FACTORS AFFECTING POLLINATION AND SEED SET OF A RARE PLANT
(SIDALCEA MALACHROIDES)

By

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FACTORS AFFECTING POLLINATION AND SEED SET OF A RARE PLANT
(SIDALCEA MALACHROIDES)

HUMBOLDT STATE UNIVERSITY

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Numerous studies have analyzed the effect of habitat fragmentation on plant pollinators and herbivores. These studies describe the decrease in pollinator services and seed set in small and isolated patches due to pollinator preference for large, contiguous patches. However, other studies have shown that small and isolated patches may escape herbivory due to dispersal limitations of many insect herbivores. *Sidalcea malachroides* (Maple-leaved checkerbloom) is a rare plant with a patchy distribution, which requires insect pollen vectors and experiences severe fruit loss to weevil larvae. To test the effect of patch size and isolation distance on pollen reception and pollen limitation, I measured pollen loads on stigmas and the difference in seed set for open and hand pollinated flowers in plants of various combinations of patch size and isolation. To examine the effect of patch reduction and isolation distance on herbivore damage, I scored the total number of damaged seeds per plant. I used multiple regression to analyze these three dependent variables against patch size, isolation distance and other potentially important variables, and separate GLM analysis to examine the effects of abiotic environmental factors. *S. malachroides* experiences a reduction in pollen reception and an increase in
pollen limitation when it occurs in isolated patches. This effect is especially pronounced in small patches. The most striking result was the lack of pollination in small patches isolated by more than 17 meters. Plants in large patches received more pollen grains, but surprisingly, degree of pollen limitation was not related to patch size. Small patches and isolated patches experience reduced damage to weevil larvae; however the effect was weak in comparison to the effect of pollinator services. Since the distribution of S. malachroides is likely limited by seed production and dispersal, and small, isolated patches do not receive adequate pollinator service, care should be taken to consider patch size and isolation distances when analyzing the status of this plant and the effects of land management activities.
I thank my advisor Mike Mesler, as well as my committee members Michael Camann, Jeffrey White, Erik Jules and Lowell Diller for their patient guidance and editing. I also thank Kazayuki and Nick for their diligence in hiking around old clearcuts with me and counting endless pollen grains. I thank Horace Burke of Texas A&M University and Robert Anderson of the Canadian Museum of Nature for the identification of my weevils. I thank the California Department of Fish and Game and especially Tony LaBlanca for their assistance in my field work by allowing use of their vehicles, fuel and computers. I thank William Bigg and Yoon Kim for assistance with my statistical analysis and Maralyn Renner and Clare Golec for technical review. I also thank Green Diamond Resource Company and Palco lumber company for allowing me access to their property as well as maps and guidance for my field work. I thank Martha Groom and others whose ideas inspired my own research. Finally, I thank my friends and family for emotional and spiritual support throughout this project.
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INTRODUCTION

For many plant species, seed set is dependent upon the number of pollen grains that are deposited on plant stigmas, and plants may experience pollen deficiencies (pollen limitation) due to a variety of factors (Proctor 1996). Patches that are sparsely distributed may experience pollen limitation as pollinators are less attracted to small or isolated patches (Groom 1998, Rathcke and Jules 1993, Shulke 2001, Sih 1987). For this reason, large patches and contiguous patches are likely to produce more seeds per plant, thereby increasing the success of populations. Herbivory may also be affected by the spatial distribution of the plants. Some plants are able to escape herbivory when they are located in small or isolated patches (Zimmerman 1980, Price 1980). Herbivores, like pollinators, prefer large floral displays, and are frequently incapable of traveling across long distances. The importance of herbivory and pollination for plant fitness, coupled with the response of pollinators and herbivores to isolation and patch size can augment potential cost-benefit scenarios in some plant species. Plants may experience a reduction in pollinator services in small or isolated patches; however, they may also escape herbivory under the same circumstances. (Groom 2001)

_Sidalcea malachroides_ (maple-leaved checkerbloom) is listed by the California Native Plant Society (CNPS) as 4.2 (plant of limited distribution) (CNDDB 2006). This species presents a unique opportunity to study the effects of patch size and isolation distance on the pollination and herbivory of a polygamous plant. Though limited in distribution, the plant is locally abundant in ruderal areas of coastal Northern California.
Plants are pollinated by a variety of insects and grazed upon by both vertebrates and invertebrates. The plant has a patchy distribution generally composed of large patches surrounded by smaller patches. Patches can also be extremely small (one or two plants) and apparently isolated from other patches (often over 100 meters). Sixteen other species of *Sidalcea*, are listed by the CNPS as either 1b or 2 (rare and endangered), however *S. malachroides* was downlisted from 1b to 4.2 in the spring of 2006. Nevertheless, due to its moderate rarity and its similarity to other rare *Sidalcea* species and disturbance related rare plants, further understanding of the effect of patch size and isolation distance on the reproductive success of the species could benefit resource management and conservation efforts.

