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Pollinators May Not Limit Native Seed Viability for Puget Lowland Prairie Restoration

ABSTRACT

Reproductive success of plants can be influenced by the rate of visitation by insects to flowers. Land managers often rely on large-scale production of native seeds in nurseries for replanting into natural environments as part of restoration strategies. This study investigated pollination of deltoid balsamroot (*Balsamorhiza deltoidea* Nutt.) and sicklekeel lupine (*Lupinus albicaulis* Douglas) at a restoration nursery compared to a Puget lowland prairie to determine if inadequate insect visitation restricts viable seed production. In 2011 and 2012, insect visitation rates and community composition were recorded for each plant species at each site. In 2012, seeds were collected from hand-pollinated and naturally-pollinated inflorescences and tested for viability. Overall visitation rates were significantly higher at the nursery than the prairie for both plant species and visiting insect communities differed between sites and years. However, pollinator limitation was not evident for either plant species at either site and visitation rate was not found to significantly influence the number of viable seeds produced. It is possible that factors other than pollinator visitation are influencing seed viability and further studies will address other factors, such as soil nutrients and seed handling practices. This study is important for land managers because it shows that although pollinator communities are different at a restoration nursery compared to a natural prairie site, overall pollination processes were not different. In fact, natural pollination by both assemblages of pollinators did not differ from forced pollination by hand. Increasing insect visitation may not significantly increase seed viability at this restoration nursery. In terms of monitoring the insect communities at both locations, weather conditions can influence visiting insect community composition so long-term data collection will be necessary to make broader generalizations about pollinator visitation at either site.

INTRODUCTION

Pollinators play a key role in the reproduction of wild plants as they are linked to viable seed production and ecosystem restoration. Pollinators and their activities thus provide an ecosystem-wide service (Kremen et al. 2007). The ability to produce viable seeds is critical for plants to be able to maintain their populations naturally. In addition, the role of pollinators needs to be better understood to improve conservation strategies, especially in threatened habitats (Fontaine et al. 2006; Mayer et al. 2011). Often land managers must understand plant-insect interactions to be able to grow successful yields of supplemental native seed in nursery settings.

Native seed from nurseries plays an important role in ecosystem restoration. Ecosystems in need of conservation attention may be stressed by factors such as invading species, fragmentation, and climate change; all of which can suppress a plant species' population size and limit its reproduction ability (McCarty 2001; Vila & Weiner 2004; Fazzino et al. 2011; Tscheulin & Petanidou 2011). Many restoration practitioners depend on native seed grown in nurseries for repopulating plant species in natural areas. Native plant nursery managers strive to produce large quantities of high quality seed to keep up with the demand.

The Center for Natural Lands Management (CNLM) relies on large scale production of native seeds for replanting into the Puget lowland prairies as part of their restoration strategy. Some years the CNLM has struggled to produce large quantities of viable seeds at Webster Nursery for certain plant species (Cheryl Fimbel, CNLM, pers. comm. 2010). The cause of this problem may be due to issues with proper seed handling or storage, inadequate environmental conditions where the plants are grown (such as soil nutrients, weather, etc.), or pollinator limitation. This study will address the latter by investigating the current state of pollination at Webster Nursery and comparing it to a Puget lowland prairie to determine if inadequate pollination is restricting viable seed production at the nursery.

When plants produce fewer viable seeds because of insufficient pollination, they are pollinator limited (Dieringer 1992; Price et al. 2008; Fazzino et al. 2011). Several aspects of pollination can influence seed viability. Rate of insect visitation can be a critical factor for the reproductive success of many plant species. Researchers found a positive relationship between insect visitation rates and pollen receipt (Engel & Irwin 2003). Differences in pollinator community structure can also affect overall pollination effectiveness (Perfectti et al. 2009).

The Puget lowland prairie ecosystem has been fragmented by coniferous forest encroachment and urban and agricultural development so that now only 3% of the original habitat remains (South Sound Prairies Working Group 2012). Re-establishing native flora has been a priority of Puget lowland prairie land managers (Stanley et al. 2008). The deltoid balsamroot (*Balsamorhiza deltoidea* Nutt.) is a species of potential concern in Washington State (Washington Natural Heritage Program 2012) and is one of the many plant species replanted into the prairies. The federally endangered Fender's blue butterfly (*Icaricia icarioides fenderi* (Macy)) occasionally feeds on another species of concern, the sickle-keel lupine (*Lupinus albicaulis* Douglas) (Wilson et al. 1997). Both plant species grow along the west coast of the United States and into Canada (USDA Natural Resources Conservation Service 2012).

