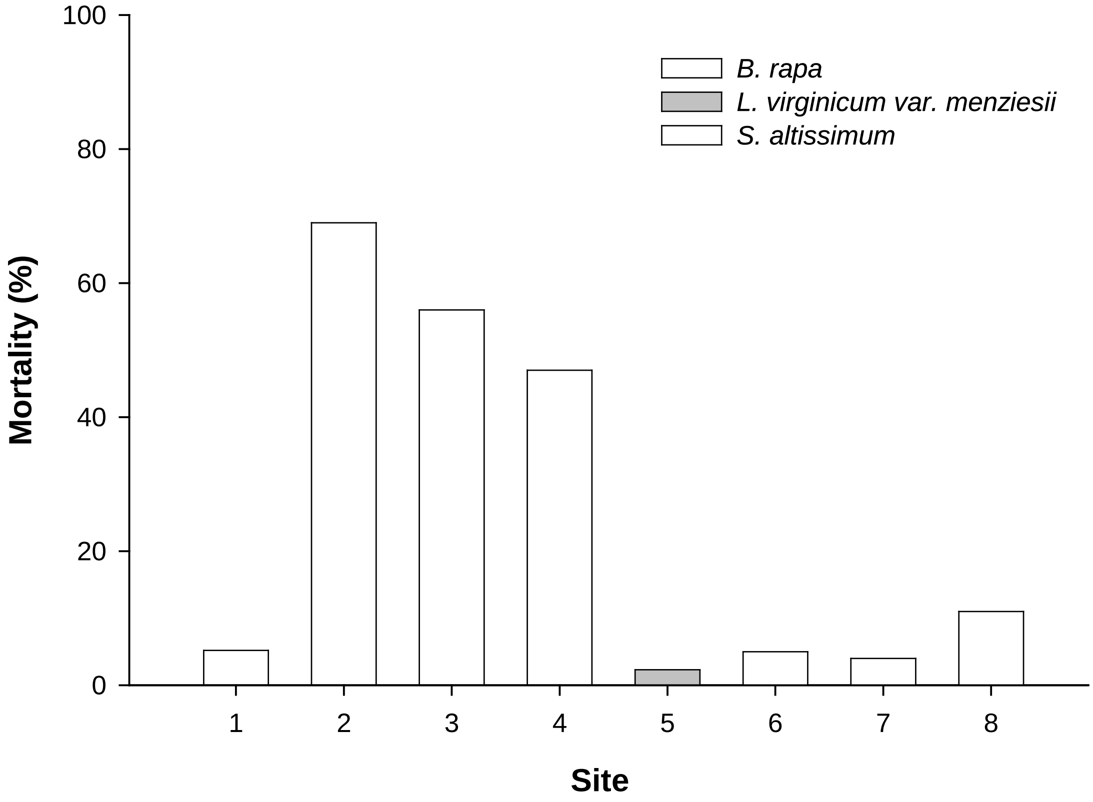

**Figure 3.6** Site-specific percent mortality of eggs and larvae related to deer herbivory, 2005-2008.

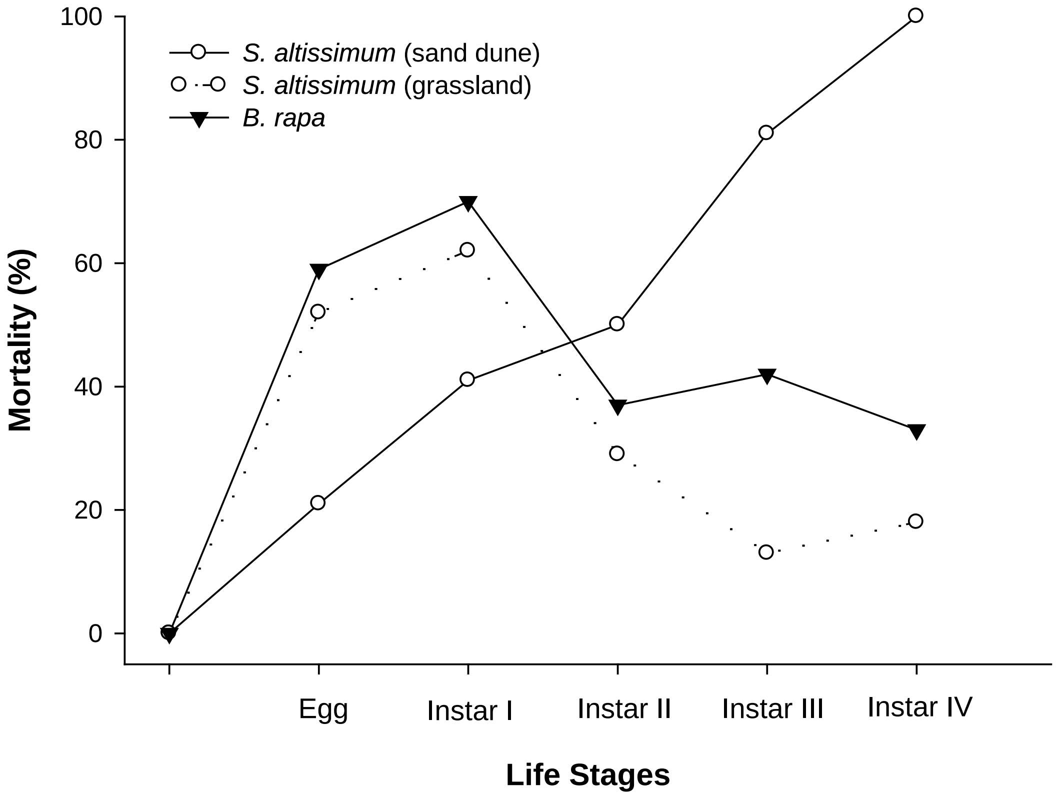

**Figure 3.7** Stage-specific mortality on *Brassica rapa* and *Sisymbrium altissimum*, 2006. Percent mortality of eggs and larvae on *Brassica rapa* (sites 1-4; n=337), *Sisymbrium altissimum* located in sand dune and sandy soils (sites 6 and 7; n=68) and *S. altissimum* located in introduced grasslands (site 8; n=341).

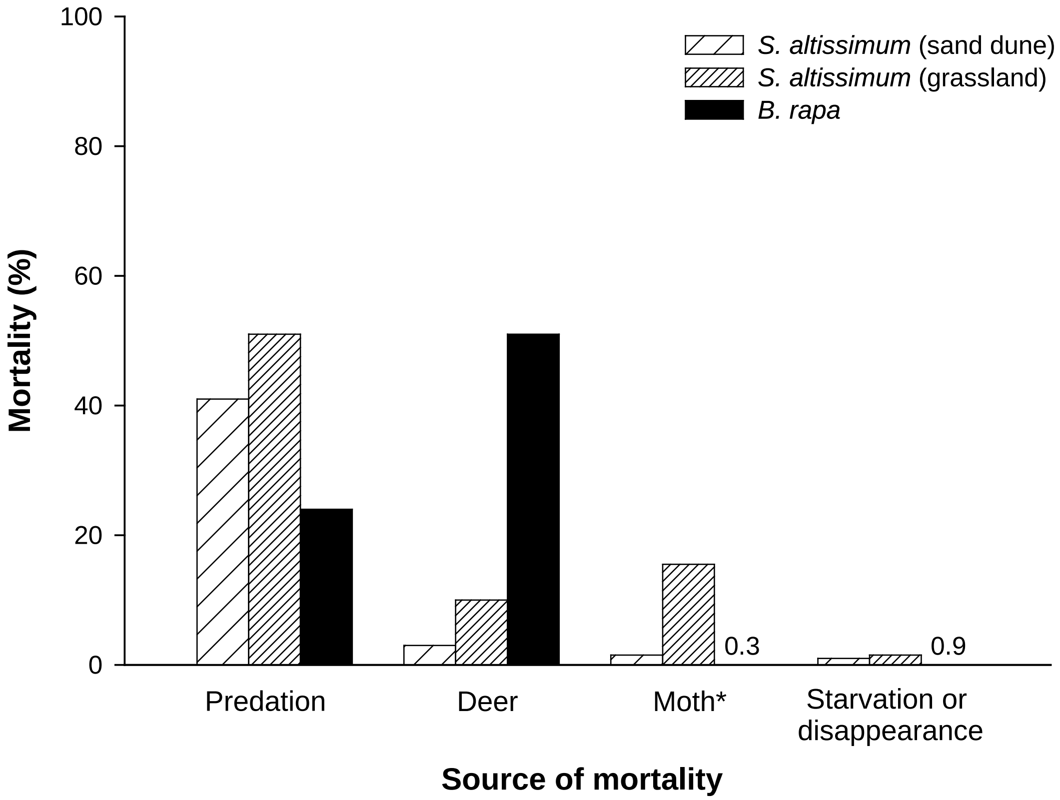

**Figure 3.8** Sources of mortality on *Brassica rapa* and *Sisymbrium altissimum*, 2006. Percent mortality of eggs and larvae on *Brassica rapa* (sites 1-4; n=337), *Sisymbrium altissimum* located in sand dune and sandy soils (sites 6 and 7; n=68) and *S. altissimum* located in introduced grasslands (site 8; n=341).

*= indirect mortality due to competition with unknown moth larvae

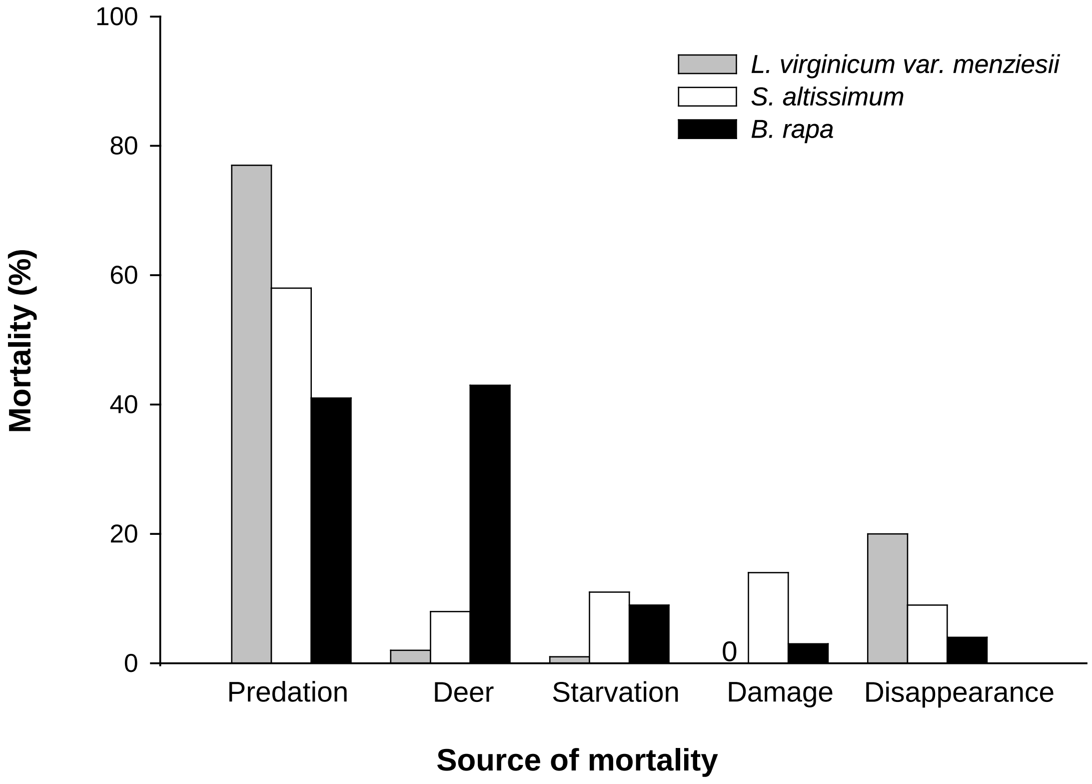

**Figure 3.9** Host-specific sources of mortality of all life stage, among all sites and host plant species, *Lepidium virginicum* var. *menziesii*, *Sisymbrium altissimum*, and *Brassica rapa* from 2005-2008 (n=1515). Unknown causes of death (n=51) and fifth-instar larvae likely to have wandered from the host plant in search of a pupation site (n=51) were not included. The highest number of death was attributed to predation (n=752). The majority of predation was limited to eggs and early instars (instar I-III) stages (n=640). Predation by spiders was observed most often (n=109) although wasps were also observed to predate on larvae. The second greatest contributor of mortality was deer herbivory (n=415).

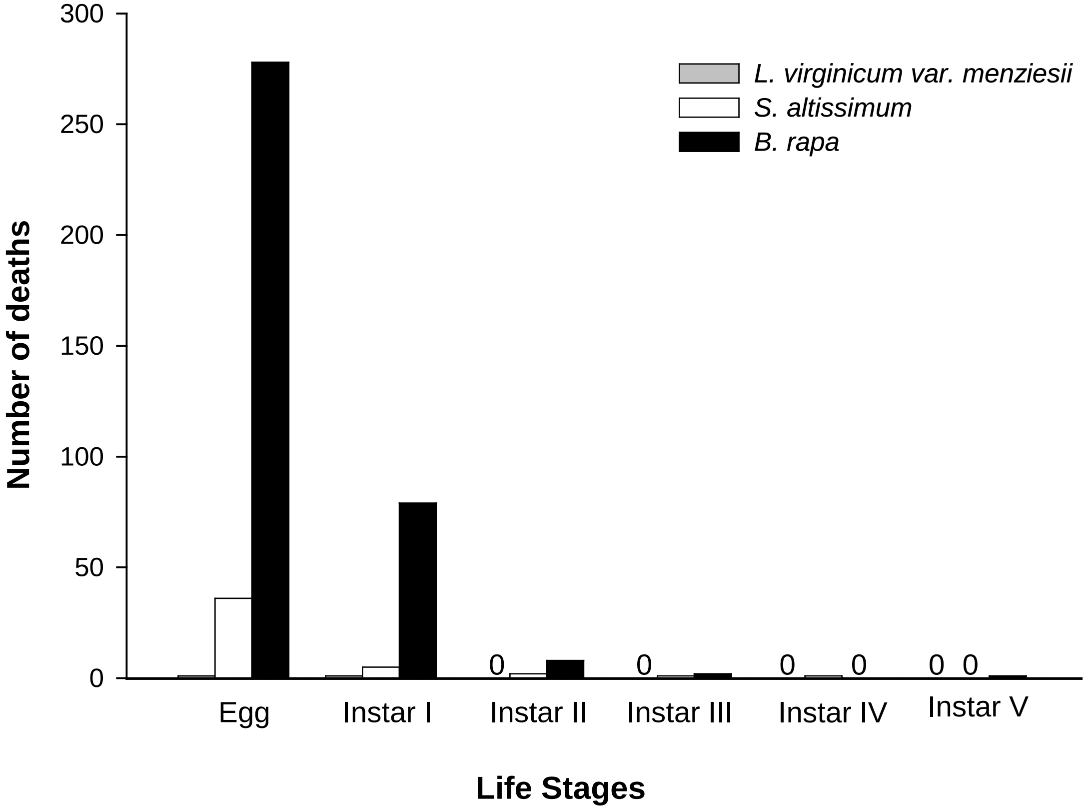

**Figure 3.10** Effects of deer herbivory on stage-specific mortality, 2005–2008 (n=415). Number of deaths due to deer herbivory among three host plant species, *Lepidium virginicum* var. *menziesii*, *Sisymbrium altissimum*, and *Brassica rapa*.

# Chapter 4

# Establishment of a Native Host Plant for Conservation of a Rare Pierid

## INTRODUCTION

The island marble butterfly, *Euchloe ausonides insulanus* Guppy and Shepard (Lepidoptera; Pieridae), is a native, rare butterfly restricted to San Juan and Lopez Islands in Washington State, USA. Populations of *E. ausonides insulanus* are highly localized and persist in small numbers, and therefore are a high conservation priority for state and government agencies. In an effort to enhance prairie remnants to support rare butterfly populations *Turritis glabra* L., tower mustard, a potential native host plant, was selected for research.

Currently, the majority of *E. ausonides insulanus* populations occupy upland grassland habitat where non-native host plant species, *Brassica rapa* L. var. *rapa*, field mustard and *Sisymbrium altissimum* L., tumble mustard, occur. Remnant patches of native prairie also occur in upland habitat and are distributed primarily along the ridgelines adjacent to introduced grasslands. Although non-native host plant species seem to have effectively replaced many of the functions formerly served by native host plant species (Pyle 2004), remnant patches of native prairie serve as important nectar and mate location sites (Lambert 2011 Chapter 2). For example, adults were observed more frequently in areas of native plants compared to areas without native plants in grassland areas without host plants (Appendix B Figure 4.4). Native prairie is also dominated by native bunch grasses that are substantially shorter in height compared to introduced grasses and may provide habitat structure complimentary to mate locating behavior (Lambert unpub. data). In another study, Severns & Warren (2008) found that the presence of native prairie (dominated by native bunch grasses and nectar plants) influenced oviposition site selection of Oregon *Euphydryas editha taylori* even though the primary larval food plant was non-native (*Plantago lanceolata*) and more abundant in introduced grassland habitat. These data compliment other research findings that habitat structure, quality and connectivity (as opposed to only host plant resources) play an important role in the distribution and abundance of many butterfly species associated with open grassland habitats (Dennis and Eales 1997, Thomas et al. 2001, Dennis and Hardy 2007, Lenda & Skorka 2010). Thus, native prairie restoration may be one important step to restoring both native nectar and host plant species on local and regional scales.