One of the potential impacts to *Sidalcea* sp. is timberland management. Since healthy populations of *S. malachroides* are frequently observed growing in recently clearcut areas or graded roads, it is possible that timberland management activities benefit the species. There are a variety of activities that occur during timberland management however, and the intensity and timing of disturbance may be important in determining whether the impact is beneficial or deleterious. Depending on specific management practices, some areas can be disturbed repeatedly within a short time frame of 5-15 years. Although the long term effects of timber harvesting activities are not clear, many plant species experience high rates of direct mortality as well as indirect effects of environmental change (Maschinski 1997, Messina 1997, Scherer 2000). One of these indirect effects may be the reduction of patch sizes and the increase in isolation distances between plants. These changes in spatial distribution have the potential to affect plant-
animal interactions such as pollination and herbivory. The current dominant paradigm in conservation at the time of this writing is to conserve the largest and most robust occurrences (patches), when impacts to a population are unavoidable during timber harvest operations or other development activities. This policy relies on the assumption that these larger, more robust occurrences are more likely to be pollinated and produce more seeds than smaller, more isolated occurrences. They are also more likely to withstand impacts from herbivory, anthropogenic impacts or competition from associated plants.

In an effort to evaluate this approach to conservation, I used multiple regression analysis to test whether pollinator services and seed set for *S. malachroides* increased in larger patches and patches with short isolation distances within a managed landscape. I specifically attempted to determine a threshold at which point patch size and isolation distance became important for pollination and seed set. I also tested whether reduced patch size and large isolation distances provided an escape from herbivory.
METHODS

Study Species

*Sidalcea malachroides* is a pioneer plant typically found in disturbed habitats along the Pacific Coast of California (Figure 1). In Humboldt County, it occurs primarily on roadsides, skid roads, recent clear-cut areas and other ruderal areas. As with other pioneer or disturbance-related species, *S. malachroides* appears to be a short-lived perennial. Populations senesce between seven to twelve years post-disturbance, and the species most likely persists across the landscape by building a large seedbank that may persist long after the population is shaded out (Maralyn Renner, electronic mail, October, 7, 2006). For similar species, seed set is an important factor in the viability of populations (Andreasen 2001), as mature plants are often quickly shaded out by competing vegetation or canopy closure.

Most western *Sidalcea* species are gynodioecious; populations consist of a mixture of female and hermaphrodite plants (Marshall 2001, Ashman 1994, Graff 1999). *Sidalcea malachroides*, however, is polygamous, and populations include perfect (hermaphrodite), staminate (male) and pistillate (female) plants (Hickman 1993). Staminate and perfect inflorescences are much larger and showier than female inflorescences (Figure 2). The populations used in this study were composed of approximately 52% female plants, 43% male and 5% hermaphrodites (*n*=5 populations, *x*=59 plants). The estimates for hermaphrodites may be conservative, as stigmas emerge after anthers have dehisced and an early census may provide a view of only anthers.
Also, only a few of the flowers on the hermaphrodite plants produce stigmas and viable ovules, and hermaphrodite plants might have been counted as males. Due to the difficulty of assessing which plants are truly hermaphrodites, as well as the low percentage of hermaphrodite plants, I used female plants in my study. Because they are obligate outcrossers, the pollen load of female plants should be especially sensitive to differences in patch size and degree of isolation.

*Sidalcea malachroides* is pollinated primarily by generalist pollinators. Insect pollinators observed on the plant include *Bombus* sp., *Apis mellifera*, *Bombylius* sp., Diptera (primarily Syrphidae) and Lepidoptera. (personal observation)

Many local *Sidalcea* species are attacked by weevils (Curculionidae) (Marshall 2001, Dimling 1992). *Sidalcea malachroides* is the preferred host of at least two species of weevil, *Macrorhoptus hispidus* (Dietz), and *Anthonomus ochreopilosus* (Dietz). Adult weevils are present on the flowers throughout the blooming season. They forage on all parts of the flower and mate on the flowers. The flowers contain six to nine seeds which are produced in a schizocarp. Weevil larvae develop inside the maturing fruit and emerge as adults. Small holes are sometimes visible in the carpel wall and larvae can be seen when the schizocarp is broken. One to three larvae may mature inside the hollow fruit. Damage to fruits can be extensive with nearly all fruits on a plant being attacked and over half of the seeds eaten (personal observation).

*Sidalcea malachroides* is also grazed heavily by numerous other invertebrate species including Lepidoptera and Coleoptera larvae, and by vertebrate species including
deer and rodents. Nectar foraging ants and predatory insects such as crab spiders are also commonly found on plants. (personal observation)

Figure 1. Range of *S. malachroides*. The species has a patchy distribution along the California coast between Del Norte and Monterey counties from 2-730 meters in elevation.
Figure 2. Photographs of female and hermaphrodite flowers. *Sidalcea malachroides* may be either female (left), male or hermaphrodite (right). Male and hermaphrodite plants have larger, showier flowers. Stigmas in hermaphrodite plants emerge from the center of the column of stamens after anthers have dehisced.

Study Area

The study was conducted on private timberlands in coastal northern Humboldt County, California. Timber harvesting operations and access restrictions limited the study to five sites ranging from the McGarvey Creek watershed, north of Orick to the Jacoby Creek watershed, south of Arcata. The sites were classified as “Upland Redwood Forest” (Holland 1986), and were approximately 304-487 meters in elevation with
moderate to steep slopes. All sites had been clearcut within the last five years and two sites had been burned after harvesting operations.