In this study, I address the following research hypotheses: 1) Insect visitation rates will be higher at the prairie site than the nursery because natural environments provide more resources and habitat for insects than environments constructed by humans. 2) There will be differences in visiting insect community composition between the nursery and prairie and between years because of differing resources and weather conditions. 3) There will be pollinator limitation at the nursery due to lower insect visitation rates and 4) Insect visitation rate will affect seed viability.

MATERIAL AND METHODS

Study Plants

To address my research questions, I focused this study on two native prairie plants, *B. deltoidea* (deltoid balsamroot) and *L. albicaulis* (sicklekeel lupine). These plants are both found at natural prairie sites and are being produced from seed by CNLM at the Webster Nursery, Tumwater, WA, USA.

Balsamorhiza deltoidea (Fig. 1 A; Asteraceae) bloomed from the last week of May to mid-June in 2011 and from May 7 to June 1 in 2012. This perennial has yellow, compact head inflorescences containing many fertile female ray flowers and bisexual disk flowers. The fruits are achenes, each with a single ovule.

Lupinus albicaulis (Fig. 1 B; Fabaceae), is a perennial and bloomed from late June to mid-July in 2011 and from May 29 to June 29 in 2012. The blue, papilionaceous flowers develop basally first in racemes. Each flower contains 10 monodelphous stamens and a one-celled pistil with an average of five ovules (indicated by the number of cells found in collected pods).

A. B.

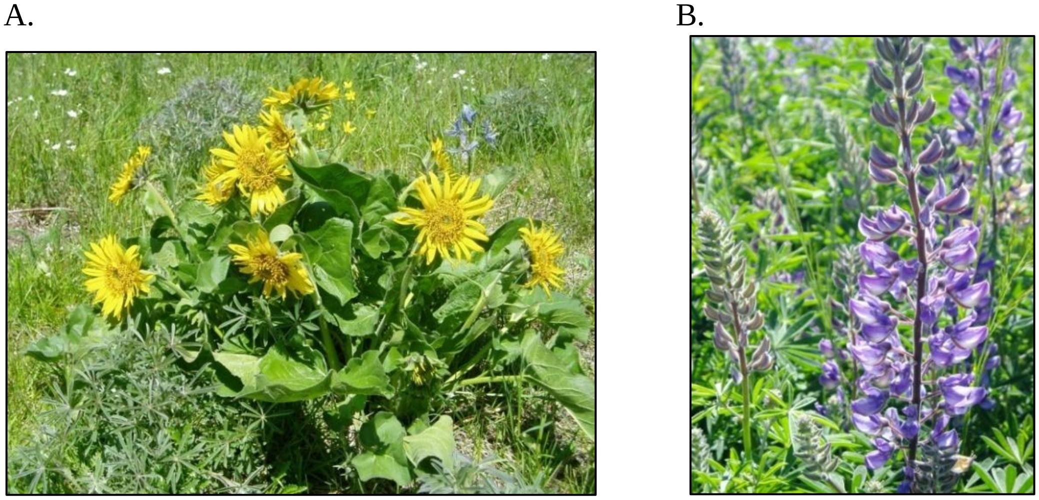


Figure 1. A= *Balsamorhiza deltoidea*, B= *Lupinus albicaulis*. The study plants found on Puget lowland prairies, Thurston Co., 2011.

Study Sites

Washington Department of Natural Resources (DNR) owns Webster Nursery (Fig. 2), which is managed by the CNLM to produce seed from native plants at a large scale for restoring Puget lowland prairies. The plants are grown outdoors in dense rows. The rows planted with *B. deltoidea* and *L. albicaulis* were last fertilized in 2008, are watered only by rain, and were not sprayed with pesticides or herbicides in 2012 (Angela Winter, CNLM nursery manager, pers. comm. 2012). Farmland, a highway, and forested areas surround the nursery.