Native prairie ecosystems are one of the most endangered ecosystems in western Washington and British Columbia. In western Washington native prairie occupies less than 3% of its historical range (Chappell et al. 2001). Such a high level of habitat loss especially on islands in the San Juan Island archipelago and Georgia Basin may have contributed to the restricted distribution and rarity of *E. ausonides insulanus* by limiting immigration between local populations. Furthermore, on a local scale small and isolated remnant prairie patches may not be a good representation of the former heterogeneous ecosystem. Given the highly invaded state of the prairies currently, it is difficult to attain a definitive understanding of how the dynamics of current *E. ausonides insulanus* populations function. This study proposes an additional native host plant, *T. glabra*, for restoration into upland grassland habitat on San Juan Island.

### *Background*

The earliest record of *E. ausonides insulanus* was a specimen collected in the 1860's from Vancouver Island (Shepard 2000). It is thought that specimens were collected in open prairie habitat described as having native forbs that provided nectar and host plant resources (Guppy and Shepard 2001). Jon Shepard (2000) suggested that *Arabis hirsuta* L. (currently *Arabis eschscholtziana* Andrz.) may have been the original native host plant for *E. ausonides insulanus*. However, *T. glabra* may be a better candidate as an alternative native host plant (see Appendix D for complete discussion).

Remnant patches of native prairie similar to the one described in historical records for Vancouver Island are found on the southern end of San Juan Island and have moderate-high percent cover of native vegetation (e.g., *Festuca roemeri, Cerastium arvense* and *Koeleria macrantha* plant community). Remnant native prairie patches provide *E. ausonides insulanus* with a variety of nectar resources (e.g., *Cerastium arvense*, *Ranunculus californicus* and *Brodiaea howellii*) and mating sites (e.g., white flowers of *C. arvense*) However, no native host plant species occur in native prairie habitat. *Lepidium virginicum* var. *menziesii* is the only native host plant species known to support *E. ausonides insulanus*. The majority of *L. virginicum* var. *menziesii* is limited to nearshore habitat between driftwood berms and open lagoons and do not overlap in distribution with species that occur in native prairie habitat. It is presumed that alterations to and reductions in native prairie drastically reduced ancestral host plant populations (e.g., *A. eschscholtziana*) in grasslands on San Juan and Vancouver Islands. Heavy sheep grazing at the turn of the 19th century likely caused a decline in native host plants populations that resulted in the extirpation of *E. ausonides insulanus* from Vancouver Island (Shepard 2000, Pyle 2004). Similarly, agriculture conversion and introduction of domestic livestock resulted in the decline and degradation of native plants in grasslands on San Juan Island (Rolph & Agee 1993, Griffin 1852 as cited in Avery 2002). However, unlike Vancouver Island, *E. ausonides insulanus* presumably persisted on San Juan Island in grasslands by switching to non-native introduced host plants (i.e., *B. rapa* and *S. altissimum*) as native host plants became increasingly rare.

Based on literature surveys and anecdotal evidence it is difficult to know what the original native host plants of *E. ausonides insulanus* were before the introduction of *B. rapa* and *S. altissimum*. However, females have been observed to oviposit on several species in the Brassicaceae family including the three primary host plants (*B. rapa*, *S. altissimum* and *L. virginicum var. menziesii*) and species such as *Sisymbrium officinale*, *Sinapis arvensis* (formerly *Brassica kaber*), *Cakile* spp. and *Brassica oleracea* (Lambert 2005, Miskelly and Fleckenstein 2007, Hanson et. al. 2009). *Turritus glabra* has been shown to support the development of *E. ausonides insulanus* under controlled laboratory conditions (J. Miskelly pers. comm.) but the development of larvae on *T. glabra* has not been tested under field conditions. Species other than the three primary host plant species have not been observed to support *E. ausonides insulanus* under field conditions due to various morphological and phenological plant traits that prevent development of the immature stages.

*Host plant suitability*

Knowledge of species-specific biology is one of the key factors that determine the success of habitat restoration for butterflies (Thomas 1995, Swengel & Swengel 2001, Wallis DeVries 2004, Schultz 2008) and is critical to the understanding of host plant use and offspring survival in *E. ausonides insulanus.* Like other species in the Brassicaceae family, *T. glabra* contains glucosinolates critical to *E. ausonides insulanus* eggs and larvae. Additionally, *T. glabra* has morphological characteristics suited to the resource requirements of *E. ausonides insulanus*. For example, *T. glabra* plant morphology is similar to host plant species *B. rapa, S. altissimum* and *L. virginicum* var. *menziesii*. Plants grow in an erect position and contain unopened, tightly clustered flower buds at the terminal.

Differences in morphological and phenological host plant traits affect *E. ausonides insulanus* oviposition site selection within the same host plant patch (Lambert 2011 Chapter 2). For example, studies on egg-laying patterns suggest that females prefer to oviposit on plants that are taller and have a greater number of racemes. Host plant height may be the most important factor that affects oviposition site selection, particularly among individual plants of *B. rapa*. For example, the height of *B. rapa* plants supporting eggs was consistently similar across four years of study (Lambert 2011 Chapter 2). Plant height is also suspected to play a role in oviposition site selection among different species of host plants that co-occur within the same host plant habitat. For example, in sand dune habitat, morphologically distinct individuals of *L. virginicum* var. *menziesii* occur in small numbers. In areas where *L. virginicum* var. *menziesii* and *S. altissimum* co-occur plants of *S. altissimum* are much taller than plants of *L. virginicum* var. *menziesii*. In four years of observational studies, eggs were never found on *L. virginicum* var. *menziesii* growing in sand dune habitat, whereas plants of *S. altissimum* were routinely observed to have eggs. Behavioral studies suggest that plants of *L. virginicum* var. *menziesii* in sand dune habitat may not be as “conspicuous” to females due to the short stature of plants compared to taller racemes of *S. altissimum* (Lambert pers. obs.). For this reason, plant height was one of the most important factors considered in the selection *T. glabra*.

Preliminary studies suggest that *T. glabra* is comparable in height to *B. rapa*. In an observational study conducted at American Camp, San Juan National Historical Park (SAJH) in April 2006, potted plants of *T. glabra* (grown in the greenhouse) were randomly placed in grassland habitat occupied by *E. ausonides insulanus*. Flower stalks of *T. glabra* ranged in height from 30 - 70 cm (based on greenhouse studies) (Lambert unpub data). An adult was observed to land and roost for several hours on flowers of a potted plant of *T. glabra* (Figure 4.1). Plant height of *T. glabra* was comparable to *B. rapa* and taller than introduced grasses (Figure 4.2). Results of these preliminary studies suggest that plant racemes that grow moderately high in grassland habitat may be accessible to adults searching for oviposition sites.

In *B. rapa*, the presence of eggs was also highly dependent on plant phenology (Lambert 2011 Chapter 2). Likewise, the phenology of *T. glabra* coincides with adult flight season and larval development. The development of flowers and fruit begin in early April and continue through July. Fruits develop at the same rate that larvae mature. This is critical to survivorship during vulnerable stages of development. Finally, host plants records for the large marble, *E. ausonides*, include *T. glabra* (formerly *A. glabra*; FNA) (Scott 1975, Opler 1999).

This preliminary research supports the supposition that *T. glabra* is a good candidate for native host plant introduction. Several factors that contribute to this assessment include host plant suitability (i.e., Brassicaceae), plant phenology coinciding with both adult flight season and larval development and morphological characteristics (e.g., tall racemes) suited to the resource requirements of *E. ausonides insulanus*. Other factors that make *T. glabra* compatible with prairie habitat include nativity and distribution (i.e., San Juan Island).

Based on previous research and preliminary studies of *T. glabra* plants placed *in situ* host plant traits are likely to influence oviposition selection where *T. glabra* and *B. rapa* co-occur. This research serves to 1) experimentally test restoration treatments to foster establishment of *T. glabra* in introduced grasslands 2) compare plant traits of *T. glabra* and *B. rapa* as they relate to *E. ausonides insulanus* oviposition site selection, 3) test whether *E. ausonides insulanus* will oviposit on *T. glabra* and 4) test whether *T. glabra* can support egg and larvae development.

### Restoration and establishment of *Turritis glabra* L.

*Turritus glabra* likely requires disturbance to establish in introduced grassland habitat and disturbance may be a necessary restoration treatment for the establishment of *T. glabra* in native prairie habitat.

Theory predicts that early successional plants are site limited (Pickett et al. 1987) and observations of *T. glabra* on San Juan Island support this supposition. Disturbance has been recognized as critical factor leading to the creation of bare ground, loose soil or light gaps, which constitute microsites in which species recruitment can occur (Mooney & Godron 1983, Pickett & White 1985). The high rate of native seedling recruitment after disturbance in experimental restoration plots conducted at American Camp (SAJH) in 2005 suggest that disturbance plus propagule input are important factors driving community composition (Lambert 2006). Additionally, species with small seeds (e.g., *T. glabra*) demand more light and may be particularly limited by lack of disturbance (Thompson et al. 1993, Lambert 2006). For these reasons, disturbance is recommended as a restoration treatment to test whether *T. glabra* will establish in introduced grassland habitat.

## METHODS

### Study site

This study took place in grassland in the Cattle Point Natural Resource Conservation Area (NRCA) located on the southern end of San Juan Island, Washington (Lat 48°27’08” Long 122° 57’ 58”).

### ***Turritus glabra*** **seedling establishment experimental study**

To experimentally test restoration treatments to foster establishment of *T. glabra*, ten pair-wise 1m x 2.5m plots were installed March 29 – April 1, 2007 (prior to the adult flight season). To ensure that adults would be present in the research area in 2007, experimental plots were established adjacent to a large patch of *B. rapa* observed to support eggs and larvae in 2006. To prevent impacts to potential pupae overwintering in grasses, plants of *B. rapa* were mapped and flagged in 2006. In addition a five meter buffer zone was designated between the edge of the *B. rapa* host plant patch and experimental plots. The perimeter of the host plant patch was 88 m in length. Experimental plots were randomly selected from the area around the perimeter of the *B. rapa* host plant patch (Figure 4.3).

Each experimental plot was subdivided into two $1m^2$ subplots (Figure 4.3). Paired plots were separated by a $0.5\ m^2$ buffer. Plots were designated as either a control (undisturbed) or treatment (disturbed). In disturbed plots (n=10), vegetation was removed and 6 inches of topsoil was turned over using a shovel. Control plots (n=10) were left undisturbed (Figure 4.3). All 20 plots (disturbed and undisturbed) were methodically sown with 100 seeds of *T. glabra* on April 1, 2007. Seeds of *T. glabra* were collected from local populations on San Juan Island in July 2006.

Experimental plots were monitored for germinants (i.e., *T. glabra* cotyledons) on April 21, 2007. Survival beyond seedling recruitment was recorded on April 11, 2008 by censusing the number of rosettes. Following seedling establishment, rosettes were monitored for reproductive stalks and flowers every 3-5 days throughout the 2008 growing season (April 14 – June 20, 2008). Plant height and the number and location of racemes on each plant were recorded to track deer herbivory. All individuals of *T. glabra* were removed at the end of the 2008 flowering season to prevent seed set.

A paired sample t-test was initially proposed to test the null hypothesis that the two population means (numbers of germinated in disturbed plots compared numbers of germinated in undisturbed plots) were equal. However, no data were collected in undisturbed plots (i.e., no germination occurred) and therefore a statistical model was unnecessary to describe study results.

### Comparative study between *T. glabra* and *B. rapa*

A comparative study was conducted to identify host plant traits (height, number of racemes and plant phenology) that may contribute to oviposition site selection in areas where both *T. glabra* and *B. rapa* occur. A subset of *T. glabra* and *B. rapa* plants were identified for study in the research area on May 5, 2008. Individuals of *T. glabra* and *B. rapa* in early stages of plant development were selected for study based on host plant traits (age class and number of racemes). Sample size was limited by the number of available host plants in age class 1 (>50 percent buds) with a single raceme. Nine individuals of *T. glabra* met research criteria. Based on these criteria, nine individuals of *B. rapa* were randomly selected from a population of twelve individuals that met selection criteria. A total of 18 plants (9 plants of each host plant species) were monitored over the course of the growing season from May 5 – June 20. Surveys were conducted every 5 – 7 days for a total of 6 census periods (May 5, 19 and 25 and June 2, 8 and 20). All host plant racemes were counted, plant height was measured and damage due to deer herbivory and below ground herbivores was recorded. The height of perennial, introduced grasses (e.g., *Agropyron repens*, *Holcus lanatus*, *Poa pratensis*) was also measured during each census period to determine the relative height of vegetation surrounding *T. glabra* and *B. rapa*. Ten inflorescences were randomly sampled within 1 m of *T. glabra* and *B. rapa* study plants.

To understand differences in host plant phenology, plants of *T. glabra* and *B. rapa* were classified according to age class (categories 1 −4) during each census period. Age class 1 represented young plants in bud and age class 4 represented senescent plants in fruit. Specifically, age classes were determined by measuring the ratio of buds to flowers and flowers to fruits. Class 1 plants contained >50 percent buds and <50 percent flowers; class 2 plants <50 percent buds and >50 percent flowers; class 3 no buds and <50 percent flowers and class 4, no buds or flowers only developing fruit.

Repeated measures ANOVA was used to test for differences in height among grasses, *T. glabra* and *B. rapa* over six census periods. Prior to running test data met assumptions of repeated measures ANOVA. Data was transformed using log(ln).

### Euchloe ausonides insulanus eggs and larvae survey

To examine oviposition preference for *B. rapa* and *T. glabra* all eggs were censused on survey plants (*B. rapa*, n=105; *T. glabra*, n=44). Censuses of eggs on all plants of *B. rapa* and *T. glabra* were conducted on April 27, May 19, 25 and June 8.

To track egg and larval survivorship on *T. glabra*, plants (n=44) were examined for eggs and larvae every 3-5 days beginning April 17 thru June 20, 2008. Plants with eggs were flagged and the location of eggs and developing larvae were tracked. Larval behavior, size (mm) and development stage (i.e., instar) were recorded.

## RESULTS

### *Turritus glabra* seedling establishment experimental study

A total of 183 seeds of *T. glabra* germinated in plots where soil was disturbed (18.3±9.97 seedlings per plot). No germinants were observed in undisturbed plots, indicating that disturbance is needed in order for recruitment to occur.

Of the 183 seeds that germinated in disturbed plots, 24% survived to maturity (i.e., developed reproductive stalks). A total of 44 plants established in disturbed plots (4.4±4.5 plants per plot) compared to 0 plants in undisturbed plots.

Almost all plants of *T. glabra* (73%) were damaged by deer or below-ground herbivores. Evidence of deer herbivory was observed on 28 plants (Figure 4.4). Five plants were browsed more than once. Although deer herbivory caused significant damage to plant racemes, browsing by deer caused very few plant deaths (n=1) compared to below-ground herbivores (n=5). The mean height of plants that were not browsed by deer (n=12) was 48±16.20cm (May 25 census period) and 52.92±5.53cm (June 20 census period).

## Comparative study between *T. glabra* and *B. rapa*

The difference in height between *T. glabra*, *B. rapa* and grasses was significant ($F_{2,25}$=5.464, P=0.011). Subsequent univariate tests showed no difference between *B. rapa* and introduced grasses but *T. glabra* was significantly shorter than *B. rapa* and introduced grasses (Tukey HSD; P=0.03, P=0.02, respectively; Figure 4.5). The mean height of *T. glabra*, *B. rapa* and introduced grasses on May 25 (during the height of female flight and oviposition) was 40.44, 56.33 and 55.56 cm respectively.

Repeated measures ANOVA indicated that there was also a significant time effect ($F_{5,21}$=24.943, P<0.001), in other words, the height of all species significantly increased over time. However, an interaction between species and time was not detected ($F_{10,42}$=0.692, P=0.726), therefore, time was not a factor that changed the relationship between species.

Similar results were found between the number of racemes in host plant species *T. glabra* and *B. rapa*. The difference in number of racemes between *T. glabra* and *B. rapa* was significant ($F_{1,16}$=4.855, P=0.043) (Figure 4.6). Repeated measures ANOVA indicated that there was a significant time effect ($F_{3,14}$=4.438, P=0.022). The number of racemes in both *T. glabra* and *B. rapa* increased over time, however, an interaction between species and time was not detected ($F_{3,14}$=1.679, P=0.217). The mean number of racemes on plants of *T. glabra* and *B. rapa* on May 25 was 1.44 and 3.78 respectively.