**Patch Selection**

The populations at my sites consisted of clusters of plants (patches) ranging from 2-200 individuals. I defined a patch as a group of plants that was isolated by at least 3 meters from other plants. I chose this criterion based upon my familiarity with the distribution of the species and also on the behavior of pollinators during preliminary investigations in the summer of 2002. For both large and small populations, pollinators tended to visit most or all of the plants in contiguous patches, however they did not visit plants more than three meters away and generally left the area after visiting plants with the patch. Smaller insects that were potential pollinators, such as tumbling flower beetles (Mordellidae), traveled from plant to plant and rarely left patches that were isolated by more than three meters.

Between the five sites, I selected fifty-nine (of approximately 400 patches) for my study. I created transects at each site and selected patches by pacing the distance determined by a random number chart. All transects followed hauling roads or skid roads, as almost all of the available *S. malachroides* occurrences were located along roadsides. I recorded distance from the road to test for any potential effects of roads. To diminish the impact of autocorrelation between patches, I did not use patches that were located within 30 meters of other study patches. (Figure 3)
Figure 3. Patch selection criteria. I selected fifty-nine focal patches using road transects and random number charts. Focal patches were located at least 30 meters from one another to reduce the impact of autocorrelation. (Black circles indicate selected patches, open circles indicate adjacent patches.)

In order to separate the potential effect of patch size and isolation distance, I attempted to sample patch sizes throughout the spectrum of isolation distances; however it proved difficult to locate patches with female plants that varied over the full range of the isolation distance parameter (Table 1). This was partially due to access restrictions that limited sites that I could sample from, but also due to the fact that almost all isolated plants observed were either male or hermaphrodite plants. Replication was very low for many of the combinations of patch size and isolation distance.
Table 1. Ranges of patch sizes and isolation distances used in pollen load and pollen limitation studies (small = 2-4 plants, medium = 5-9 plants, large = 10-49 plants, very large = 50+ plants).

<table>
<thead>
<tr>
<th>Isolation Distance</th>
<th>Single Plants</th>
<th>Small</th>
<th>Medium</th>
<th>Large</th>
<th>Very Large</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-5 meters</td>
<td>3</td>
<td>4</td>
<td>6</td>
<td>3</td>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td>5.1-10 meters</td>
<td>8</td>
<td>4</td>
<td>6</td>
<td>3</td>
<td>2</td>
<td>23</td>
</tr>
<tr>
<td>10.1-20 meters</td>
<td>5</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>20.1+ meters</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>14</td>
<td>14</td>
<td>7</td>
<td>5</td>
<td>59</td>
</tr>
</tbody>
</table>

Focal Plant Selection

I chose one focal female plant in each patch for my studies. To do this, I established the perimeter of each patch and located the female plant nearest the center of the patch. For each focal plant, I determined patch size and isolation distance. I measured isolation distance from the focal plant to the nearest plant in the adjacent patch (Figure 4). I then counted the number of pollen donor plants in the patch (male and hermaphrodite), area of patch in meters (to calculate density), number of plants in nearest patch, number of pollen donor plants in nearest patch and number of heterospecific blooming species within ten meters. The number of plants and pollen donors in the nearest patch was recorded to test for an interaction between nearest patch and patch size. Any interaction might indicate that my patch criteria was inaccurate and patches scale is much larger in terms of insect visitation. The number of heterospecific plants was recorded to test for any possible effect of large heterospecific floral displays attracting...
insects versus the floral displays provided by the *S. malachroides* patch. I recorded the number of racemes on the focal plant as a measure of site quality, as well as aspect, canopy cover, hydrology (soil moisture), slope, shrub layer density and burn treatment. Aspect and canopy cover were recorded due to their potential to affect air temperature and subsequently insect behavior. Hydrology, slope, shrub layer density and burn treatment were chosen based on observations of more robust patches occupying habitats that were more mesic, flat and previously burned with lower shrub layer density. I did not take any precautions to control vertebrate or invertebrate herbivory, and some loss occurred from both sources.

**Figure 4.** Focal plant selection criteria. Intrapatch isolation distance was not measured, however patch density was recorded and tested as a metric of average intrapatch isolation distance.
Pollen Load Study

I measured pollen loads on stigmas from each focal plant as a direct measure of pollen load and analyzed the effect of patch size and isolation distance on pollination. To assure proper species identification, I collected pollen of associated blooming plants and determined that *S. malachroides* pollen is large and easily distinguished from the pollen of nearby plants (Appendix B). Preliminary field investigations showed that stigmas emerge and separate into multiple distinct lobes. When they first emerge, these lobes are translucent-white; as they mature and senesce, they become pink and then finally red. I selected only translucent-white stigmas with fully separated lobes for collection since pink-red stigmas are brittle and pollen has dried up or fallen off.