Figure 2. Webster Nursery, Tumwater, WA, 2011

The US Department of Defense manages Johnson Prairie (Fig. 3), a natural prairie site on Joint Base Lewis-McChord. Johnson is one of the few remaining natural Puget lowland prairie sites, and is located near Rainier, WA. This prairie receives frequent horseback riding, hunting, and off-road driving activity, though less military training activity than other prairie sites located on the base (Stinson 2005). This site was burned in August, 2011 for restoration purposes. Coniferous forest borders this prairie site.

Figure 3. Johnson Prairie, Thurston Co., 2012

Visitation Rates

The methods used for this study were adapted from procedures used to calculate visitation rates in many other studies. Arroyo et al. (1982) first recorded the number of visits to a know number of flowers for a set time interval. Others (Arroyo et al. 1985, Inouye & Pyke 1988, Berry & Calvo 1989, McCall & Primack 1992) replicated this method to allow comparisons among studies (Kearns & Inouye 1993). Boyd (2004) used this method to calculate visitation rates and combined those with pollen deposition values as a measurement of pollinator effectiveness.

For this study, plots were selected to collect visitation rate data for both study plant species in 2011 and 2012. Plot locations were selected randomly at Webster Nursery, and plot locations at Johnson Prairie were selected randomly from patches of plants with similar floral densities as found at the nursery. Floral density was calculated for each plot by counting the number of inflorescences of the focal species in bloom and dividing that number by the area of the plot (3 m^2).The selected patches contained few other flowering species to reduce the chance that floral competition would be a confounding factor. In 2011, six plots were selected for *B. deltoidea* and 16 plots were selected for *L. albicaulis* at each location and observed once (*B. deltoidea* n=6, *L. albicaulis* n=16). Differences in number of observations made were due to sampling time constraints. In 2012, 30 plots were selected at each site for *B. deltoidea*, and each observed once (n=30). After sampling *B. deltoidea* it was noted that visitation rates can vary throughout the bloom period, so the experimental design was changed for *L. albicaulis* in 2012. Recorded visits to flower patches for three rounds of timed intervals can be used to calculate a mean number of visits per flower per hour (Tscheulin & Petanidou 2011). Ten plots were selected at each site and each sampled three times for *L. albicaulis* in 2012 (n=10).

Observations took place during peak flowering times on three days for each plant species between May 20 and July 6 in 2011. In 2012, observations took place between May 8 and June 21 on six days for *B. deltoidea* and five days for *L. albicaulis*. Each observation period lasted 10-minutes. All observations were made between 1000 and 1530 hours. Sampling dates were chosen to be as close together as possible on days with similar temperature, cloud cover, and wind conditions within an optimal range for insect activity (temperatures ranging from 9 to 27 ^{0}C, clear to cloudy skies with shadows present, and still air to light breeze). I assumed all flowers in bloom were receptive to pollen.

Visiting insects were grouped into morphotypes: small dark bees (Halictidae, Colletidae: Hylaeinae, Apidae: Xylocopinae, and Andrenidae), large dark bees (*Andrena* sp. and Colletidae), green metallic bees (*Agapostemon* sp.), cuckoo bees (Apidae: Nomadinae), honey bees (*Apis mellifera*), bumblebees (*Bombus* sp.), flies (Diptera), ants (Formicidae), wasps (Hymenoptera: Apocrita), and beetles (Coleoptera). The only category that was further identifies into species categories was the bumblebee category as they were easily identified to species in the field. The number of visits made by each insect type was recorded during each ten-minute period. A visit was recorded only if the insect landed on the reproductive parts of a flower in an inflorescence. If an insect appeared to be "nectar robbing," where there was no potential for pollen transfer, the visit was discounted. Nectar robbing was rarely observed in this study.

Two-sample Wilcoxon tests were used to compare the overall mean visitation rate and visitation rate of each insect group at the nursery to the prairie for each plant species because the data were not normally distributed. The data were first logarithmically transformed. Analyses were conducted using R statistical package (www.r-project.org) and an alpha = 0.05.