Most plants of *T. glabra* and *B. rapa* were browsed by deer.  A greater number of *B. rapa* plants were browsed by deer (95%) than plants of *T. glabra* (87%) (Figure 4.7). Evidence of deer herbivory of *B. rapa* was observed at all 5 census periods, and in 4 of 5 census periods for *T. glabra*. The timing of deer herbivory differed between *T. glabra* and *B. rapa*. The majority of *T. glabra* plants were browsed early in the season. Forty-seven percent of *T. glabra* plants were browsed on May 19 (compared to 24% of *B. rapa*). The peak of deer browsing on *B. rapa* occurred several weeks later. Twenty-nine percent of *B. rapa* plants were browsed on June 8 (compared to 7% of *T. glabra*) (Figure 4.7).

*Turritus glabra* and *B.rapa* were similar in age class for the first three census periods, from May 5 – May 25 (age class 1 and 2). However, trends in age class differed in the last three census periods from June 2 – June 20. On June 2 very few plants of *T. glabra* had buds as opposed to flowers compared to plants of *B. rapa* that had similar numbers of buds and flowers (differences in age class 2 and 3) (Figure 4.8). Similarly, by June 20, most plants of *T. glabra* had developed fruits and were without buds or flowers (age class 4) compared to more than half of the plants of *B. rapa* that had flowers and a small number of buds (age class 2 and 3) (Figure 4.8).

### Euchloe ausonides insulanus eggs and larvae survey

A total of 24 eggs (2-April 27, 5-May 19, 17-May 25 and 0-June 8) were observed on all plants of *B. rapa* within the study site (n=105). A single greenish-white egg was observed on *T. glabra* (n=44) (Figure 4.9). Egg development and survivorship on *T. glabra* was tracked. The egg developed and changed colors from greenish-white to orange (May 20), red (May 25) and brown (June 2). The egg hatched and the first-instar larva (1.5 mm in length) was observed feeding on flower petals on June 4 (Figure 4.9). The larva was not found on June 8 and was presumed to have been predated.

## DISCUSSION

The results of this study suggest that the establishment of *T. glabra* is highly dependent upon the input of seed and open ground (i.e., disturbance) and that the persistence of *T. glabra* is likely to depend on the reduction of competition from competitive introduced grasses and exclusion of deer.

Findings also confirm that *E. ausonides insulanus* will oviposit on plants of *T. glabra* if they are present. Females may initially lay few eggs on *T. glabra* compared to more robust non-native host plants such as *B. rapa* but an increase in the number of native host plants may eventually shift resource use to *T. glabra*. For example, although only one egg was observed on *T. glabra*, very few oviposition sites were available to females on the date the egg was laid May 19th (14 available oviposition sites) compared to *B. rapa* (5 eggs were observed on 193 available oviposition sites). Thus the ratio of eggs to available oviposition sites was higher on *T. glabra* compared to *B. rapa* (7.1% of available oviposition sites of *T. glabra* were occupied compared to 2.6% of available oviposition sites of *B. rapa*). This data suggests that *T. glabra* may be a suitable host plant for *E. ausonides insulanus* where both species co-occur although further study is needed to verify these results.

## Turritus glabra establishment

The establishment of *T. glabra* requires both disturbance and seed input. Disturbance was found to be one of the critical factors leading to the creation of open ground required for *T. glabra* germination. In disturbed soil treatment plots where *T. glabra* seeds were added both germination (183 seedlings) and recruitment (44 established plants) were significantly higher than in undisturbed plots (0 seedlings and 0 established plants). Thus, *T. glabra* requires disturbance to promote recruitment.

In this study soil disturbance stimulated *T. glabra* germination, but fire may be used as an alternative method to promote recruitment. For example, in native prairie habitat where *T. glabra* is established (e.g., Yellow Island) local populations have been observed to increase in abundance following fire (A. Lambert pers. obs., P. Green pers. comm.). In addition, several studies conducted in Puget Sound prairies have found that other native forb species (e.g. *Camassia* spp., *Ranunculus* spp. and *C. arvense*) increase following prescribed burning (Schuller 1997, MacDougall 2002, Dunwiddie 2003, Andreu 2005). Therefore, prescribed burning may be one strategy to maintain populations of *T. glabra* in native prairie restoration in upland habitat on San Juan Island following propagule input. Coordinated efforts may be needed to create conditions that enable *T. glabra* to regenerate while at the same time protect plants that become occupied by *E. ausonides insulanus* eggs and larvae. For example, unoccupied patches of *T. glabra* may be burned while patches that support eggs and larvae are protected to avoid mortality of pupae overwintering in vegetation surrounding host plants. The design of an appropriate burn strategy may involve a combination of temporal and spatial parameters that mitigate short-term impacts to host plant habitat in order to maximize *E. ausonides insulanus* population growth. Research documenting the effects of prescribed burning on populations of endangered butterfly species, Fender's blue butterfly, *Icaricia icarioides fenderi* Macy and regal fritillary, *Speyeria idalia* Drury, underscore the importance of management strategies that balance prescribed burning with efforts to protect critical host plant habitat (Schultz and Crone 1998, Swengel 1996).

In native prairie habitat it may be that managers can establish populations of *T. glabra* with a combination of seed input and fire treatments but in introduced grassland habitat efforts to restore *T. glabra* may be more resource intensive and complex.  Although this study did not directly test the effects of competition, in cases where habitat is dominated by introduced grasses (e.g., *Agropyron repens*, *Poa pratensis* and *Agrostis tenuis*) establishment of *T. glabra* will be limited by a combination of factors such as lack of seed input, site limitation (i.e., lack of disturbance) and competition for resources. In order to restore sustainable populations of *T. glabra*, native prairie species will need to be restored in tandem with *T. glabra*. Plans to restore *T. glabra* within a matrix of native bunch-grass species (e.g., *Festuca roemeri, Danthonia californica* and *Koeleria macrantha*) may aid in reducing competition. Unlike the thick impenetrable mat of rhizomes created by many introduced grasses, native bunch grasses have fibrous root systems that allow native forbs (such as *T. glabra*) to occupy the space between grasses (Lambert 2006). *Turritus glabra* and native bunch grasses may also partition resource requirements. For example, mature plants of *T. glabra* have a relatively deep root system with a single main root axis that likely draws resources from below fibrous root soil profiles. Furthermore, *T. glabra* is a biennial or sometimes short-lived perennial and once established plants may persist for several years in native prairie habitat where competition is minimal.

Long term establishment of *T. glabra* will most likely require reduction of introduced grasses (i.e., competition) and exclusion of deer and below-ground herbivores. For example, deer damaged 64% of all *T. glabra* plants including several plants browsed more than once over the course of the season (Figure 4.4 and 4.7). Deer fed on buds, flowers and stems of *T. glabra* effectively removing the reproductive parts of plants and reducing the height of plants by several centimeters (2-32cm). Deer herbivory was observed to stimulate the growth of buds and flowers on new racemes in *B. rapa*. However, new growth on *T. glabra* was slow and varied in response to deer herbivory. For example, in the comparative study between *T. glabra* and *B. rapa*, 14% of *T. glabra* plants regenerated new racemes in response to deer herbivory compared to 75% of *B. rapa* plants. In another study, deer herbivory was found to limit the establishment of native bulbs (e.g., *Triteleia hyacinthine, Allium cernuum, Brodiaea coronaria* and *Camassia quamash*) more than competition from introduced perennial grasses (Gonzales & Arcese 2008).

Deer herbivory may also directly contribute to *E. ausonides insulanus* mortality. For example, in a four year demographic study of *E. ausonides insulanus*, deer herbivory was found to be the greatest contributing factor of egg mortality on *B. rapa*. It was found that more than 58% of egg mortality on *B. rapa* was due to deer herbivory (n=478; Lambert 2011 Chapter 4). Thus, if *T.*

*glabra* is used as an alternative native host plant for *E. ausonides insulanus* it will be necessary to protect plants from being browsed by deer especially during the period of egg and larval development (i.e., May and June).

### Variation in oviposition on *T. glabra* and *B. rapa*

Morphological and phenological comparisons between *T. glabra* and *B. rapa* showed that certain traits may influence oviposition choice. The comparative study results indicated that *T. glabra* was significantly shorter in height that *B. rapa* and introduced grasses (Figure 4.5) and may have contributed to the low number of eggs observed on *T. glabra* (n=1) compared to *B. rapa* (n=24). In another four year study of *B. rapa*, a significant relationship was found between host plant height and egg-laying patterns. Plants of *B. rapa* with eggs were significantly taller than plants without eggs (Lambert 2011 Chapter 2). These combined results suggest that host plant height may contribute to *E. ausonides insulanus* oviposition selection in habitat where two (or more) potential host plant species are available and that taller host plant species (e.g., *B. rapa*) may receive greater numbers of eggs. Similarly, Masumoto et al. (1993) found that in a closely related butterfly species, yellow-tip, *Anthocaris scolymus* Butler, the number of eggs and larvae on *T. glabra* increased significantly on host plants that were taller, growing in low densities and surrounded by sparse vegetation (as opposed to dense vegetation cover) (characteristics defined as “host plant conspicuousness”). It may be that the disproportionate number of eggs found on *B. rapa* relates to the “conspicuousness” of tall racemes and thus females approach taller plants compared to smaller less “conspicuous” host plants (i.e., *T. glabra*). Other factors that may contribute to “host plant conspicuousness” are plant size (number of racemes) and abundance (density). Plants of *B. rapa* had significantly greater numbers of racemes than *T. glabra* (Figure 4.6) and were twice as abundant at the study site (105, 44, respectively).

Under certain conditions *T. glabra* may be more conspicuous to *E. ausonides insulanus* searching for oviposition sites. For example, *T. glabra* racemes grow moderately high (48±16.20 cm without deer herbivory) compared to leaves of native bunch grasses (approximately 18 cm) and will likely be accessible to adults searching for oviposition sites within a matrix of native bunch grasses and forbs (i.e., native prairie plant community). Further, Kozloff (2005) described the height range of *T. glabra* to be 50–80cm, thus, *T. glabra* may grow taller under certain conditions.

A subset of 9 individuals of *T. glabra* and 9 individuals of *B. rapa* plants were selected for comparative study based on host plant traits and refer to the study results for mean number of racemes, host plant phenology and deer browsing in the remainder of this section.

Trends in host plant phenology (i.e., average age class) were similar in *T. glabra* and *B. rapa* between May 19 and May 25 (Figure 4.8) at the time when females were most abundant and searching for oviposition sites (Lambert 2011, Chapter 2 Figure 4.7). This suggests that the timing of host plant development of *T. glabra* is consistent with the timing of *E. ausonides insulanus* oviposition. During this time frame, *T. glabra* is similar to *B. rapa* in that *T. glabra* produces unopened, tightly clustered flower buds required for oviposition.

Despite similarities early in the season, *T. glabra* and *B. rapa* differed in host plant phenology later in the season. For example, between census periods May 25 and June 2 (7 days) most plants of *B. rapa* remained the same age class 2 (plants <50 % buds and >50% flowers), in contrast to *T. glabra* that increased from age class 2 (plants <50 % buds and >50% flowers) to age class 3 (no buds and <50% flowers) (Figure 4.8). In other words, buds and flowers *T. glabr*a matured at a faster rate than *B. rapa*. These differences may be important to the development and survival of eggs and early instar larvae. Ostensibly, larvae can complete development on *T. glabra* under controlled conditions (J. Miskelly pers. comm.) but it is unknown whether *T. glabra* can support the development of *E. ausonides insulanus* in the field. To better understand the synchrony of larval development and *T. glabra* phenology more research is needed. The flowering phenology of *T. glabra* would likely be influenced by microclimates and site conditions, and future experiments should explore a range of such conditions.

Almost all plants censused in this study were browsed by deer (87% of *T. glabra* and 95% of *B. rapa*) and evidence of new deer herbivory was observed at every census period. Thus, deer herbivory is likely to alter host plant dynamics and consequently impact *E. ausonides insulanus* oviposition site selection. Deer preferentially browse buds and flowers of host plants and indirectly compete with ovipositing females for host plant resources. Other research has shown deer herbivory to significantly affect island flora, fauna and ecosystems processes (Cote et al. 2004, Gaston et al. 2008, Martin et al. 2010) and thus it is not surprising that relatively high abundance of deer on San Juan Island (Davison pers. comm.) corresponds with the high rate of deer damage to *E. ausonides insulanus* host plants (i.e., *B. rapa* and *T. glabra*).

The percentage of plants browsed by deer was similar in *T. glabra* (87%) and *B. rapa* (95%) but the timing of deer herbivory differed between plant species. The peak of deer herbivory on *T. glabra* occurred mid-season (May 19) compared to deer herbivory on *B. rapa* that occurred later in the season (June 8) (Figure 4.7). The timing of deer herbivory is important because it influences the availability of oviposition sites (i.e., clustered flower buds on a single raceme) on different host plant species. For example, on May 19 evidence of deer browsing was observed on 7 of the 9 *T. glabra* plants that were compared for plant traits. This reduced the number of available oviposition sites to as low as 3 racemes on 2 plants. In comparison, only 4 of the 9 monitored plants of *B. rapa* were damaged by deer providing an upward of 17 available oviposition sites (or racemes) between 5 plants. Although the average number of racemes per plant differed between *T. glabra* and *B. rapa* and contributed to the difference in available oviposition sites between species, preferential deer browsing on *T. glabra* early in the season considerably reduced the number of potential oviposition sites. For this reason, protecting *T. glabra* from deer browsing, especially early in the season is critical to *E. ausonides insulanus* oviposition site selection.

### Development of egg and larva on *T. glabra*

This is the first study to document the use of an additional native host plant for conservation of a rare pierid. The observation of an egg on *T. glabra* suggests that *T. glabra* may have been one of the historic native host plants of *E. ausonides insulanus*. The phenology of *T. glabra* coincides with the adult flight season and egg and early instar larval development. For example, the reproductive stalks of *T. glabra* begin to develop in early May during which time a single egg was first observed on flower buds (May 19) and subsequently hatched into a first-instar larva (June 4) (Figure 4.9). The use of *T. glabra* by *E. ausonides insulanus* is not surprising considering host utilization in *Euchloe* is broad and that most species use several related host plant species for oviposition. In an evolutionary context, oviposition choice is more restricted than larval tolerance of food plants and thus the incorporation of non-native host plants is relatively common in *Euchloe* (especially the use of non-native Brassicacea introduced by the way of agriculture) (Shapiro 1984). For example, non-native host plant range expansion coincided with range expansion of *Euchloe Olympia* Edwards, Olympia marble, from the Manitoulin Island area in eastern Canada to western Quebec (Laberry et al. 1998). Anecdotal evidence suggestions that *E. ausonides insulanus* also shifted in distribution and abundance due to the expansion of *B. rapa* on San Juan Island. Thus, research shows that novel host plant relationships with native host plant species (i.e., *T. glabra*) are also possible.

While it is suspected that females would lay eggs on *T. glabra* in native prairie habitat if plants were present and available to ovipositing females, it is unknown whether *T. glabra* could support the development of eggs and larvae in the field. Although variation in oviposition preference is heritable and responsive to selection, females may lay eggs on host plants that do not maximize survival or development (Renwick and Chew 1994). More field study is necessary to better understand the phenological relationship between developing larvae and *T. glabra* maturation. Future studies may consider alternate field sites. It is suspected that many site related factors influence trends in mortality including factors related to differences in habitat (e.g., sand dune and introduced grasslands). For example, percent mortality due to starvation and disappearance was substantially lower on plants of *S. altissimum* located in grassland habitat compared to *S. altissimum* located in sand dunes (Lambert 2011, Chapter 3 Figure 4.8). Stressful environmental conditions (low precipitation and high temperatures) may have caused *S. altissimum* to develop at a faster rate in sand dune habitat. Whereas *S. altissimum* located in grassland habitat may have access to higher levels of soil moisture which provides more reliable resources to developing larvae. Another consideration may be establishing study sites in areas where adults have been routinely observed early in the flight season. For example, adults were observed flying in the area of the redoubt at American Camp as early as April 8, 2004. The redoubt (transect 1) was also one of the first sites where adults were observed in 2005 (April 27), 2006 (May 2), 2007 (April 27) and 2008 (April 25) (see Appendix B).

## CONCLUSION

The introduction and establishment of *T. glabra* may provide an important host plant resource for *E. ausonides insulanus* but more study is warranted to confirm whether *T. glabra* can support the development of larvae under field conditions. In summary, the findings conclude that *T. glabra* may be a good candidate for native host plant introduction. Several factors contribute to this assessment including host plant suitability (i.e., plants contain glucosinolates), plant phenology (coinciding with the timing of female oviposition) and morphological characteristics (e.g., relatively tall plant racemes). Other factors that make *T. glabra* compatible with prairie restoration include nativity, distribution (i.e., San Juan Island) and life history traits. The structure afforded by native prairie species is also likely to reduce competition between *T. glabra* and introduced grasses (provided that *T. glabra* is planted with native bunch-grasses as part of on-going prairie restoration efforts). The results of this study also conclude that the establishment of *T. glabra* requires disturbance and seed input and that the exclusion of deer may be necessary to the long-term establishment of *T. glabra*.