I collected stigmas to measure pollen loads between May 4, 2004 and June 9, 2004, which is the peak blooming period of the plants. To collect the stigmas, I systematically chose the tallest blooming raceme on each focal plant to avoid any bias in pollinator preference. I selected five flowers with receptive stigmas, collected stigmas and mounted them on slides with fucshin gel (Kearns 1993). After mounting the stigmas, I used a compound microscope to count both conspecific and heterospecific pollen grains.
Pollen Limitation Study

To compliment my pollen load study, I also measured the degree of pollen limitation using fruit counts. I chose two racemes on the focal plant (not used in the pollen load study) for the pollen limitation study. The racemes were of similar position and height on the plant to avoid any bias due to pollinator preference or resource allocation in the plant. I supplemented pollen on five flowers of one raceme (treated) and marked five flowers on the other raceme for open pollination (control). I later determined the difference in fruit set between the two treatments. I attempted to use flowers of similar age with white-translucent stigmas and fully separated lobes. Occasionally I was forced to use flowers with slightly young or pink stigmas. To supplement pollen, I brushed the stigmas with pollen from plants within the same patch and marked the calyx of selected flowers with a Sharpie® maker. Since supplemented flowers had no pollen limitation, a large difference in fruit set between supplemented and open flowers in a given plant would indicate pollen limitation.

Between August 1 and August 15, 2004, I returned to collect fruits from each of the five treated and control flowers on focal plants. Fourteen of 58 focal female plants were lost due to mammalian herbivory, competition by adjacent species and logging operations. Most of these were either single plants or in small patches, however I lost one in a medium-sized patch and one in a large patch. The isolation distances of the plants which were lost ranged from 5.8 meters to 61.0 meters. This loss reduced my ability to test some combinations of patch size and isolation distance (Table 2).
Table 2. Ranges of patch sizes and isolation distances used in pollen limitation and seed predation study (small=2-4 plants, medium = 5-9 plants, large = 10-49 plants, very large = 50+ plants).

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<th>Medium</th>
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<th>Very Large</th>
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<td>3</td>
<td>3</td>
<td>6</td>
<td>3</td>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td>5.1-10 meters</td>
<td>5</td>
<td>3</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td>10.1-20 meters</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>20.1+ meters</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
<td>8</td>
<td>13</td>
<td>6</td>
<td>5</td>
<td>45</td>
</tr>
</tbody>
</table>

In the lab, I counted the number of mature, aborted, and damaged seeds in each fruit. I based the classification of mature and aborted seeds on the size, shape and color of seeds. Seeds were either mature (1.5-2 mm) or aborted (<1.5 mm). Seeds scored as aborted had other defining characteristics such as a darker color, irregular shape and rougher texture and often appeared to be decaying. Seeds may have been aborted due to lack of pollination or some other reason such as lack of water or nutrients or weevil damage.

Seed Predation Study

Although both vertebrates and invertebrates graze heavily upon S. malachroides leaves and stems, the most direct impact to reproductive success is seed predation by weevil larvae. In order to measure the effect of isolation distance and patch size on seed predation, I counted the number of damaged seeds in each fruit collected in the pollen limitation study. I recorded whether damage was a hole in seeds, partially eaten seeds or
fully consumed seeds. Damaged seeds included both mature and aborted seeds, and it was often difficult to assess damage to aborted seeds due to their small size. I also recorded the number of weevils or weevil larvae present in fruits as well as the species of adult weevil.

Data Analysis

For each of my three studies (pollen load, pollen limitation and seed predation) I performed an individual univariate regression analysis with the independent variables. I then performed multiple regression for all three studies to assess the separate and independent effect of variables.

I used the log of the mean number of *S. malachroides* and heterospecific pollen grains per plant as my dependent variables for the pollen load study. For the pollen limitation study I used mean difference of mature seeds between treated and control flowers. I used mean damaged seeds in the seed predation study. My independent variables included isolation distance (meters), patch size (number of plants in patch), size of nearest patch, number of heterospecific blooming species, and raceme number (Table 3). Although I recorded the pollen donors (hermaphrodite and male) within the focal and nearest adjacent patch, as well as patch density, both factors were highly collinear with patch size and were not used in the analysis (Pearson correlation coefficient=.990, \( p<.001 \)).

I screened my data for normality, outliers and potential multicollinearity using NCSS. I then performed stepwise linear regression with NCSS to determine the variables
for my final models. Alpha levels were set at .05 to add and .20 to remove variables. Minimum root mean square error was set at .015 with a maximum of 20 iterations. Finally, I performed multiple regression using the selected variables. Due to the presence of outliers in my data set, which violated the assumption of normally distributed residuals in least squares regression, I used robust regression. It essentially down-weights or completely removes various observations. I chose Huber’s robust method because it is currently the most frequently recommended in regression text books (Hintze 2004). To test for any effects of various abiotic factors on pollination, seed set or herbivory, I also performed a General Linear Modeling analysis. I used the Tukey-Kramer method on each of the dependent variables using the following categorical independent variables; aspect and canopy cover (due to their potential to affect air temperature and subsequently insect behavior), as well as slope, hydrology, shrub layer density and burn treatment (based on observations of more robust patches occupying habitats that were more mesic, flat and previously burned with lower shrub layer density). To test for any effects of various abiotic factors on pollination, seed set or herbivory, I also performed a General Linear Modeling analysis. I used the Tukey-Kramer method on each of the dependent variables using the following categorical independent variables; aspect and canopy cover (due to their potential to affect air temperature and subsequently insect behavior), as well as slope, hydrology, shrub layer density and burn treatment (based on observations of more robust patches occupying habitats that were more mesic, flat and previously burned with lower shrub layer density).
Table 3. Variables used in the pollen limitation, pollen load and seed predation studies. Dependent variables for each study were analyzed against isolation distance (meters), patch size, adjacent patch size, number of heterospecific blooming plants, and raceme number.