Visiting Insect Communities

Community analysis was performed to examine characteristics of the visiting insect communities. R statistical package was used to perform two-sample Wilcoxon tests to find differences in visiting insect morphotype richness, evenness, and diversity between the study sites and years for both plant species (alpha = 0.05). PC-ORD statistical software (http://home.centurytel.net/~mjm/pcordwin.htm) was used to run all other community analyses. Shannon's diversity index (H') and Simpson's diversity index (D) were used to calculate visiting insect morphotype diversity for each plant species. The total number of visits made by each morphotype was summed from all observations to compare community composition and total number of visits made to each plant species each year. Permutative multivariate ANOVAs and non-metric multidimensional scaling (NMDS) ordinations were used to determine if insect communities differed between sites for each plant species in each year (alpha = 0.05). Indicator Species Analysis was performed to find evidence for preferences of insects for certain environmental conditions (alpha = 0.05). Multiresponse permutation procedures (MRPP) were used to determine if temperature, wind speed, or cloud cover (as ranked categorical variables) influenced the community structure of visiting insects (alpha = 0.05).

Pollinator Limitation

Procedures for this pollinator limitation experiment were adapted from methods used by Fazzino et al. (2011) who compared seed set from naturally-pollinated *B. deltoidea* inflorescences to hand-cross-pollinated inflorescences to investigate pollinator limitation. In 2012, a subset of 10 plots for *B. deltoidea* at each site was selected randomly from the visitation rate plots, and all plots from the *L. albicaulis* visitation rate observations were used for the seed set experiment. Two similarly sized plants were chosen within each plot for *L. albicaulis*. On the first plant, four inflorescences of similar size were marked with thread before the styles matured. A bag made out of tulle was placed over one inflorescence to exclude all insect visitations to test for autogamy (unassisted self-pollination). A second inflorescence was also bagged and then hand-pollinated using pollen from flowers on the same plant to mimic geitonogamy (pollinator-assisted self-pollination). A third inflorescence was left uncovered and hand cross-pollinated and a fourth inflorescence was left uncovered to be naturally pollinated. On the second plant, one inflorescence of a similar size to the others was marked and left to be naturally pollinated as a control comparison to rule out differences in resource allocation in the treated plant. Only hand-cross-pollination and natural pollination treatments were applied to one plant per plot for *B. deltoidea*, as Fazzino et al. (2011) documented that this species is self-incompatible and does not reallocate resources in this kind of experiment.

After setting up the plots, hand-pollination treatments were applied every other day until the stigmas shriveled. I then covered all the inflorescences with a coarser mesh bag to prevent seed predation. When the fruits matured, I collected the inflorescences and extracted and counted the seeds. For *L. albicaulis*, I also counted flowers (indicated by pedicel scars), ovules, and pods (fruits).

A tetrazolium assay was used to test the seeds for viability using procedures adapted from the International Seed Testing Association (2012). Ten seeds were randomly selected from each inflorescence for *B. deltoidea*, and all seeds from the *L. albicaulis* inflorescences were tested. *Balsamorhiza deltoidea* seeds were soaked in warm water for four hours, and *L. albicaulis* seeds were soaked for 24 hours. A 1% aqueous solution of 2,3,5-triphenyltetrazolium chloride was prepared and the pH was adjusted to 6.8. All seed coats were pierced before soaking the seeds in the tetrazolium solution. After four hours, I examined the embryos for the red staining that indicates viability.

Because these data were not normally distributed, I used two-sample Wilcoxon tests to compare the number and percent of viable seeds produced by the inflorescences of each treatment group for each plant species at each site. These analyses were done using R statistical software (alpha=0.05). The seed number data were first logarithmically transformed, and the percent viable seed data were first arcsine square root transformed. To determine if there was pollinator limitation for either plant species at Webster Nursery or Johnson Prairie, I compared the number and percentages of viable seed produced by the hand-pollinated inflorescences to the naturally pollinated inflorescences. For *L. albicaulis*, I also compared number of seeds per flower, seeds per ovule, seeds per pod, and pods per flower for each treatment.

Visitation Rate vs. Viable Seed Production

Finally, using R statistical package (alpha=0.05), I investigated whether or not different variables affected viable seed production. I used simple linear regression to determine if insect visitation rate affected number or percent viable seed of the naturally pollinated inflorescences for *B. deltoidea*, and number, percent viable, seeds per flower, seeds per ovule, seeds per pod, and pods per flower for *L. albicaulis*. I also used simple linear regression to determine if inflorescence diameter or plant volume affected the percentage or number of viable seeds produced by *B. deltoidea*.