**Figure 4.1** *Euchloe ausonides insulanus* adult resting on container plant of *Turritis glabra*, American Camp, SAJH, April 2006. Plants were propagated at the UW Botanic Garden. Seeds were collected from Yellow Island. Container plants were placed in *E. ausonides insulanus* habitat for approximately 6 hours to photograph plant *in situ*. Unexpectedly, an adult landed on *T. glabra* while taking photographs.  The adult was observed roosting for several hours.

**Figure 4.2** Container plants of *Turritus glabra* placed *in situ* to investigate the structure of *T. glabra* in introduced grassland habitat. The yellow inflorescences of *Brassica rapa* is represented in the background.

**Figure 4.3** Experimental plots for *Turritus glabra* experiment (1m x 2.5m) installed March 29 – April 1, 2007. Meter tape indicates the perimeter of the host plant patch. Vegetation was removed and soil turned in treatment plots (n=10). Control plots (n=10) were left undisturbed (as indicated by the white PVC).

**Figure 4.4** Evidence of deer browsing on *Turritus glabra*. Seventy-three percent of all *T. glabra* plants were damaged by deer. Five plants were browsed more than once.

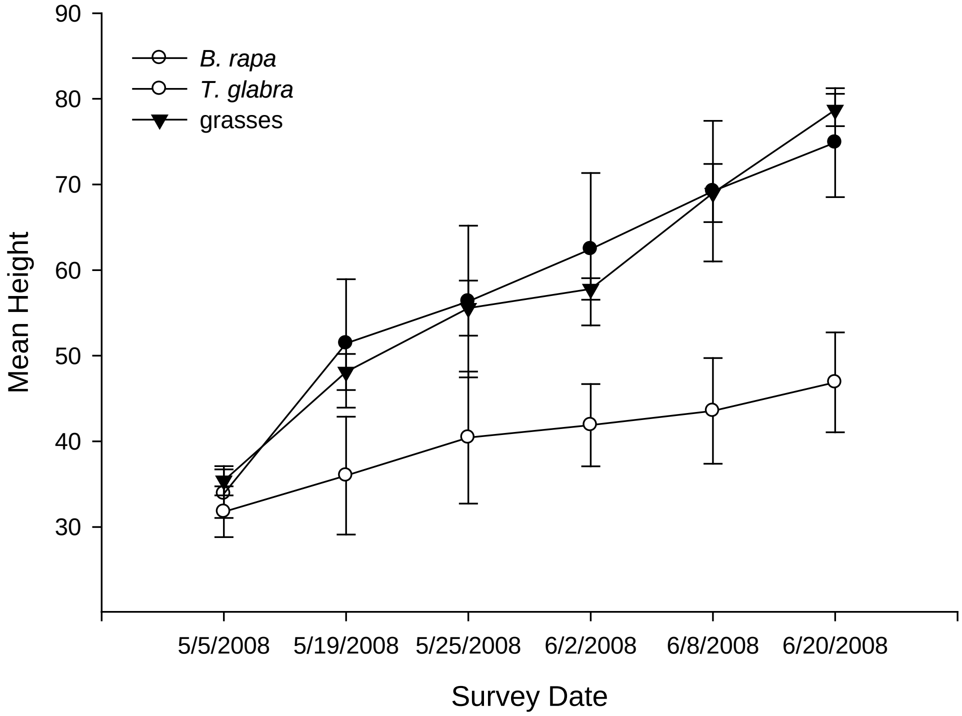

**Figure 4.5** Mean height of *Turritus glabra*, *Brassica rapa* and introduced perennial grasses at Cattle Point Natural Resource Conservation Area (NRCA). A significant difference in height between *T. glabra*, *B. rapa* and grasses was detected (F2,25=5.464, P=0.011). Subsequent univariate tests showed no difference between B. rapa and introduced grasses but *T. glabra* was significantly shorter than *B. rapa* and introduced grasses (Tukey HSD; P=0.03, P=0.02 respectively). The mean height of *T. glabra*, *B. rapa* and introduced grasses on May 25 was 40.44, 56.33 and 55.56 cm respectively.

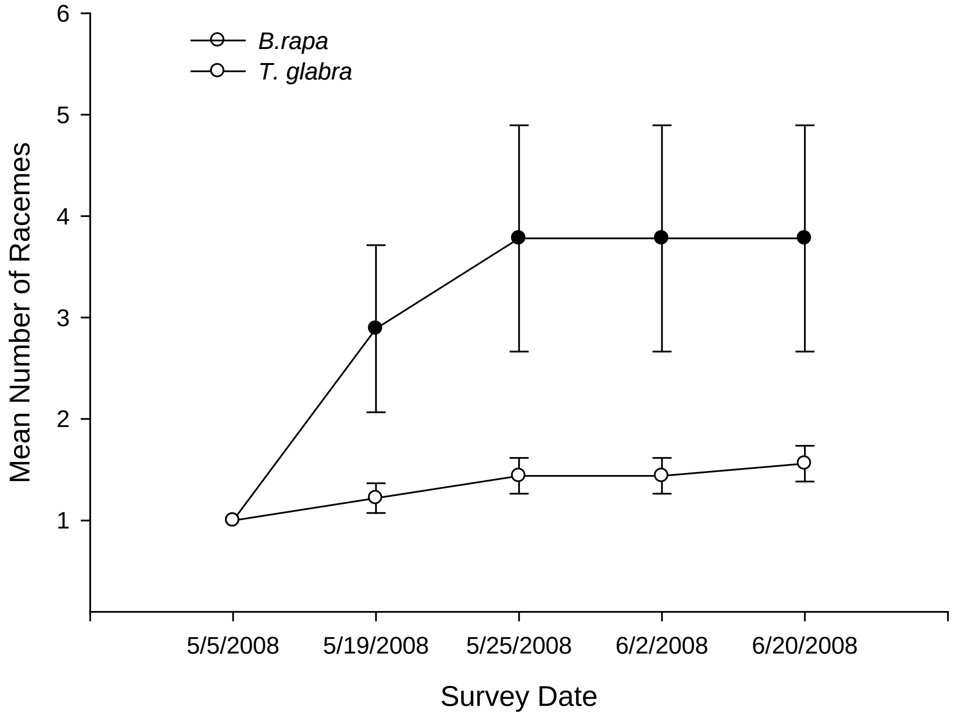

**Figure 4.6** Mean number of racemes on host plant species *T. glabra* and *B. rapa* at Cattle Point Natural Resource Conservation Area (NRCA). A significant difference in number of racemes between *T. glabra* and *B. rapa* was detected ($F_{1,16}$=4.855, P=0.043).

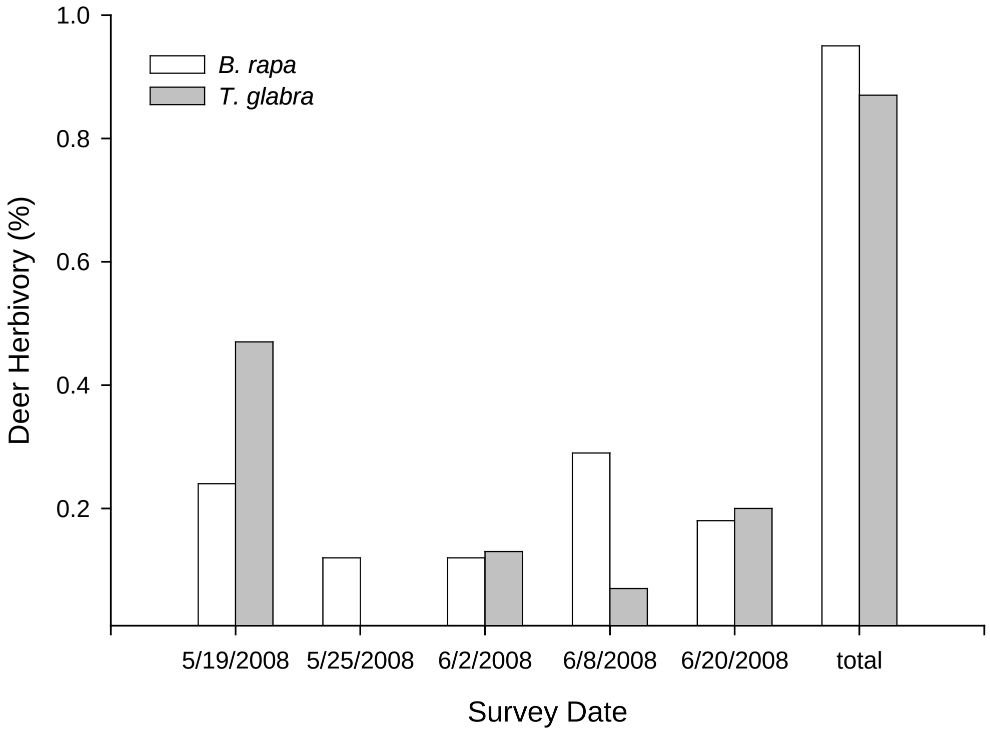

**Figure 4.7** Percent deer herbivory of *Turritus glabra* and *Brassica rapa* at Cattle Point Natural Resource Conservation Area (NRCA). Ninety-five percent of *B. rapa* plants were browsed by deer compared to 87% *T. glabra* plants. The highest percent deer herbivory of *T. glabra* occurred on May 19. Comparatively, the majority of deer herbivory of *B. rapa* occurred several weeks later on June 8.

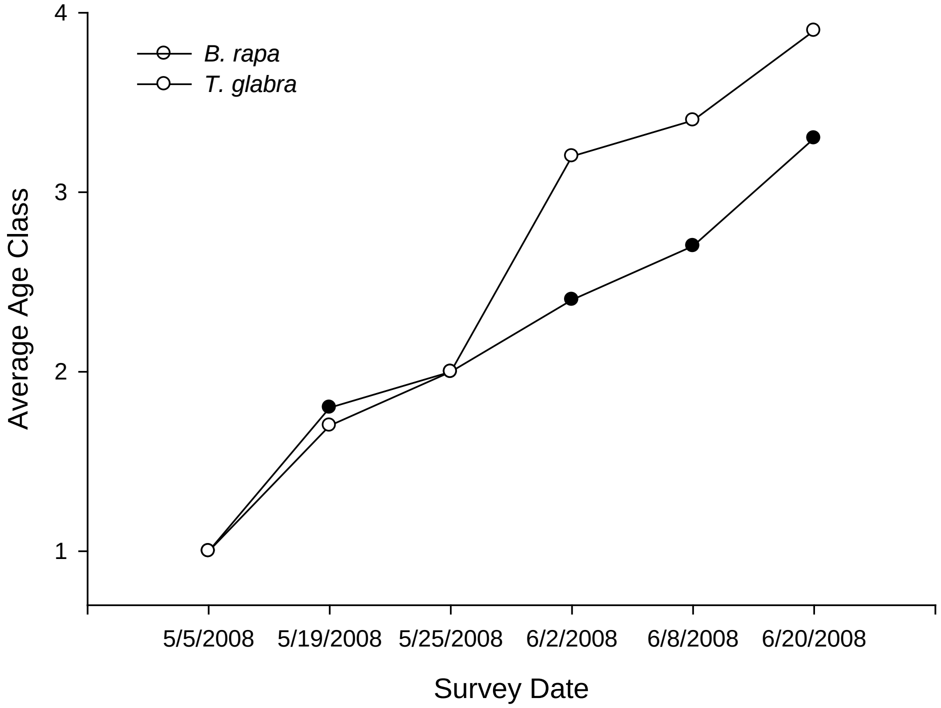

**Figure 4.8** Average age class of *Turritus glabra* and *Brassica rapa* from May 22 – June 20, 2008. Trends in age class differed in the last three census periods from June 2 – June 20. On June 2 very few plants of *T. glabra* had buds compared to plants of *B. rapa* that had similar numbers of buds and flowers (differences in age class 2 and 3).

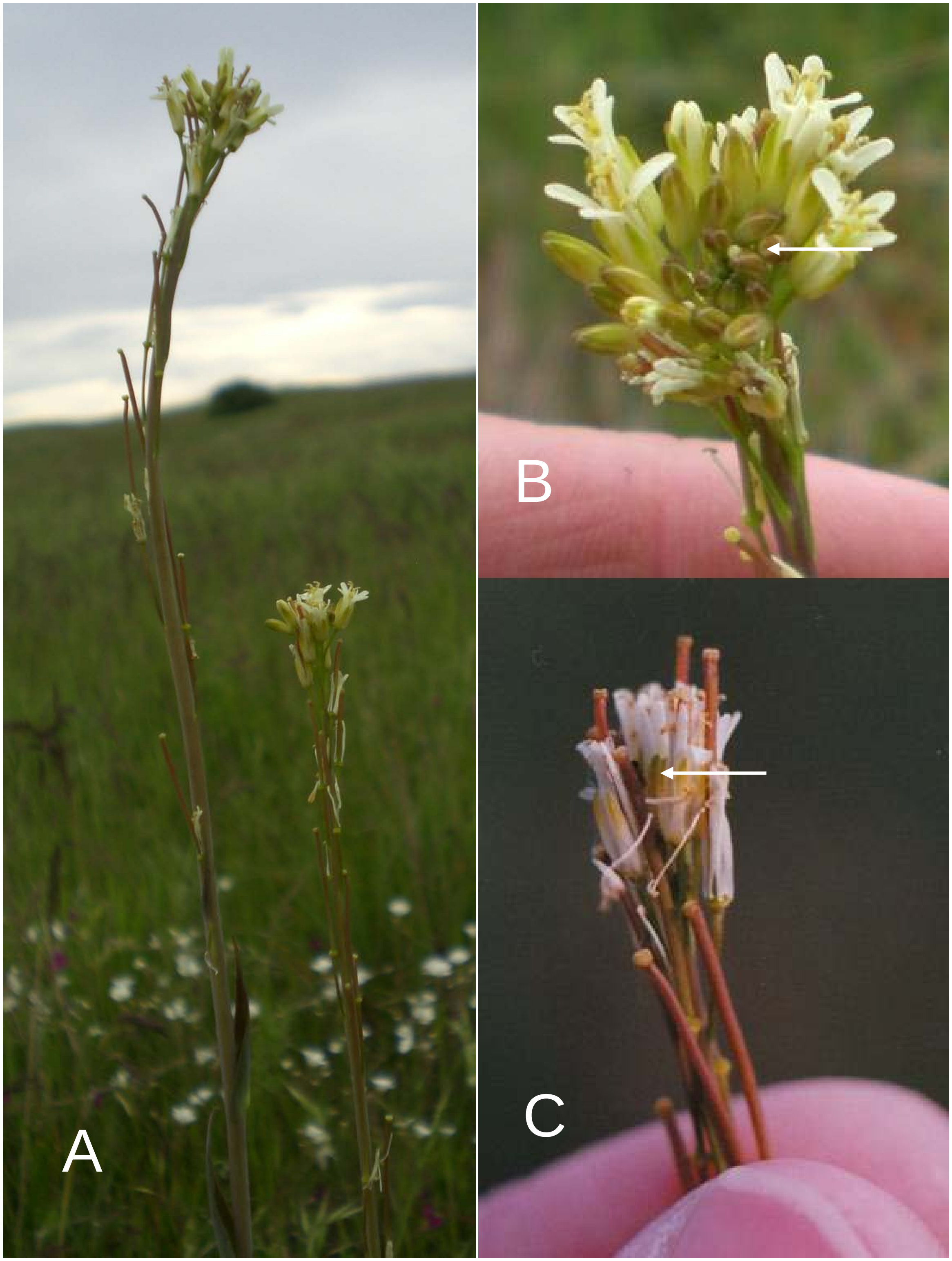

**Figure 4.9** *Euchloe ausonides insulanus* egg and larva on *Turritus glabra*. **A**) Flowering plants of *T. glabra* at Cattle Point Natural Resource Conservation Area (NRCA). The white inflorescences of *Cerastium arvense* is shown in the background. **B**) Greenish-white egg observed on May 19, 2008. **C**) First-instar larva (1.5 mm in length) observed on flower petals on June 4.

# Chapter 5

# Conservation and Management of *Euchloe ausonides insulanus*

## INTRODUCTION

The island marble butterfly, *Euchloe ausonides insulanus* Guppy and Shepard (Lepidoptera; Pieridae), is a native, rare butterfly restricted to San Juan and Lopez Islands in Washington State, USA. Populations of *E. ausonides insulanus* are highly localized and persist in small numbers, and therefore are a high conservation priority for state and government agencies. The majority of known individuals are located at American Camp, San Juan Island National Historical Park, on the southern end of San Juan Island. The population at American Camp is thought to be vulnerable because of the subspecies low population numbers and narrow distributional range.

This paper is based on research findings conducted from 2005–2008. I discuss key ecological issues related to the conservation and management of *E. ausonides insulanus* including potential impacts of climate change, host plant patches dynamics, disturbance, topographic and habitat heterogeneity and significant mortality factors that likely contribute to overall population abundance. Table 5.1 summarizes conservation issues and potential management strategies in support of the *E. ausonides insulanus* population at American Camp, San Juan Island National Historical Park. An experimental approach to management actions may be necessary to better understand the ecological mechanisms that contribute to population abundance. It should be noted however, that population abundance may not be an adequate measure of long term success and that conservation strategies should also consider factors that prevent the population from reaching a threshold beyond which it may not be able to recover.