<table>
<thead>
<tr>
<th>Study</th>
<th>Pollen Load</th>
<th>Pollen Limitation</th>
<th>Seed Predation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dependent Variable</td>
<td>Mean # <em>S. malachroides</em> Pollen Grains</td>
<td>Mean Difference of Mature Seeds</td>
<td>Mean Total Damaged Seeds</td>
</tr>
</tbody>
</table>
RESULTS

Pollen Load

Pollen reception declined in patches with large isolation distances, and plants isolated by more than 17 meters were not pollinated (univariate regression, $r^2=17.3\%$, $t=-3.45$, $p=.001$). The effect of isolation distance on pollen reception was most pronounced for single plants and plants in very small patches (Figure 5). In contrast, plants in large patches received more pollen grains (univariate regression, $r^2=8.8\%$, $t=2.33$, $p=.023$). Most of the sampled patches were small and not isolated. (Table 4)

The number of heterospecific pollen grains received by stigmas increased with both patch size and the number of heterospecific blooming plants (multiple regression $r^2=22.93\%$, $F=8.331$, df=2, $p<.001$). Plants in very large patches received more conspecific pollen and fewer heterospecific pollen grains (Figure 6). Although I was not able to directly test pollinator behavior or effectiveness, generalist pollinators such as bumblebees, honeybees and butterflies were more commonly observed in large, contiguous patches. Within the large patches, these generalist pollinators traveled within and amongst S. malachroides plants. They were not observed at S. malachroides patches if Ceonothus sp., Rubus sp. or other plants with large nectar rewards were present. In contrast, hoverflies and beetles were more commonly observed in small, isolated patches and frequently traveled between different species.
Table 4. Variables affecting mean number of S. malachroides pollen grains found on stigmas. (multiple $r^2=36.7\%$, $F=7.838$, $df=4$, $p<.001$)

<table>
<thead>
<tr>
<th>Independent Variable</th>
<th>Regression Coefficient</th>
<th>$r^2$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolation Distance</td>
<td>-0.0196</td>
<td>12.4%</td>
<td>0.002</td>
</tr>
<tr>
<td>Patch Size</td>
<td>0.0073</td>
<td>7.9%</td>
<td>0.012</td>
</tr>
<tr>
<td>Adjacent Patch Size</td>
<td>0.0102</td>
<td>7.6%</td>
<td>0.014</td>
</tr>
<tr>
<td>Heterospecific Blooming Plants</td>
<td>0.1187</td>
<td>6.5%</td>
<td>0.023</td>
</tr>
</tbody>
</table>

Figure 5. The relationship between isolation distance, patch size and pollen receipt of *Sidalcea malachroides* (SIMA). Patch size (number of plants) and isolation distance of the focal plant patch was tested against mean pollen loads of five flowers on focal plants.
Figure 6. Relationship of mean heterospecific versus conspecific pollen grains. Large patches received greater amounts of conspecific pollen and less heterospecific pollen.

Pollen Limitation

The difference in seed set between open and hand pollinated flowers (degree of pollen limitation) varied as a function of isolation distance and raceme number (Table 5). The degree of pollen limitation increased with isolation distance (Figure 7) and, surprisingly, also with increasing raceme number. There was no detectable relationship between pollen limitation and patch size.
Table 5. Variables which affected the mean difference between mature seeds for hand pollinated versus open pollinated flowers. (multiple $r^2=49.5\%$, $F=20.58$, $df=2$, $p<.001$)

<table>
<thead>
<tr>
<th>Independent Variable</th>
<th>Regression Coefficient</th>
<th>$r^2$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolation Distance</td>
<td>0.046</td>
<td>40.0%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Raceme Number</td>
<td>0.004</td>
<td>9.9%</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Figure 7. The relationship between degree of pollination limitation and isolation distance. Pollen limitation was measured as the mean difference in the number of seeds produced by hand and open pollinated flowers in focal plants.
Seed Predation

Weevil larvae were common and often caused extensive damage. Up to three larvae were encountered in many mature fruits and larvae generally damaged both the fruit wall and seeds. Over half of the 3,117 seeds collected for the pollen limitation study were damaged by weevil larvae ($x = 56.6\%$ seeds damaged) Fifty-six of the 214 fruits collected had damage to 100% of the seeds they contained. *Macrorhoptus hispidus* was present in all sites surveyed, but *A. ochreopilosus* was positively identified only at the Fickle Hill and McGarvey Creek sites. Other invertebrate herbivores were also observed on plants, including butterfly and ladybird beetle larvae, leaf miners, slugs and snails, and leaf footed bugs (Coreidae). Adults of many beetle species were observed mating and possibly ovipositing on plants. Vertebrate herbivory was also common, and entire plants were sometimes consumed. The sole seed predators were weevil larvae. The mean number of damaged seeds for both control and hand pollinated flowers decreased with isolation distance, but increased with patch size (Figure 8) (Table 6).
Figure 8. Relationship between seed predation by weevils, isolation distance and patch size.