RESULTS

Visitation Rates

Insect visitation rates differed between Webster Nursery and Johnson Prairie, both overall and for many of the insect groups in both years. In 2011, overall visitation rates were significantly higher at Webster nursery than at Johnson prairie for *L. albicaulis* (W=57, $n_1=n_2=16$, P=0.0078), but not for *B. deltoidea* (W=8, $n_1=n_2= 6$, P=0.1255). In contrast, in 2012 overall visitation rates were significantly higher at Webster nursery than at Johnson prairie for both *B. deltoidea* (W=169, $n_1=n_2=30$, P<0.0001) and *L. albicaulis* (W=11, $n_1=n_2=10$, P=0.0036). Webster nursery also had significantly higher visitation rates than Johnson prairie for specific insect morphotypes visiting each of the plant species in both years (Tables 1 & 2).

Table 1. Results of two-sample Wilcoxon tests comparing insect visitation rates at Webster nursery and Johnson prairie for *B. deltoidea*

	2011 ($n_1=n_2=6$)		**2012 ($n_1=n_2=30$)**	
Insect Morphotype/Species	**W**	**P**	**W**	**P**
Small Dark Bees	-----	-----	**348.5**	**0.0326**
Large Dark Bees	**2.0**	**0.0124**	435.0	0.3337
Green Metallic Bees	15.0	0.4047	420.0	0.1608
Cuckoo Bees	-----	-----	420.0	0.1608
Honey Bees	15.0	0.4047	-----	-----
Bumblebees (total)	21.0	0.4047	**244.0**	**0.0008**
Bombus sitkensis	-----	-----	**335.5**	**0.0266**
Bombus mixtus	21.0	0.4047	465.0	0.3337
Bombus vosnesenskii	-----	-----	**343.0**	**0.0289**
Bombus melanopygus	-----	-----	405.0	0.0815
Bombus flavifrons	-----	-----	465.0	0.3337
Flies	27.0	0.0740	449.5	1.000
Ants	21.0	0.4047	-----	-----
Beetles	-----	-----	435.0	0.5703

Significant results are in bold. All significant results indicate higher visitation rates at Webster nursery than at Johnson prairie.

Table 2. Results of two-sample Wilcoxon tests comparing insect visitation rates at Webster nursery and Johnson prairie for *L. albicaulis*

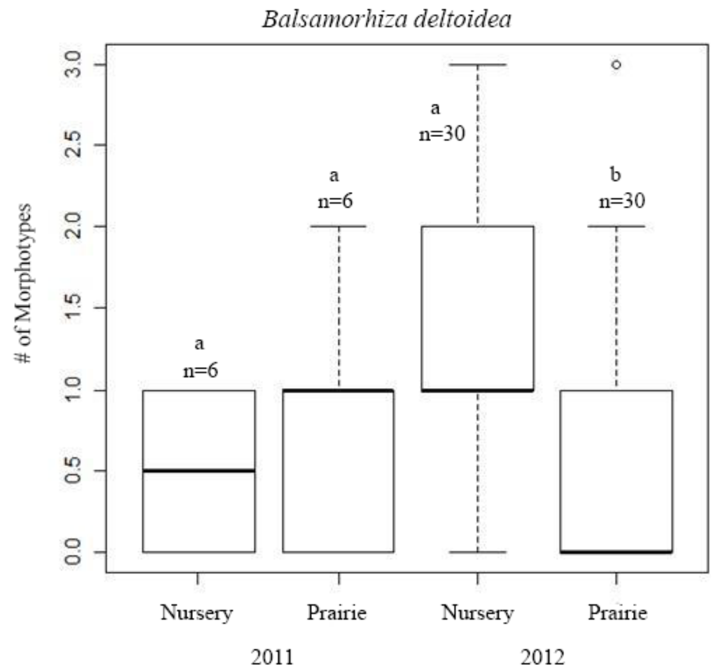
	2011 (n_1=n_2=16)		2012 (n_1=n_2=10)	
Insect Morphotype/Species	**W**	**P**	**W**	**P**
Small Dark Bees	164.5	0.0988	72.0	0.0666
Large Dark Bees	126.0	0.9216	46.0	0.7280
Bumblebees (total)	**54.0**	**0.0054**	**2.0**	**0.0002**
Bombus sitkensis	147.5	0.4102	45.0	0.5842
Bombus mixtus	**29.5**	**0.0001**	**0**	**1.0000**
Bombus vosnesenskii	**76.0**	**0.0353**	**25.0**	**0.0149**
Bombus melanopygus	136.0	0.3485	45.0	0.3681
Flies	152.0	0.0800	55.0	0.3681
Wasps	120.0	0.3485	**-----**	**-----**
Beetles	120.0	0.3485	65.0	0.0779

Significant results are in bold. All significant results indicate higher visitation rates at Webster nursery than at Johnson prairie.