### Potential impacts of climate change

Butterflies are particularly vulnerable to climate change because of their sensitivity to changes in environmental conditions such as temperature and precipitation (Erhardt & Thomas 1991), and have experienced range shifts induced by warming global temperatures (Parmesan 1996, Parmesan et al. 1999, Parmesan & Yohe 2003, Thomas 2005). Additionally, climate causes shifts in host plant phenology and can affect growth, survival, development and fecundity of butterfly species (Watt & Woiwod 1999, Dixon 2003, Parmesan 2006). Short-term trends in *E. ausonides insulanus* phenology and abundance may be related to year-to-year fluctuations in temperature (Appendix B Table 2). For example, in 2004, a total of 270 adults were observed on transects and the mean temperature in April was 10° C. In contrast, in 2008, only 63 adults were observed on transects and the mean temperature in April was 6.7° C. Wetter, cooler conditions in early spring may reduce the amount of time females have to lay eggs and therefore contribute to reduced fecundity. Several species of pierids have been found to have a limited amount of flight time and subsequently limited oviposition opportunities due to poor weather conditions (Ives 1978, Jones 1977, Jones et al. 1987, Jones & Ives 1979, Courtney & Duggan 1983, Kingsolver 1983, Courtney 1986, Doak et. al. 2006).

Future climatic conditions are also predicted to have an effect on plant phenology, growth and survival (Estrella et al.2007, Pen˜uelas et al.2009, Yang & Rudolf 2010, Singer & Parmesan 2010). Butterfly species are particularly sensitive to changes in plant communities because of intricate ecological and evolutionary associations with food plants at multiple life stages (Ohgushi 1992, Hellmann 2002, Hellmann et al. 2008, Forrest & Miller-Rushing 2010, Chuine 2010). *Euchloe ausonides insulanus* is supported by both native host plant species, *Lepidium virginicum* var. *menziesii* (DC) Hitchc, tall peppergrass, and introduced host plant species, *Brassica rapa* L. *var. rapa*, field mustard, and *Sisymbrium altissimum* L., tumble mustard. *Lepidium virginicum* var. *menziesii* is the only native host plant species known to support *E. ausonides insulanus*. Extant populations of *L. virginicum* var. *menziesii* occur in nearshore habitat between driftwood berms and open lagoons on the south end of San Juan Island. Periodic disturbance (shifting sand and gravel) is needed to maintain *L. virginicum* var. *menziesii* habitat. However, intense and frequent disturbance may be detrimental to *E. ausonides insulanus* pupae and host plant habitat. As climate warming continues the intensity and frequency of off-shore storm surges is expected to increase (Bindoff et al. 2007) and overwintering pupae and *L. virginicum var. menziesii* habitat are likely to be affected. For example, a high tide windstorm on February 2006 shifted driftwood barriers and deposited large volumes of gravel on the lagoon side of the beach thus altering the structure and function of the backshore environment. Due to extensive shoreline disturbance (including the movement and removal of driftwood berms) the number of *L.*

*virginicum* var. *menziesii* plants was reduced by more than 50% at one research site (Chapter 3 Table 5.1; Figure 5.1). Flooding and reshaping of the backshore and lagoon environment also likely caused significant mortality to overwintering pupae.

Butterfly species endemic to islands may be even more vulnerable to changes in climate because of limitations in distribution, especially those species whose habitat is also compromised by anthropogenic change or rise in sea level (Chown et al. 2008, New 2008). For example, global sea level is expected to increase an average of 19 inches by 2100 (IPCC 2007) thus contributing to the reduction and potential elimination of *L. virginicum var. manziesii* habitat. However, adults were observed to disperse from *L. virginicum var. manziesii* habitat to upland *B. rapa* host plant habitat at sites where backshore environments were located adjacent to open grassland habitat and host plants were located within distance that females could disperse (approximately 1km) (Lambert upub. data). Thus, field studies suggest that adults can disperse to alternate host plant habitat (i.e., grasslands) if principal host plant habitat is scarce, difficult to find, or if habitat has been heavily disturbed.

The establishment of additional native host plant (e.g., *Turritus glabra*) in upland grasslands (e.g., in areas of prairie restoration) (see Chapter 4) may buffer impacts to *L. virginicum var. menziesii* in the event that storm surges continue to alter nearshore habitat or rise in sea level flood native host plants. Additionally, in the event that climate warming causes shifts in host plant phenology (i.e., *B. rapa, S. altissimum* and *L. virginicum var. menziesii*), an additional native host plant may provide an additional phenological niche for *E. ausonides insulanus*. Furthermore, the number of eggs laid within host plant species may change considerably from year to year in response to changes in host plant quality and quantity (Parmesan et al. 1999, Hellmann 2002, Thomas et al. 2004, Wilklund & Friberg 2009). Increasing the diversity of host plant species across different types of habitat (nearshore, introduced grassland, native prairie and sand dune) may support population changes that are related to host plant distribution and abundance. Finally, year to year changes in weather patterns are also one of the leading factors causing local population extinctions of butterflies (e.g., Ehrlich et al. 1980) and habitat heterogeneity may be critical to the long-term survival of *E. ausonide insulanus.* Adult dispersal from *L. virginicum var. menziesii* habitat into adjacent grasslands after heavy disturbance to *L. virginicum var. menziesii* plants in 2006 illustrates the importance of maintaining a diversity of high quality host plant habitats.

### Population structure and host plant dynamics

Studies on the family Pieridae have been particularly influential to the development of population dynamics and host plant interactions in ecology (see review by Courtney 1986). The management and conservation of the *E. ausonides insulanus* should consider the application of these areas of study for the protection and conservation of *E. ausonides insulanus*. For example, understanding the population structure of *E. ausonides insulanus* at the local level is critical to designing conservation strategies that target restoring host plant habitat (see Chapter 2 Host Plant Patch Study). Deciding on the appropriate number, spatial arrangement and size of host plant patches to be created or enhanced at American Camp in important to the success of habitat restoration projects that aim to increase the abundance of the *E. ausonides insulanus*. For example, restoring host plant habitat using a *metapopulation* design would likely entail planting relatively few patches of host plant patches spaced at large distances apart from one another to ensure demographic independence. However, restoration plans that incorporated a *patchy population* design may install patches of host plants in close proximity to one another to facilitate movement between patches (see Chapter 2 for detailed description of patch size and density).

*Euchloe ausonides unsulanus* follows an overall metapopulation structure but local populations (i.e., American Camp) are distributed in a more patch-like fashion. Metapopulations have generally been described as having individuals that exhibit strong site fidelity and little dispersal capabilities (low vagility) (Smith & Green 2005). This type of behavior results in discrete local breeding populations connected by little migration (Harrison 1989, Hanski 1999b, Hanski & Simberloff 1997). Based on distributional surveys by Hanson et al. (2010), *E. ausonides insulanus* is likely to have several discrete populations that exhibit metapopulation dynamics. For example, at least two populations located north of American Camp and on Lopez Island are believed to be breeding populations (Hanson et al. 2010).

Historically, metapopulation structure has been used to describe flight patterns of butterflies which have limited host plant finding abilities 50m from host plant patches (Harrison 1989).

In contrast, *E. ausonides insulanus* move distances of 100m or more between patches of host plants (Lambert unpub. data). This suggests that at on a local scale, *E. ausonides insulanus* does not exhibit a metapopulation structure as documented in other butterfly species (Harrison 1989, Smith & Green 2005). On a local scale, *E. ausonides insulanus* exhibits behavior characteristic of a “patchy” population structure. Two important behavioral mechanisms are associated with patchy population dynamics, 1) behavioral avoidance that prevent an over abundance of eggs from being laid on single host plants (i.e., “egg load assessment”) and 2) the occurrence of disproportionate numbers of eggs on the edges of host plant patches (i.e., “edge effect”) (Shapiro 1981)(see Chapter 2). Both behaviors are closely associated with *Euchloe* spp. that feed on brassicaceous host plants (Shapiro 1975, Scott 1975, Shapiro 1981, Shapiro 1984, Karban & Courtney 1987). For example, *E. ausonides insulanus* is an obligate inflorescence feeder limited by temporal variations in plant phenology. It is therefore critical that females select appropriate ovipositing sites that can support the development of larvae. To avoid larval starvation (and/or intraspecific competition) females lay single greenish-white eggs on unopened terminal flower buds.

The concept of the “edge effect” also supports the theory that local populations at American Camp are more likely to be patchily distributed than to adhere to a strict metapopulation structure. This is a phenomenon whereby host plants that occur on the periphery of host plant patches receive a disproportionately high number of eggs (Shapiro 1984) (see Chapter 2 for discussion on “edge effect” and host plant density). The distribution of eggs on “edges” of host plant patches may be related to host plant dynamics. Host plants are generally contagiously distributed (Whittaker 1975). Patches are relatively dense and uniform in the center and plants on the edges are scattered at low density. The distribution of host plants may influence the egg laying patterns of *E. ausonides insulanus*.

The “edge effect” behavior may have also evolved as an avoidance mechanism in response to predators or parasitiods that may be found in more dense areas of host plant habitat (located in the center of patches). For example, Dempster (1969) found that an increase in the density of plants increased the abundance of predators of *P. rapae*. He concluded that reducing the number of plants in experimental plots greatly reduced the abundance of predators and increased the survival of *P. rapae*. Levels of parasitism have also been known to fluctuate between host populations and habitat patches. Wiklund and Ahrberg (1978) attribute these differences to habitat preferences of parasitoids. They suggest that habitat preference of parasitoids may be narrower compared to those of the host. This implies that eggs laid outside of dense (more central) habitat may escape parasitism.

Monitoring the population structure of *E. ausonides insulanus* including the spatial arrangement and size of *E. ausonides insulanus* populations and host plant patches may help to inform conservation management. For example, the results from the *Host plant patch study* (Chapter 2) provide managers with several ways to think about the spatial structure of host plants (i.e., patch size, connectivity and host plant density) and how that relates to egg densities (see Chapter 2). The results of the patch study may be useful for understanding how to maximize the number of eggs at different scales. Number of eggs may be measured per square meter or per host plant. The percent of plants occupied by eggs may also be useful to patch design depending on the goals of restoration. Identifying the rate at which newly disturbed habitat are colonized by host plants and subsequently occupied by *E. ausonides insulanus* may help to predict the number of host plant patches that need to be protected and maintained. Thus, data on the length of time host plant patches are occupied may be useful for future conservation management actions.

### Managing disturbance

Disturbance is a key factor that maintains populations of primary host plants of *E. ausonides insulanus*. Brassicaceous annuals, *B. rapa, S. altissimum,* and *L. virginicum var. menziesii* are the primary host plants of *E. ausonides insulanus*. Because they are early successional species they readily colonize areas that have been disturbed. *Brassica rapa* and *S. altissimum* are weedy non-native species and are particularly prone to colonize exposed soil disturbed by wind (i.e., sand dunes) or small mammals (i.e., grasslands). This is due impart because of the reproductive strategy of weedy species. For example, seeds of *B. rapa* can survive in the soil for extremely long periods of time (Holm et al. 1997). *Brassica rapa* and *S. altissimum* are adapted to temperate climates and germinate readily in sandy soils at American Camp (although *B. rapa* is found more often in grasslands and *S. altissimum* in sand dune areas).

Host plants tend to be widely distributed because species follow an early successional plant strategy and regenerate in areas of disturbance. Where there is disturbance (and significant seed bank) host plants attain high densities and then die out over time. *Euchloe ausonides insulanus* must disperse from regions having declining plant populations to areas where the host plants are colonizing. For this reason, *E. ausonides insulanus* may be described as a "colonizing species".

The creation of bare ground, loose soil and light gaps (in dense grasslands) is necessary for recruitment where seeds are dormant in the soil. In addition, seeds disperse from dense patches (parent plants) into open sites that have been recently disturbed (Mooney & Godron 1983, Pickett & White 1985). In grassland habitat *B. rapa* colonizes areas of over-turned soil caused by small mammals (e.g., *Oryctolagus cuniculus*, European rabbit, single-entrance mounds separate from main warren). The amount of soil disturbance by small mammals is often relatively small (<0.5m in diameter). In patchy, small areas of soil disturbance, *B. rapa* plants are generally robust (Lamber pers. obs.). The presence of large plants of *B. rapa* may be related to the absence of intraspecific competition (Crawley 1997). The size and robustness of host plants is associated with larger egg loads and more vigorous host plants are more likely to support developing eggs and larvae (Lambert 2011 Chapter 2). For this reason, small mammals may be important patch creators of *B. rapa* host plant habitat in dense introduced grasslands. The distribution of mounds influences the distribution of host plants and subsequently egg laying patterns of *E. ausonides insulanus*. Although mounding by small mammals may influence host plant dynamics in dense grassland habitat, mounds associated with extensive warrens occupied by large numbers of *O. cuniculus* was not observed to support host plant habitat.

Understanding host plant ecology and population dynamics is important to planning for management activities that may cause some level of disturbance. For example, it may be important to minimize disturbance as much as possible during the construction phase of Cattle Point Road to ensure that *E. ausinides insulanus* does not recruit in host plant habitat that will eventually be destroyed. Over the study period, *B. rapa* and *S. altissimum* were observed growing along the margins of Cattle Point Road. For this reason, plant species will likely colonize disturbed areas created from road construction activities.

Construction activities should be carefully planned in advance in an effort to limit the creation of ‘sink’ populations of butterflies. ‘Sink’ populations are described as having a negative effect on overall population fecundity (Hanski 1997, Boughton 1999). Due to the rarity of *E. ausonides insulanus*, any habitat that is being occupied by eggs or larvae and is disturbed prior to adult emergence (e.g., mowing, trampling) may be considered a ‘sink’ (see *Stage-specific survivorship* Chapter 3 Figure 3.4). Temporary piles of soil or fill created in the vicinity of construction sites or along roadways may make *E. ausonides insulanus* especially vulnerable to ‘sink’ dynamics because adults lay the majority of their eggs on isolated plants often found in newly disturbed areas (Cromartie 1975, Shapiro 1984 and Lambert 2011 Chapter 2). Planning in advance for construction activities that may create some type of disturbance may prevent colonization of weedy non-native host plants and thus prevent the creation of ‘sink’ populations of *E. ausonides insulanus*.

In the long-term, prescribed disturbance treatments may be important to the persistence of *E. ausonides insulanus* occupying *B. rapa* grassland habitat. If introduced grasses stabilize soils and prevent germination and establishment of *B. rapa* then local populations of *E. ausonides insulanus* could be extirpated from grassland habitat at American Camp. For example, Crawley and Brown (1995) found that in the absence of disturbance rapid secondary succession led to the local extinct of populations of *Brassica napus* ssp. *oleifera*. Although this is not likely to happen within a short time frame, as grasslands become more stabilized host plant patches may be reduced to numbers inadequate to support viable butterfly populations. Different disturbance techniques (i.e., raking, hand removing, upturning topsoil) may be tested to identify host plant response. Pilot studies that take minimal resources to implement (i.e., meter square test plots) may inform future management applications.

### Topographic and habitat heterogeneity

Topographic and habitat heterogeneity may be critical to the long term perseverance of *E. ausonides insulanus*. The persistence of *E. ausonides insulanus* may in fact be related to the varied topography and availability of multiple host plant species in different habitat types within American Camp. American Camp supports the largest population of *E. ausonides insulanus* and may have historically provided safe sites for the population during times of human disturbance and environmental variability. Topographic and habitat heterogeneity support *E. ausonides insulanus* in different ways.

Surface topography (slope and aspect) and landscape features that have topographic relief (slopes, bluffs, sand banks or driftwood berms) are critical to the dispersal and movement of *E. ausonides insulanus*. For example, movement between *B. rapa* patches located on south-facing slopes at American Camp is aided by off-shore wind. In open grassland habitat females fly < 1 meter from the ground in wide circles (100– 500 meters or more), moving in the direction up a slope and returning down slope eventually returning to the original host plant patch (i.e., foray searching) (Lambert unpub. data). Topographic relief is also important to mating and dispersal. *Euchloe ausonides insulanus* males flew approximately 1.5 meters above the ground along ridgelines, bluffs, road-cuts, trail edges, fence-lines and shrub/forest edges to reach mate locations. Males also patrol bluffs and driftwood berms in search of females (Lambert unpub. data). Out of a total of 759 observations of adults, only one adult was observed to fly across denuded rabbit warrens centrally located in American Camp (Chapter 2; Lambert unpub. data). The relatively flat rabbit warrens lack vegetation structure and may act as a barrier to movement of adults dispersing from grassland habitat to sand dune habitat. For this reason, the addition of landforms having topographic relief may be considered in the management of the rabbit area in the future. For example, small patches of host plant habitat (*S. altissimum*) have recently established in areas where rabbits have been reduced significantly in number (Lambert pers. obs.). The addition of landforms having topographic relief (e.g., small hummocks or dunes) may facilitate butterfly movement into extant patches of host plant habitat in the rabbit area thus enabling adults to disperse between grassland and sand dune habitat.