Table 6. Both isolation distance and patch size affected the total number of damaged seeds on treated and control plants. (multiple $r^2=23.1\%, F=6.319, df=4, p=.004$) Treated and control flowers were summed and the mean number of damaged seeds was used as a dependent variable.

<table>
<thead>
<tr>
<th>Independent Variable</th>
<th>Regression Coefficient</th>
<th>$r^2$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolation Distance</td>
<td>-0.068</td>
<td>8.2%</td>
<td>0.040</td>
</tr>
<tr>
<td>Patch Size</td>
<td>0.037</td>
<td>12.9%</td>
<td>0.011</td>
</tr>
</tbody>
</table>
Environmental Factors

General Linear Modeling (GLM) analysis revealed that in addition to patch size and isolation distance, a series of environmental factors also affected pollen reception. Specifically, xeric ridges received higher pollination (Table 7). Although pollinators were abundant in large burned patches, and burned patches appeared to have better floral displays, burn treatment did not account for a significant amount of variation in pollination.

Fruit damage by weevils increased in burned areas, areas with eastern and southern slopes and with moderately dense shrub cover for both treated and control plants (Table 8).
Table 7. The effects of abiotic environmental conditions on pollen reception.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Adj MS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspect</td>
<td>7</td>
<td>8.22</td>
<td>2.88</td>
<td>0.02</td>
</tr>
<tr>
<td>Burn Treatment</td>
<td>1</td>
<td>1.21</td>
<td>0.42</td>
<td>0.52</td>
</tr>
<tr>
<td>Shrub Density</td>
<td>2</td>
<td>5.85</td>
<td>2.05</td>
<td>0.14</td>
</tr>
<tr>
<td>Slope</td>
<td>2</td>
<td>0.78</td>
<td>0.27</td>
<td>0.76</td>
</tr>
<tr>
<td>Overhead Canopy</td>
<td>2</td>
<td>0.29</td>
<td>1.15</td>
<td>0.33</td>
</tr>
<tr>
<td>Hydrology</td>
<td>1</td>
<td>13.48</td>
<td>4.71</td>
<td>0.04</td>
</tr>
<tr>
<td>Error</td>
<td>43</td>
<td>2.86</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>58</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 8. The effects of abiotic environmental conditions on seed predation by weevils (General Linear Modeling).

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Adj MS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burn Treatment</td>
<td>1</td>
<td>34.34</td>
<td>5.46</td>
<td>0.026</td>
</tr>
<tr>
<td>Shrub Density</td>
<td>2</td>
<td>55.61</td>
<td>8.84</td>
<td>0.001</td>
</tr>
<tr>
<td>Aspect</td>
<td>7</td>
<td>25.56</td>
<td>4.06</td>
<td>0.003</td>
</tr>
<tr>
<td>Hydrology</td>
<td>1</td>
<td>17.80</td>
<td>2.83</td>
<td>0.103</td>
</tr>
<tr>
<td>Overhead Canopy</td>
<td>2</td>
<td>9.03</td>
<td>1.43</td>
<td>0.254</td>
</tr>
<tr>
<td>Error</td>
<td>31</td>
<td>6.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION

Pollen Load and Pollen Limitation

My results demonstrate the effect of isolation distance on the reproductive success of *S. malachroides*, however the effect of patch size is less clear. Plants in large patches received more pollen grains, however pollen limitation was not related to patch size. In addition, plants in isolated patches received less pollen grains and also experienced greater pollen limitation. Although isolation and reduced patch size may have lowered reproductive success, both factors appeared to provide some escape from damage to fruits by weevil larvae.

The positive relationship between patch size and pollen reception presumably reflected pollinator response to large attractive floral displays and local concentrations of nectar and pollen (Rathcke 1983, Sih and Baltus 1987, Jennersten 1988, Krannitz and Maun 1991). Consistent with this view, pollen reception also increased with the number of heterospecific blooming plants in a plot and with the size of adjacent *S. malachroides* patches, both of which contributed to larger displays. Generalist pollinators such as honeybees, bumble bees and butterflies appeared to be more common in large patches, and I mainly observed small hoverflies and beetles on plants in small, isolated patches. As expected, plants in larger patches received more conspecific and heterospecific pollen grains.
The increase in pollen reception for large patches did not translate into decreased pollen limitation. This confusing result most likely reflected the fact that seed production is complicated. Several factors in addition to number of pollen grains received can determine the number of seeds produced by a plant (Stephenson 1981, Willson and Price 1980, Gorchov 1988). Factors that might uncouple pollen load and degree of pollen limitation in *S. malachroides* fall into two categories: factors that make it difficult to measure the true degree of pollen limitation regardless of patch size, and factors that correlate with patch size.