Surface topography may also influence host plant phenology. For example, host plants spatially arranged on north and south facing slopes flower at different times and duration (Lambert pers. obs.). Currently most of *E. ausonides insulanus* population exists on south facing slopes. This is likely a result of environmental factors that have created and sustained open grassland south of the ridgeline. However, patches of host plant habitat do occur in grasslands on north facing slopes. In fact, adult surveys conducted from 2005–2007 show that *E. ausonides insulanus* occupy over 40% of available host plant habitat in grasslands occurring on north facing slopes (see Chapter 4, Table 5.1). Open grassland host plant habitat on north facing slopes may provide safe sites for *E. ausonides insulanus* during times of extreme disturbance (see Potential Impacts of Climate Change) or year-to-year fluctuations in weather. The relationship between stages of butterfly development and the timing of host plants is complex. Rainfall and temperature are moderated by seasonal patterns that influence the phasing of host plant flowering. The presence of host plants on both north and south facing slopes may provide *E. ausonides insulanus* with oviposition sites (i.e., buds and flowers) at different times of the season. Several studies have documented the pitfalls of topographic homogeneity. For example, Hellmann et al. (2003) found that the population growth rate of *Euphydryas editha* in more heterogeneous landscapes were 50% greater than in more homogeneous areas concluding that topographically homogeneous habitat patches may be more vulnerable to extinction. Long-term surveys of *E. editha* also found that populations in homogeneous habitats were the first to go extinct (Hanski et al. 2004).

Long-term conservation goals might include the removal of encroaching shrubs and restoration of host plants in areas adjacent to extant butterfly populations on north facing slopes. This would ensure that host plant habitat would be available to adults using host plant habitat located on north facing slopes if host plants on south facing slopes ever became unavailable (or unsuitable for oviposition) due to seasonal changes in weather.

In addition to topographic heterogeneity, habitat heterogeneity may be critical to maintaining *E. ausonides insulanus* populations over the long-term. Variable weather conditions is one of the leading factors causing population extinctions (Ehrlich et al. 1980) and maintaining several species of host plants that span different types of habitat (grasslands, sand dunes and tidal lagoon) may sustain some individuals through inclement periods of weather.

In order to maintain a diversity of host plant habitats conservation strategies may include the protection of extant populations of host plants *B. rapa*, *S. altissimum* and *L. virginicum var. menziesii*, especially populations shown to support high larval survivorship (see Chapter 4). The introduction of an additional native host plant compatible with prairie habitat and ability to meet the resource requirement of *E. ausonides insulanus* may also help to diversify host plant habitat and contribute to the long-term conservation goals of *E. ausonides insulanus* (see discussion on *Turritus glabra*, Chapter 4 and Appendix D).

## Mortality factors

Based on demographic data collected from 2005–2008, *E. ausonides insulanus* overall survivorship of eggs to instar IV was 12% (n=1617 eggs tracked). Survivorship was comparable to both common and rare *Pieris* spp. but considerably less than *Euchloe hyantis* (26.9 % [n=52; Karban and Courtney 1987]). Relatively low survivorship is likely attributed to multiple factors including the high rates of mortality in egg and instar I stages (Chapter 3 Appendix C). Low survivorship in early stages of development is common in many butterfly species and in general, the trend in low survivorship in early stages in *E. ausonides insulanus* was similar to trends found in other pierid species (Courtney 1986). However, egg and instar I survivorship was particularly low in *E. ausonides insulanus* (54% and 26%, respectively) and was mainly attributed to predation and deer herbivory.

A significant portion of plants studied over the four year study were browsed by deer and evidence of deer herbivory was observed at almost every study period. Deer herbivory reduced *E. ausonides insulanus* abundance by indirectly reducing availability of oviposition sites (Lambert 2011 Chapters 3) and by direct consumption of eggs and larvae. Over the course of four years of study, death due to deer consumption of eggs and larvae accounted for a large percentage of total mortality; 415 individuals were eaten by deer, 26% of all eggs tracked (1617 eggs tracked) (Lambert 2011 Chapter 3). For this reason, conservation and protection of *E. ausonides insulanus* may require the exclusion of deer from host plant patches that are susceptible to deer browsing (e.g., in short distance from forest cover), especially plants occupied by multiple eggs and larvae. Deer herbivory was observed to vary according to spatial and temporal scales and therefore an experimental approach may be necessary to better understand the relationship of deer herbivory to *B. rapa* and *S. altissimum* host plant dynamics.

The highest survivorship of eggs to instar IV was found on local populations of the only known native host plant, *L. virginicum* var. *menziesii* (Lambert 2011 Chapter 3). For this reason, it may be important to protect native host plant habitat from human disturbances such as trampling and removal of driftwood from *L. virginicum* var. *menziesii* habitat. In 2008, a large driftwood shelter was constructed in *L. virginicum* var. *menziesii* habitat at site 8. The removal of driftwood to create human shelters alters the structure and functions of backshore environments. Driftwood barriers stabilize sand on the low energy side of the barrier. Driftwood protects *L. virginicum* var. *menziesii* from tidal flooding and wave action and provides plants with nutrients from decomposing wood and shoreline detritus. When driftwood is removed *L. virginicum* var. *menziesii* plants are likely to be buried. Plants of *L. virginicum* var. *menziesii* are also inadvertently trampled during the construction of beach shelters (Figure 5.2). For this reason, preventing the construction of driftwood shelters near host plant habitat may be necessary.

## CONCLUSION

Numerous processes can lead to extinction and many of the processes discussed here (e.g., disturbance, host plant availability etc.) can operate at different temporal and spatial scales. This research shows that a combination of factors likely influence overall low population numbers and local population extinctions related to patch dynamics in E. ausonides insulanus. For example, multiple factors may have contributed to the local extinction of E. ausonides insulanus at site 8 which include habitat loss caused by an unprecedented storm in 2006, human impact in 2008, reduction in host plant quality (lack of oviposition sites) and predation of eggs and larvae. Thus, managers should consider both multiple management strategies to maintain and increase abundance of E. ausonides insulanus at American Camp.

**Table 5.1** Summary table of conservation issues and management strategies for the protection and conservation of *E. ausonides insulanus*.

| Conservation Issue | Management Strategy |
|---|---|
| Off-shore storms and damage to overwintering pupae and *L. virginicum var. menziesii* host plant habitat | Maintain a diversity of high quality host plant habitat. Establish additional native host plant in prairie habitat |
| Significant changes in size of host plant patches coupled with low *E. ausonides insulanus* abundance | Identify the rate at which host plant patches are colonizing newly disturbed habitat. Maximize the number of eggs in restored host plant patches. |
| Potential creation of 'sink' populations due to human mediated disturbance (i.e., construction activities) | Minimize disturbance as much as possible during construction activities especially in areas adjacent to occupied host plant habitat. Protect high quality native host plant habitat (i.e. *Lepidium* populations in lagoon areas) from driftwood removal and trampling. |
| Low recruitment of *B. rapa* in introduced grasslands | Prescribe small scale disturbances in grassland (based on pilot study) |
| Limited adult movement between host plant habitat in open grasslands and sand dunes (i.e., lack of dispersal in rabbit area) | Create topographic relief in habitat previously occupied by rabbits. Protect and enhance host plant habitat on north facing slopes. |
| Variable year-to-year weather conditions (i.e., periods of low temperature and high precipitation during flight season) coupled with low *E. ausonides insulanus* abundance | Maintain a diversity of host plant habitats. Protect extant populations of host plant habitat. Establish additional native host plant in prairie habitat. |
| Deer consume buds and flowers of host plants and eggs and larvae | Exclude deer from host plants occupied by eggs and larvae (based on experimental study) |

**Figure 5.1** *Lepidium virginicum* var. *menziesii* research site, May 29, 2005 and May 29, 2006. High tide windstorm in February 2006 shifted driftwood barriers and deposited large volumes of gravel on top of *L. virginicum var. menziesii* plants reducing the number of *L. virginicum var. menziesii* host plant habitat by 50% in 2006.

2008

**Figure 5.2** *Lepidium virginicum* var. *menziesii* research site, April 26, 2008. Driftwood shelter built in host plant habitat. Removal of driftwood alters the structure and functions of backshore environments that support *L. virginicum var. menziesii* host plant habitat. Plants of *L. virginicum var. menziesii* are also inadvertently trampled during the construction of driftwood shelters.

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# Appendix A

## Place names of research study sites

Research study sites where life history and morphology of eggs and larvae of *Euchloe ausonides insulanus* were studied at American Camp, San Juan Island, 2005–2008. Study site numbers refer to research areas where demographic surveys were conducted (see Chapter 3 Figure 3.1). Although demographic surveys were not conducted in 2008 at study site 1, egg dispersion and egg load studies were conducted at site 1 from 2005–2008 (see Chapter 2 Figure 2.4).

| **Study site number** | **Research study site** | **Host plant** | **Year(s) surveyed** |
|---|---|---|---|
| 1 | Slope South of Redoubt* | *Brassica rapa* | 2005-2008 |
| 2 | Old Town Lagoon Grassland* | *Brassica rapa* | 2005-2008 |
| 3 | West End Uplands | *Brassica rapa* | 2006, 2007 |
| 4 | Cattle Point NRCA-East Meadow | *Brassica rapa* | 2006 |
| 5 | Jakle's Lagoon* | *Lepidium virginicum var. menziesii* | 2005-2007 |
| 6 | Dunes and South Beach* | *Sisymbrium altissimum* | 2005-2007 |
| 7 | Cattle Point NPS-NRCA Border | *Sisymbrium altissimum* | 2006 |
| 8 | Redoubt | *Sisymbrium altissimum* | 2006 |

* Research study sites where development time of eggs and larvae (instars I-V) were studied in 2005 and 2006 and larval size was measured in 2005.

# Appendix B

## Relative adult abundance of *Euchloe ausonides insulanus*, 2004-2008

This report summarizes the results of field surveys conducted at American Camp, San Juan National Historical Park from 2004−2008.

### Study site

This study was conducted at American Camp, in open habitat located on the southern end of San Juan Island, Washington. American Camp is part of the San Juan National Historical Park managed by the National Park Service (NPS). Coastal habitat unique to *E. ausonides insulanus* includes grasslands, sand dunes and tidal lagoons. All habitat types are distinguished by the presence of host plants , *Brassica rapa* var. *rapa*, *Sisymbrium altissimum* and *Lepidium virginicum* var. *menziesii*, full sunlight and some type of topographic relief such as bluffs, ridges or dunes.

Grassland is the dominant feature at American Camp and includes both introduced grasses and remnant native prairie along the main ridge. *Brassica rapa* occurs most often in introduced grasslands. *Sisymbrium altissimum* is the dominant host plant in sand dune habitat. The only known native host plant, *Lepidium virginicum var. menziesii* inhabits backshore habitat between lagoons and tidal shoreline.

### Methods

Relative adult abundance surveys were conducted across habitat types in areas with and without host plants in an effort to monitor behavior and movement between patches. Host plants patches were mapped and digitized every year from 2004-2007 using ArcView GIS 3.2. Shape files were created from NPS digital orthophotos, American Camp 1997, and are available at the NPS North Cascades Office. GPS coordinates of adult transects were also recorded and provided to NPS managers.

Relative adult abundance surveys follow methods outlined by Pollard and Yates (1993). Pollard and Yates methods were chosen over mark-release methods because of the rarity of the species. Survey techniques are based on observational, low-impact methods that use set transects to monitor relative abundance over time (Pollard 1977). Sixteen belt transects (200m x 30m wide) were established in open habitat in American Camp in 2004 including introduced grassland, remnant prairie, sand dune and tidal lagoon habitat (Figure 1).

Transect locations were selected based on host plant surveys conducted in 2003 and 2004. To examine if adults were present in areas without host plants, eight transects were established in grassland sites without host plants and measured against transects with host plants. To control for adult behavior associated with topography (i.e., dispersal and mate location) transects were selected in areas similar in slope and aspect. Transects 2, 5, 6 and 7 were placed in grassland sites with *B. rapa*; transects 9, 10, 11 and 12 in grassland sites without host plants and 1, 3, 4 and 8 in grassland sites with native forbs and grasses but without host plants. In addition to transects located in grassland habitat, transect 14 was placed in sand dune habitat north of South Beach and transect 13 was established between shoreline and lagoon berms at Old Town Lagoon (Figure 1). In 2005, two additional transects were established in *L. virginicum var. menziesii* host plant habitat. Transect 15 was located at Jakle's Lagoon and transect 16 at Third Lagoon (Figure 1).

Following Pollard and Yates methods, the number of butterflies observed while walking each transect at a consistent pace (200 m in 10 minutes) was recorded during optimal flight conditions (>14° C, <12 mph wind speed and relatively low percent cloud cover). The number of butterflies observed on transects was recorded every 6-9 days (the estimated lifespan of an adult) over the course of the flight season (April–June). Transects were surveyed randomly, such that data was not collected consecutively in the same order at the same time of day to minimize any bias due to repeated sampling.

## Analysis

Butterfly numbers were estimated to create an index. The index provides a measure of presence/absence of adults occurring in different habitats at American Camp as well as general trends in the numbers of adults over time (2004–2008).

Total adult abundance is not an absolute measure of population. Rather, the data comprise an index of abundance with which to compare annual results. An index of five years (2004–2008) is limited to interpretation due to natural climate cycles (e.g. El Nino) and variation in environmental conditions. Establishing an index was a means to observe patterns of population fluctuations in the long-term and was one of the goals of the proposed monitoring scheme. Short-term objectives included detecting changes in occupancy of host plant habitat and use of different habitat types (e.g., grassland habitat without host plants).

## Results and discussion

A decline in total numbers of adults was observed from 2004–2008 (Figure 2). In 2004, 270 adults were counted on transects compared to 63 in 2008. These data suggest a downward trend in relative adult abundance from 2004–2008.

Over the five year survey period, the greatest number of adults was observed on transect 7 (164 adults; Table 1). Transect 7 contained one of the largest patches of *B. rapa*. Host plant density was consistently patchy within the transect site offering adults sufficient edge habitat. Host plant habitat was also bordered by a relatively abrupt south facing slope. The site gradient combined with dispersed patches of host plant habitat provided ample topographic relief for patrolling males and edge habitat for ovipositing females (see Chapter 2 for discussion on host plant density and oviposition site selection).

The population decline observed on transect 15 (Jakle’s Lagoon) from 2005 (11 adults) to 2008 (0 adults) (Table 1) is noteworthy because the research site was surrounded by forest and open water and therefore may have limited immigration/emigration of adults. A combination of factors likely contributed to the local extinction of adults at Jakle’s

Lagoon including predation of eggs (Chapter 3) and mortality of overwintering pupae due to flooding caused by a high tide wind storm in 2006 (Chapter 5 Figure 5.2).

A downward trend in peak abundance was recorded from 2004-2007 (Table 2; Figure 3). For example, there was a 50% decrease in the numbers of adults at peak abundance in 2007. Fifteen adults were reported on May 18, 2007 compared to 32 adults observed on May 10, 2006. However, an increase in peak abundance was reported in 2008. Twenty-three adults were reported on May 24, 2008 compared to 15 adults on May 18, 2007.

The 2008 adult flight season was the shortest flight season recorded (36 days) over the course of five years of adult surveys at American Camp (Table 2; Figure 3). In 2008, adults were first observed on transects on May 16 and last observed on transects on June 20 (Table 2). Although the first observation of an adult on transects did not occur until May 16, an adult was observed off-transect in the area of the redoubt at American Camp on April 25 (Miskelly & Potter 2009). The earliest sighting of an adult in 2008 however, was observed several miles north of American Camp at Pear Point Gravel Quarry. On April 19, two adults were netted and marked for mark-recapture study (Peterson 2009). Habitat features at Pear Point Quarry were significantly different than host plant habitat located in American Camp. At Pear Point Quarry host plants were scattered on steep south-facing slopes with little vegetative cover. The site was also relatively protected from wind. Thus ambient air and soil temperature may have been higher than at American Camp. Warm temperatures early in the flight season may have triggered early emergence of adults at Pear Point Quarry which suggests that the timing of adult eclosion may be influenced by microclimatic conditions.

Adults were not observed on all transects every year (Table 2). In 2004 and 2005, adults were present in grasslands without host plants (transects transects 9, 10, 11 and 12) but absent from these transects from 2006-2008 (Figure 4). The occurrence of adults on transects without host plants (or native forbs) may be related to overall adult abundance. Adults may be more likely to disperse from host plant habitat when numbers are high. Greater abundance of adults may have also increased the likelihood that adults were observed. Interestingly, adults were observed on transects with native forbs (without host plants) every year from 2004–2008. This suggests that native forbs may be important resources for adults. Native forbs provide nectar and are mating sites for adults (see Chapter 2 Figure 2.1).

**Table 1** Number of adults observed on transects at American Camp, San Juan Island National Historical Park, San Juan Island, Washington, 2004−2008.

| Transect Number | 2004 | 2005 | 2006 | 2007 | 2008 | Total Adults |
|---|---|---|---|---|---|---|
| 1 | 16 | 17 | 12 | 5 | 1 | 51 |
| 2 | 43 | 4 | 12 | 5 | 9 | 73 |
| 3 | 17 | 7 | 1 | 4 | 1 | 30 |
| 4 | 2 | 1 | 0 | 0 | 0 | 3 |
| 5 | 47 | 31 | 21 | 12 | 12 | 123 |
| 6 | 16 | 4 | 6 | 4 | 6 | 36 |
| 7 | 57 | 34 | 37 | 16 | 20 | 164 |
| 8 | 9 | 4 | 0 | 1 | 0 | 14 |
| 9 | 2 | 2 | 0 | 0 | 0 | 4 |
| 10 | 5 | 1 | 0 | 0 | 0 | 6 |
| 11 | 1 | 1 | 0 | 0 | 0 | 2 |
| 12 | 5 | 0 | 0 | 0 | 0 | 5 |
| 13 | 38 | 41 | 9 | 5 | 1 | 94 |
| 14 | 12 | 33 | 22 | 18 | 13 | 98 |
| 15 | — | 11 | 4 | 1 | 0 | 16 |
| 16 | — | 3 | 1 | 0 | 0 | 4 |

**Table 2** Adult phenology, total adult abundance, peak abundance and mean temperature on transects at American Camp, San Juan Island National Historical Park, San Juan Island, Washington, 2004−2008. Weather data acquired from Friday Harbor Airport (FHA) was collected by the Automated Surface Observation System and is maintained by the Federal Aviation Administration.

| Year | First observ. of adults | Start observations of adults on transects | End observations of adults on transects | Total adults | Peak abund. | April mean tempC (FHA) | Mean temp C on transect |
|---|---|---|---|---|---|---|---|
| 2004 | 8-Apr | 8-Apr | 19-Jun | 270 | 8-May | 10 | 24.3 |
| 2005 | 8-Apr | 20-Apr | 28-Jun | 194 | 5-May | 9.4 | 22.9 |
| 2006 | 10-Apr | 3-May | 12-Jun | 125 | 10-May | 8.9 | 23.4 |
| 2007 | 18-Apr* | 28-Apr | 19-Jun | 71 | 18-May | 8.3 | 20.9 |
| 2008 | 19-Apr** | 16-May | 20-Jun | 63 | 24-May | 6.7 | 19.5 |

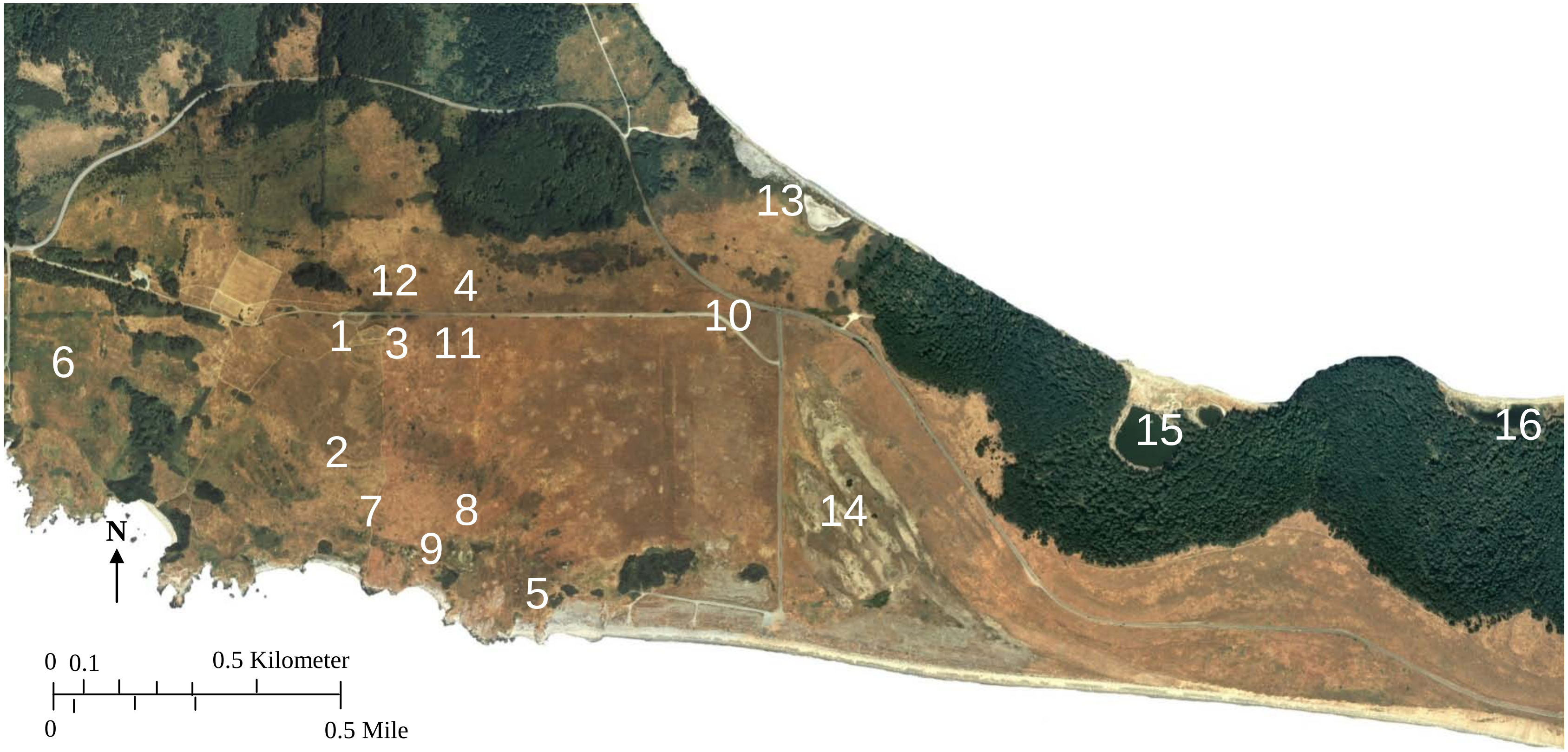

**Figure 1** Location of belt transects (1−16) at American Camp, San Juan Island National Historical Park, San Juan Island, Washington. Belt transects were 200m x 30m wide

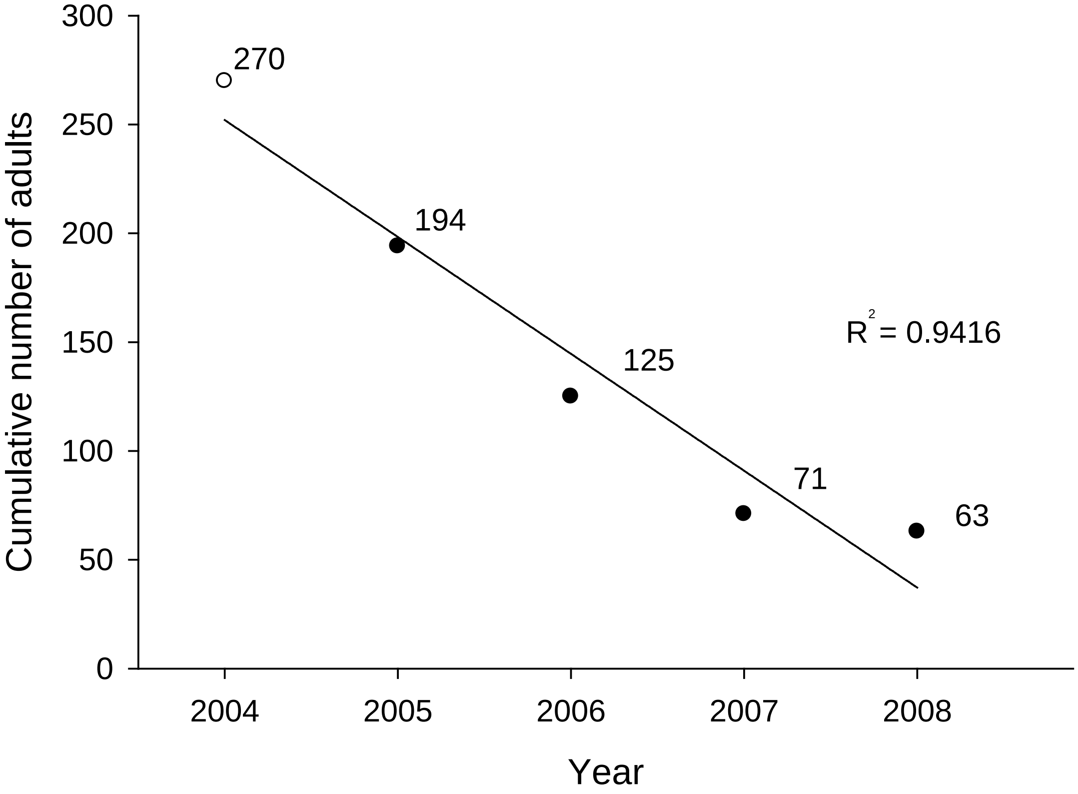

**Figure 2** Relative adult abundance, 2004−2008. Points correspond to the number of adults observed on transects 1-16 located at American Camp, San Juan Island National Historical Park, San Juan Island, Washington.

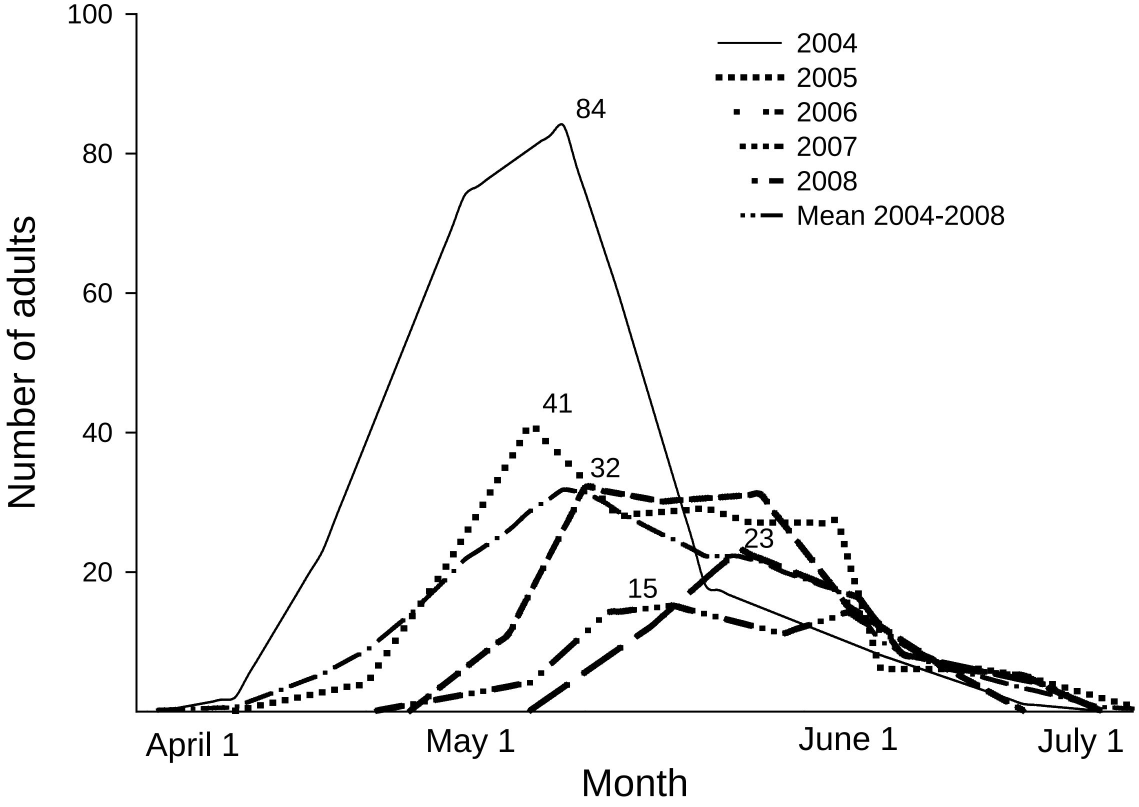

**Figure 3** Trends in adult phenology and abundance, 2004-2008. Points on abundance curves indicate the number of adults observed on transects during the peak of the flight season for each corresponding year.

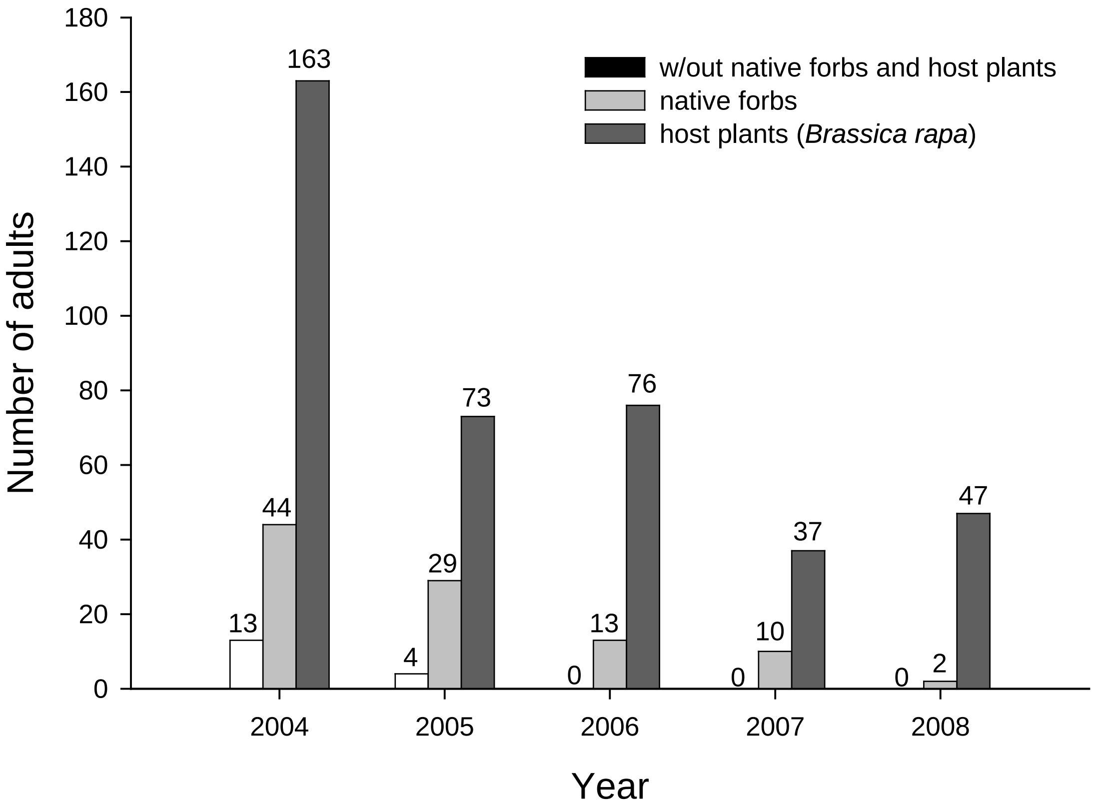

**Figure 4** Number of adults observed on transects in grassland without native forbs and host plants (tansects 9, 10, 11 and 12), grassland with native forbs (transects 1, 3, 4 and 8) and grassland with host plants (*B. rapa*) (transects 2, 5, 6 and 7).

# Appendix C

## Description of direct and indirect factors that contribute to *Euchloe ausonides insulanus* egg and larval mortality

| Mortality Factors | |
|---|---|
| Predation | Direct observation of spider predation and evidence of spider activity<br>Direct observation of wasp predation<br>Disappearance of eggs and instars I-III likely attributed to predation |
| Deer | Deer consumption of racemes occupied by eggs and larvae |
| Starvation | Senescence of host plant<br>Placement of eggs on senscent flower petals<br>Larvae consumes all edible plant tissue |
| Damage | Floral damage caused by unknown moth species<br>Damage to host plant by physical factors (i.e., wind)<br>Wilted host plant material caused by below ground herbivores |
| Disappearance | Disappearance of instar IV likely walked off host plant |
| Unknown | Desiccated eggs and larvae |

# Appendix D

## An evaluation of the suitability of two potential native host plant species

The purpose of this section is to assess the suitably of two native host species, *Turritis glabra* L. and *Arabis eschscholtziana* Andrz. in the restoration of *E. ausonides insulanus* populations. It is first necessary to begin with a review of the taxonomy, nativity and distributional range of these species to address previous misconceptions concerning taxonomy and nativity in both the primary and secondary literature. Secondarily, the suitability of each potential host species is examined. *Turritis glabra* and *Arabis eschscholtziana* are both native species and are found within the historic and current distributional range of *E. ausonides insulanus* and posses traits (Table 1) that may be compatible with *E. ausonides insulanus* larval development.

Jon Shepard suggested that *Arabis hirsuta* L. (currently *Arabis eschscholtziana* Andrz.) may be the original native host plant for *E. ausonides insulanus*. In his 2000 report titled <u>Status of Five Butterflies and Skippers in British Columbia</u> he states “*Arabis hirsuta*, which occurs on the island and south through the Puget trough and Willamette Valley, is the presumed larval foodplant of the island marble.” It is also possible that a related species, *Turritis glabra* L. (formally *Arabis glabra* L. Bernh.) served as a host plant for *E. ausonides insulanus* prior to the establishment of non-native host plant species because it shares similar morphological and life history traits, overlaps in distribution (sometimes found at the same collection site) and harbors similar chemical requirements for the development of *E. ausonides insulanus* (i.e., glucosinolates).