Factors that affect the ability to measure the true degree of pollen limitation regardless of patch size include variation in pollen availability and quality, stigma receptivity, and available abiotic resources. The internal regulation and abortion of ovules can be caused by abiotic factors such as water, light and/or nutrient availability (Willson and Price 1980, Stephenson 1984). It is difficult to determine whether ovule development was halted due to a lack of adequate pollen for open pollinated flowers or a dearth in some other resource. Accurate detection of pollen limitation also depends on the application of a large number of pollen grains to receptive stigmas of treated plants. Unfortunately I was not always able to secure a large number of grains for hand pollination, and I may have occasionally used stigmas that were too old or too young.

Other sources of variation in pollen limitation in both large and small patches were not artifacts of techniques used, but likely correlated with patch size. These factors include variation in ovule number between flowers, abortion due to herbivory and interference of heterospecific pollen grains on stigmas. These sources of variation in
seed production, which were probably not correlated with pollination treatment, created corresponding variation in pollen limitation.

Flowers in large patches tended to receive more heterospecific pollen, presumably because they are mainly visited by generalist pollinators. An increase in heterospecific pollen reception may have depressed seed production via interference with the performance of conspecific pollen. Such a reduction in seed production would increase measured levels of pollen limitation, in spite of receipt of ample conspecific pollen. Weevil larvae consumed more seeds per fruit in large patches and this increase in seed consumption in large patches may have also affected my ability to accurately measure pollen limitation. Finally, pollen limitation may also have been greater than expected in large patches because plants in these patches tended to be more robust. Favorable growing conditions may have elevated the maximum number of seeds plants could produce. Under these circumstances, an increase in pollen reception in large vs. small patches might not result in a proportionate decrease in pollen limitation. Potentially, plants in small patches that receive fewer grains would be less pollination limited because they are capable of producing fewer seeds.

The increase in pollen limitation with raceme number is perplexing, as plants with more racemes would be expected to attract more pollinators. Pollen receipt did not increase with raceme number, however, suggesting that the increase in pollen limitation with raceme is likely the result of within-plant competition for resources.

Isolation distance affected both pollen receipt and seed set; patches that were isolated by more than 17 meters were not pollinated. For the populations I studied,
isolated patches were generally small, creating the circumstance least likely to attract efficient pollinators. An isolation distance of seventeen meters is a surprisingly short threshold for pollinator services given the foraging ranges of most pollinators. Throughout portions of its range, *S. malachroides* occupies more open habitats and pollination may be attained at greater distances, due to an increase in visibility for pollinators. The areas incorporated in my study were fairly dense, however, and varied topographically, and plants that were more than 17 meters away from a given patch were often not visible. The failure of pollination in very small, isolated patches accounted for much of the positive relationship between isolation distance and pollen limitation.

Seed Predation by Herbivores

Both a reduction in patch size and an increase in isolation distance appear to provide some escape from seed predation. This effect was pronounced for small, isolated patches which receive little seed predation. Only four of the fifty-nine plants in my study escaped weevil predation entirely, and these were in very small, isolated patches. Other studies have also detected an effect of patch size and isolation distance on seed predation (Brody 1997, Ehrlen 1996 and 1997). The most parsimonious explanation for this pattern is the abundant open flowers and/or developing fruits available in large patches (Herrera et. al 2002). Herbivory can pose a threat to rare plant species (Pavlik and Manning 1993, Pavlik et. al 1993, Louda and McEachern 1995, Helenurm 1998, Timmerman-Erskine and Boyd 1999), and this threat can occur through a decrease in net seed production. My study suggests that small patch size and increased isolation creates a tradeoff whereby the
decrease in seed predation in small, isolated patches may mitigate lower pollen receipt in these patches and any subsequent decrease in net seed production.

Additional Factors

Like many *Sidalcea* species (Glad 1994, Fernau 1998, Dimling 1992), *S. malachroides* appears to tolerate a wide range of habitats, especially in recently disturbed areas. The critical question is whether differences in abiotic resources affect population dynamics. Reproductive success in plants is often limited by environmental factors such as water, light and nutrient availability, due to direct effects of resource availability and also indirect effects of pollinators abundance and behavior. Pollen reception was higher for plants on xeric ridges, apparently due to the open space that increases both sunlight and visibility. Seed predation by weevils increased in burned areas and areas with moderately dense shrub cover and in areas with southern or eastern aspects. Shrub cover may have protected herbivores or allowed for greater dispersal. Populations in burned areas appeared to be more robust and were typically on open ridges. The effect of burning on herbivore damage was more likely due to a correlation with these variables which affect insect habitat and behavior. Southern and eastern facing aspects are generally warmer and increase activity in insects. *Sidalcea malachroides* plants were more robust and showy in burned patches, and I expected an increase in both pollinator services and seed set in burned patches. Studies have shown that reproductive success is generally higher in burned sites due to a decrease in competition and increase in nutrient availability (Menges 1995, Hunter 1986), however there was no relationship between
burn treatment and pollen reception or pollen limitation. In general, I expected that
abiotic factors would have greater impact on pollen receipt, pollen limitation and
herbivory. This result may be due to insufficient statistical power related to unbalanced
categorical data or it may reflect the greater importance of biotic factors compared to
abiotic factors in the success of this disturbance-related species.