There has been much confusion concerning identification of *Arabis hirsuta* specimens collected from the San Juan Island Archipelago. For example, there are numerous herbarium records that identify specimens as *A. hirsuta* collected from the San Juan Islands (except for San Juan Island proper) and we now know that all of these specimens where misidentified according to David Giblin, the WTU herbarium collections manager and Ihsan Al-Shehbaz, the authority on the family Brassicaceae and genera within this family (e.g., *Arabis* and *Turritis*; see FNA). In fact, Dr. Shehbaz states “all previous accounts that list *Arabis hirsuta* L. from North America are wrong” (Ihsan Al-Shehbaz pers. comm.). *Arabis hirsuta* is native to Europe and adjacent Asia and is not found in North America (Ihsan Al-Shehbaz pers. comm.). There are two native *Arabis* species that were misidentified as *A. hirsuta*, *A. pycnocarpa* Hopkins and *A. eschscholtziana* Andrz. and occur throughout the Pacific Northwest. *Arabis pycnocarpa* consists of two varieties, both of which do not occur in the distributional range of *E. ausonides insulanus* (see FNA distributional maps) and is therefore not considered as a potential host plant for *E. ausonides insulanus*. However, *A. eschscholtziana* has been collected from many islands in the throughout the San Juan Archipelago and in some cases found to co-occur with *T. glabra* (D. Giblin, pers. comm.). All prior herbarium collections listed as *A. hirsuta* or *A. hirsuta* var. *eschscholtziana* within the distributional range of *E. ausonides insulanus* are *A. eschscholtziana* (D. Giblin pers. comm.).

*Arabis glabra* L. Bernh. has recently been reclassified as *Turritis glabra* L. (FNA). Molecular data fully supports Linnaeus (1753) original descriptions and organization of this species. The genus *Arabis* belongs to the tribe Arabideae, whereas *Turritis* belongs to the tribe Camelineae (Al-Shehbaz et al. 2006) although *Turritis* will eventually be classified into a tribe of its own (Al-Shehbaz pers. comm.). Secondary literature describing the origin of this species is misleading. For example, Atkinson and Sharpe (1985) cite *T. glabra* as a weedy, introduced species. In contrast, current literature suggests that *T. glabra* is a circumboreal species, native to North America, southwest Asia, and north Africa (Hitchcock and Cronquist 1973, FNA 2011).

*Turritis glabra* and *Arabis eschscholtziana* are both native species and are found within the historic and current distributional range of *E. ausonides insulanus*. Also, these species are documented to occur in similar locations within the San Juan Island range. For example, both species have been collected on Boulder Island, Yellow Island and the San Juan Island National Wildlife Refuge and Wilderness Area (East Sucia #7; see Tables 2 and 3). In general, differences in morphological characters distinguish the two species, but botanists have commonly confused the two species in the past (L. Vorobik pers comm.). It may be that in overlapping populations hybridization occurs which can make identification difficult. In spring 2006, Dr. Linda Vorobik examined voucher specimens of *T. glabra* collected from Yellow Island and greenhouse plants (grown from seed collected on Yellow Island in 2004) and confirmed the identification of *T. glabra* based on traits such as flower color (creamy white to pale yellow vs. pinkish white), flower size (smaller vs. larger), sepal morphology (non-gibbous base vs. gibbous base) and fruit shape (erect, roundish, compressed to stem vs. flat, semi-erect, at slight angle away from the stem). Dr. Al-Shehbaz (pers. comm.) suggested using similar key characteristics to differentiate between *T. glabra* from *A. eschscholtziana*:

"The species differs from *A. eschscholtziana* by having nearly four-angled (instead of typically flattened) fruit, pale yellowish (vs. white flowers), totally glabrous and glaucous (vs. non-glaucous and sometimes sparsely hairy) middle and upper portions of the stem, and often clavate instead of slender stigmas." Morphological characteristics of the two species are summarized in Table 1.

Since the publication of Shepard's report (2000) *A. hirsuta* (*A. eschscholtziana*) has generally been accepted as the original native host plant for *E. ausonides insulanus* although there is no empirical evidence to support this claim. There are is some evidence to support Shepard's assumption. For example, it has been widely documented that the relatively common butterfly, *Euchloe ausonides* Lucas, large marble, utilizes species of *Arabis* throughout its range (Opler 1974) and primarily *Arabis* in British Columbia (Guppy and Shepard 2001). It is therefore likely that the closely related subspecies, *E. ausonides insulanus* exploited *A. eschscholtziana* (and perhaps *T. glabra*) before *E. ausonides insulanus* was extirpated from BC in 1908. Although Shepard's reference to the genus *Arabis* is most likely correct, his supposition at the species-level (i.e., *A. eschscholtziana)* may be narrow. It is likely that he presumed that *Arabis eschscholtziana* was the original native host plant because it is commonly cited as the only native *Arabis* species occurring in the Puget Trough, San Juan Islands (Atkinson and Sharpe 1985).

Based on literature surveys and anecdotal evidence it is difficult to know whether *A. eschscholtziana* was the original native host plant of *E. ausonides insulanus*. However, it is likely that *E. ausonides insulanus* utilized both species of *Arabis* and *Turritis* in the past and would likely oviposit on *A. eschscholtziana* and *T. glabra* if present in grasslands on San Juan Island today (A. Lambert unpub. data; Figure 1). Both species have been collected in the Puget Trough area over the last century and have been found throughout the San Juan Island region. While there are no records of *A. eschscholtziana* collected on San Juan Island proper two populations of *T. glabra* have been found on San Juan Island in two locations (A. Lambert unpub. data). Specimens collected off of Westside road, near the San Juan Island County Land Bank, where confirmed to be *T. glabra* by L.A. Vorbik (Table 3).

*Turritis glabra* and *A. eschscholtziana* are both found in similar habitats. However, according to herbarium records *Turritis glabra* may exploit a wider range of habitat types (including open sand). Based on descriptions of herbarium specimens, *A. eschscholtziana* typically occurs on rocky bluffs and slopes several hundred feet above sea level. Although *Turritis glabra* occurs on rocky bluffs, it is also commonly observed in early successional habitats such as open stony or sandy grasslands. The habitat types under which *T. glabra* commonly occur may be better suited to open grassland habitat currently supporting *E. ausonides insulanus*. In addition, Dr. David Giblin, also observed *T. glabra* more often and in greater numbers than *A. eschscholtziana* during Washington Territory University (WTU) collecting trips throughout the San Juan Islands from 2005-2009 (D. Giblin pers. comm.). *Arabis eschscholtziana* has been described as a “minor” species, occurring in small numbers. Chris Chappell, botanist with Washington Department of Natural Resource, observed *A. eschscholtziana* “occasionally in shallow-soiled balds…occurring in small populations or sparse (it is a minor species…)” (Chappell comm. with Potter pers. comm. 2005). Based on personal observations, herbarium site descriptions and conversations with local expert botanists, individuals of *T. glabra* occur in greater numbers at collection locations. Patches of host plants containing a moderately abundant number of individuals (i.e., populations of *T. glabra*) thus would be more likely to sustain healthy populations of *E. ausonides insulanus*. Finally, because *T. glabra*, commonly known as tower mustard, is taller (50-80cm) than *A. eschscholtziana* (40-50cm) it is likely that *T. glabra* would be more accessible to adults searching for oviposition sites if it were growing among introduced grasses (Figure 2) or native bunch grasses. For these reasons, *T. glabra* may be a more appropriate candidate for recruitment of *E. ausonides insulanus* in upland grassland habitat in areas ideal for native prairie restoration.

**Table 1** Morphological characteristics of *Turritis glabra* L. and *Arabis eschscholtziana* Andrz.

| **Trait** | ***Turritis glabra* L.** | ***Arabis eschscholtziana* Andrz.** |
|---|---|---|
| Flower color | pale yellowish, white-creamy | white-pinkish |
| Stigma | often clavate | mostly slender |
| Sepal base | non-gibbous | typically gibbous |
| Fruit | four angled to roundish | typically flattened |
| Seed arrangement | biserate | uniserate |
| Mature inflorescence | fruits erect, compressed to stem | fruits semi-erect, pedicels somewhat angled away from stem |
| Stem | glabrous and glaucous | non-glaucous and sometimes sparsely hairy |
| Height | 50-80 cm tall | 40-50 cm tall |

*Characteristics are based on discussions with Dr. L.A. Vorobik and Dr. I. Al-Shehbaz in 2005 and from Hitchcock and Cronquist 1973, Kozloff 2005 and Flora of North America accessed on line January 2, 2011.

**Table 2** Selected collection records of *Arabis eschscholtziana* Andrz. (formally *Arabis hirsuta* var. *eschscholtziana* (Andrz.) Rollins) from the WTU on-line database, January 2, 2011. *Arabis eschscholtziana* is currently listed as *Arabis hirsuta* var. *eschscholtziana Arabis glabra* or *A. hirsuta* in the WTU database as of January 2, 2011. For more details on each herbarium specimen and full collection records of *Arabis eschscholtziana* visit: http://biology.burke.washington.edu/herbarium/collections/vascular/search.php

| **Herbarium** | **Collection Yr., Month, Day** | **Determiner** | **Collector** | **State** | **County** | **Locality** | **Site Description** | **Specimen Details** |
|---|---|---|---|---|---|---|---|---|
| 1. WTU | 2009 Sep 25 | David Giblin | Amy Dearborn | WA | San Juan | San Juan Islands National Wildlife Refuge and Wilderness Area. Blind Island. Approximately 0.5 kilometers west of Castle Island and 0.25 kilometers off south end of Lopez Island. | Rocky outcrops and meadow-like areas with well developed soils. Mix of native forbs and native grasses. | Uncommon, a few stalks. |
| 2. WTU | 2008 Apr 25 | D. Giblin | Megan A. Jensen | WA | San Juan | East Sucia #7 | The largest island in the chain. Forested with Arbutus, Juniperus, Pseudotsuga, with shrub understory. | Occasional, scattered on east end. |
| 3. WTU | 2005 Apr 26 | D. Giblin | Peter Dunwiddie | WA | San Juan | San Juan Islands National Wildlife Refuge and Wilderness Area. Boulder Island. | Open grassy 4 acre(?) island with rock/cliff shores. Only trees are several dozen stunted Pseudotsuga menziesii. | Rare. |
| 4. WTU | 2005 May 29 | D. Giblin | John Floberg | WA | San Juan | San Juan Islands National Wildlife Refuge and Wilderness Area. Boulder Island. | Open grassy 4 acre (?) island with rock/cliff shores. Only trees are several dozen stunted Pseudotsuga menziesii. | Rare, rock ledges, edge of turf. |
| 5. WTU | 2000 May 15 | | P. F. Zika | WA | King | Steep south slope of Rattlesnake Ledge, circa 3 miles south of North Bend. | Moist gravel at base of volcanic cliffs; with Aquilegia, Cerastium arvense, Sedum oreganum. | White flowers, fruits erect or spreading. |
| 6. WTU* | 1991 Apr 28 | P. Dunwiddie | R. Johns | WA | San Juan | Yellow Island, along hill trail above cabin on W end. | Meadow with Fritillaria and Ranunculus. | |
| 7. WTU | 1984 Jun 9 | Scott R. Atkinson | Fred Sharpe | WA | San Juan | Orcas Island. Mt. Constitution. Summit, below lookout. | Rocky open slopes; with Polygonum nuttallii. | Infrequent perennials. |
| 8. WTU | 1908 Jun 7 | L. A. Vorobik | J. B. Flett | WA | King | Alki Point. | | |
| 10. WTU | 1904 Jun 20 | | W. H. Lawrence | WA | San Juan | Stuart Island. | Dry ground; open places. | |

* Trinomial not listed in WTU database, but assumed to be *A. eschscholtzianan* (*A. hirsuta* var. *eschscholtzianan).*

**Table 3** Selected collection records of *Turritis glabra* L. (formally *Arabis glabra* (L.) Bernh.) form the WTU on-line database, January 2, 2011. *Turritis glabra* is currently listed as *Arabis glabra* in the WTU database as of January 2, 2011. For more details on each herbarium specimen and full collection records for *T. glabra* visit: http://biology.burke.washington.edu/herbarium/collections/vascular/search.php

| Herbarium | Collection Yr., Month, Day | Determiner | Collector | State | County | Locality | Site Description | Specimen Details |
|---|---|---|---|---|---|---|---|---|
| 1. WTU | 2009 May 27 | D. Giblin | Phil Green | WA | San Juan | San Juan Islands National Wildlife Refuge and Wilderness Area. Flower Island, east of Lopez Island. | Several hectare, 50' high bedrock island. A mix of grassy ledges, shrub thickets, a few trees. | Several widely scattered plants, edge of shrub thickets. |
| 2. WTU | 2008 Sep 26 | D. Giblin | Peter W. Dunwiddie | WA | San Juan | San Juan Islands National Wildlife Refuge and Wilderness Area. East Sucia #7. | The largest island in the chain. Forested with Arbutus, Juniperus, Pseudotsuga, with shrub understory. | Frequent in grassy openings. |
| 3. WTU | 2005 Apr 26 | D. Giblin | Joseph Arnett | WA | San Juan | San Juan Islands National Wildlife Refuge and Wilderness Area. Aleck Rock. South of Lopez Island. | Grassy meadow with rocky outcrops. | |
| 4. WTU* | 2005 Apr 28 | L. A. Vorobik | A.M. Lambert | WA | San Juan | Yellow Island | rocy prairie | Abundant but scattered, creamy yellow flrs., roundish fruits compressed to stem |
| 5. WTU | 2005 Apr 28 | David Giblin | Ben Legler | WA | San Juan | San Juan Islands National Wildlife Refuge and Wilderness Area. Castle Island. | Steep, cliffy island with many rock ledges, cracks and beaches. | Few scattered clumps on ledges. |
| 6. WTU* | 2005 Apr 29 | L. A. Vorobik | A.M. Lambert | WA | San Juan | San Juan Island, San Juan County Land Bank, off of Westside road | Steep, west-facing, rocky slope | Sparse, flowers creamy yellow, fruits compressed to stem and roundish, stem glaucous |
| 7. WTU | 2005 Apr 29 | David Giblin | Jessie Johanson | WA | San Juan | San Juan Islands National Wildlife Refuge and Wilderness Area. Rum Island. | Island is narrow and runs north-south direction with forested area in center. | Small group of plant on rocky ledge; east side of island; fruits. |

*Personal collection not yet accessioned into the WTU.

**Table 3** continued.

| Herbarium | Collection Yr., Month, Day | Determiner | Collector | State | County | Locality | Site Description | Specimen Details |
|---|---|---|---|---|---|---|---|---|
| 8. WTU | 2005 Apr 29 | David Giblin | John Floberg | WA | San Juan | San Juan Islands National Wildlife Refuge and Wilderness Area. Boulder Island. | Open grassy 4 acre (?) island with rock/cliff shores. Only trees are several dozen stunted Pseudotsuga menziesii. | Rare. |
| 9. WTU | 2004 Apr 30 | Ben Legler | Peter W. Dunwiddie | WA | Island | Whidbey Island, Ebey's Landing National Historic Preserve Bluff Trail. 2 KM north from main parking lot. Along Trail and nearby woods at top of bluff. | Sandy, west-facing, exposed bluff dominated by grasses, Achillea, Vicia; wind-swept Pseudotsuga forest with Ribes, Berberis. | Seeds biseriate. |
| 10. WTU | 2004 Apr 30 | Ben Legler | Ben Legler | WA | Island | Whidbey Island, Ebey's Landing, at border between state park and the Nature Conservancy property circa 1/8 mile south of Perego's Lagoon. | Eroding base of bluff adjacent to high tide line; dominated by grasses. | Flowers cream; common in grass on slope; seeds biseriate. |
| 11. WTU | 1984 Jun 9 |  | Scott R. Atkinson | WA | San Juan | Orcas Island, Mount Constitution, Little Summit, S of main summit. | Rocky meadows. | Infrequent perennials with creamy-white flowers. |
| 12. WTU | 1939 Apr 29 | L. A. Vorobik | W. C. Muenscher | WA | Whatcom | Chuchanut Island. | On grassy ledge. |  |
| 13. WTU | 1936 Jun 8 | L. A. Vorobik | Harold W. Smith | WA | Island | Goose Rock, Deception Pass State Park, Whidbey Island. | Meadows. |  |
| 14. WTU | 1931 Apr 25 | L. A. Vorobik | J. William Thompson | WA | Island | Goose Rock, Whidbey Island. | Open rocky slopes. |  |
| 15. WTU | 1904 Jun 27 | L. A. Vorobik | W. H. Lawrence | WA | San Juan | Stuart Island. | Dry stony soil, open fir woods. |  |
| 16. WTU | 1897 May 29 | L. A. Vorobik | N. L. Gardner | WA | Island | Whidbey Island. | Waste ground. |  |
| 17. WTU | 1888 May 16 | L. A. Vorobik | C. V. Piper | WA | King | Seattle. |  |  |

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# DEDICATION

To my daughter, Josephine Flora.
You were born of butterflies.