Although I did detect relationships between the variables I studied, in general the
$r^2$ values for my analyses were modest. The low $r^2$ values were likely due to the large
amount of variation in the natural environment as well as my small sample size and
inability to evenly sample all combinations of patch size and isolation distance. Although
multicollinearity was not detected for patch size and isolation distance, and power levels
were acceptable, the results were influenced by the great number of small, contiguous
patches sampled. Future studies would benefit from a balanced orthogonal design of
patch sizes and isolation distances. Further studies including pollen thresholds for seed
set, seed viability, plant longevity and the effects of sex structure are needed to fully
understand the reproductive success of $S. \text{malachroides}$.

The density of pollen donors in patches may also have influenced both pollen
receipt and seed predation for plants, but I could not analyze this variable due to the
collinearity of pollen donors with patch size. Other studies have demonstrated a
relationship between pollen receipt and the density of pollen donors (Agren et al. 1986,
House 1992, Elmqvist et al. 1988). Predation can also be higher in patches containing a
higher proportion of male plants (Marshall 2001), but only if the weevil species present
eats pollen. Sex-biased seed predation was evident in populations of $S. \text{hendersonii}$
where *Anthonumus melancholicus* (Dietz) was observed and not in populations with only *Macrorhoptus sidalcea* (Sleeper), which is most likely due to the pollen foraging behavior of *A. melancholicus* (Marshall 2001). For the hermaphrodite plants collected in 2004, damage was also greater for female plants than hermaphrodite plants (Appendix A). This increase in damage may be due to the greater number of possible ovules and mature seeds in female plants (Appendix A). For *S. malachroides*, weevils appear to forage on pollen, but prefer female plants for egg deposition and are more attracted to patches with a higher percentage of female plants.
CONCLUSIONS

*Sidalcea malachroides* experiences a reduction in pollinator services and increased pollen limitation in small, isolated patches. In contrast, large dense patches receive more pollen as well as more damage to seeds by weevil larvae. As large, dense patches receive greater amounts of herbivory, net seed production may not be higher in these patches. Curiously however, many studies have shown that even prolific seed damage does not translate into an effect on population dynamics because seed production often exceeds habitat availability for seedling recruitment (Harper 1977, Louda 1989 and 1995). Herrera (2002) discovered that there is an interaction between pollinators and herbivores on seedling recruitment in *Helleborus foetidus*. His results suggested an ecological pathway favoring the evolution in pollinator-dependent plants suffering from intense herbivory, whereby plants evolve traits to maximize pollination while reducing herbivory. Although large, non-isolated patches receive greater seed predation, it is unknown whether or not this damage causes reduced seedling recruitment thereby affecting population dynamics.

The implications for management of the species in regard to this tradeoff for patch size and isolation distance are not entirely clear. On the one hand, large, dense patches may be more important to conserve due to the possibility of more prolific seed production. On the other hand, small, isolated patches may also be important due to their ability to avoid herbivory. Metapopulation dynamics and gene flow further confound the issue as to which occurrences are more important to preserve during impact assessment.
and mitigation guidelines. Furthermore, it is often difficult to assess the status of small populations. Most large occurrences on timberlands are observed in areas that were recently harvested, where small occurrences are observed in areas that were harvested between five to seven years previously. This is seemingly due to a decrease in population size after the regrowth of associated vegetation, however this has not been confirmed at the time of this writing. To fully ensure appropriate management strategies more work is needed to quantify the longevity of individual plants, populations and their associated seedbanks.

*Sidalcea malachroides* is a fairly short-lived perennial species that likely depends on seed set and large seed banks. Pollen limitation due to isolation distance and prolific herbivory may be contributing to the rarity of the species. As it is the oldest and most genetically unique *Sidalcea* species in North America (Andreasen 2001), care should be taken when assessing impacts and viability of this species.
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**PERSONAL COMMUNICATIONS**

Renner, M. 2005 Personal Communication. PALCO, Science Department, P. O. Box 712, 125 Main St., Scotia, California, 95565.
Figure 9. Damage to seeds was greater for treated ($x=3.89$) and untreated ($x=1.76$) female plants than hermaphrodite plants ($x=0.63$). (ANOVA $df=56$, $F=3.89$, $p<.001$)
Figure 10. Mean ovule number was greater for female plants ($\bar{x}=7$) than hermaphrodite plants ($\bar{x}=6.1$). (ANOVA $df=56$, $F=4.45$, $p=.016$)
Figure 11. Preliminary field investigations revealed that hermaphrodites are self-compatible, however seed set means are lower than those of female plants. Hermaphrodite plants had greater seed set ($\bar{x}=4.6$) than untreated plants ($\bar{x}=2.48$), and less seed set than treated plants ($\bar{x}=6.07$). Treated female plants had the greatest seed set, which indicated that pollen limitation occurs in the species. (ANOVA $df=56$, $F=19.65$, $p<.001$)
Figure 12. *Sidalcea malachroides* pollen and heterospecific pollen.
Figure 13. *Anthonomus ochreopilosus.*
Figure 14. *Macrorhoptus hispidus.*
Figure 16. Fruit (schizocarp) of *S. malachroides* containing weevil larvae inside.