Visiting Insect Communities

Characteristics of visiting insect community composition varied between sites and years. There was no significant difference in morphotype richness for visiting insects on either plant species between Webster nursery and Johnson prairie in 2011 (*B. deltoidea*: W=22.5, n_1=n_2=6, P=0.4760; *L. albicaulis*: W=109.5, n_1=n_2=16, P=0.4790), but there was increased insect richness at Webster Nursery for both plant species in 2012 (Figure 4A: *B. deltoidea*: W=189, n_1=n_2=30, P=<0.0001; 4B: *L. albicaulis*: W=24, n_1=n_2=10, P=0.0491).

A.



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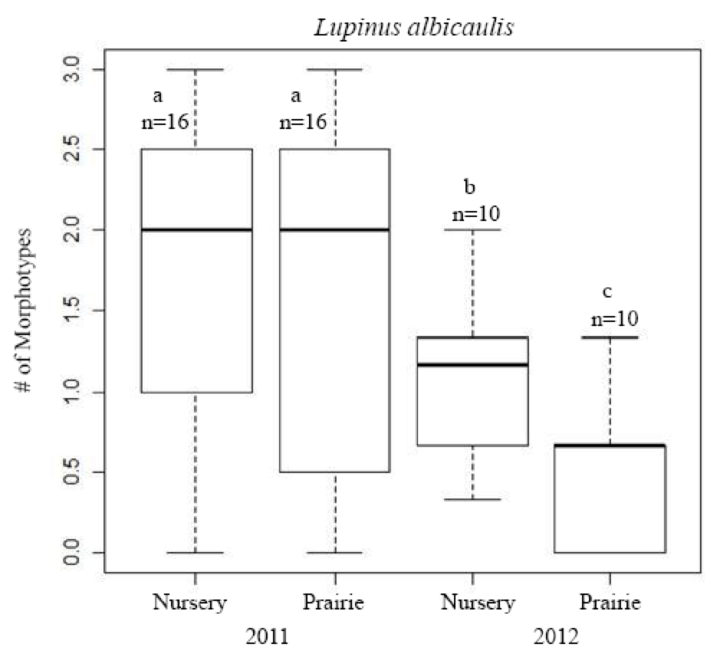


Figure 4. Visiting insect morphotype richness for A) *Balsamorhiza deltoidea* and B) *Lupinus albicaulis* at Webster nursery and Johnson prairie in 2011 and 2012. Different letters above bars indicate a significant difference between sites and years.

Balsamorhiza deltoidea had a significantly more even distribution of visiting insect morphotypes at Webster nursery in 2012 ($W=299$, $n_1=n_2=30$, $P=0.0060$), however no significant difference was found in morphotype evenness between Webster nursery and Johnson prairie in 2011($W=21$, $n_1=n_2=6$, $P=0.4047$) (Table 3). There was no significant difference found in visiting insect morphotype diversity between Webster nursery and Johnson prairie in 2011 for *B. deltoidea* (H': $W=21$, $n_1=n_2=6$, $P=0.4047$; D: $W=21$, $n_1=n_2=6$, $P=0.4047$), but diversity was significantly higher at Webster nursery in 2012 (H': $W=301$, $n_1=n_2=30$, $P=0.0067$; D: $W=302$, $n_1=n_2=30$, $P=0.0071$) (Table 3). *Lupinus albicaulis* had a significantly more even distribution of visiting insect morphotypes at Johnson prairie in 2012 ($W=362.5$, $n_1=n_2=30$, $P=0.0475$), and no siginifcant difference was found in morphotype evenness between Webster nursery and Johnon prairie in 2011($W=135$, $n_1=n_2= 16$, $P=0.8025$) (Table 3). There was no significant difference found in visiting insect morphotype diversity between Webster nursery and Johnson prairie in either year for *L. albicaulis* (2011- H': $W=127.5$, $n_1=n_2=16$, $P=1.0000$ D: $W=122$, $n_1=n_2=16$, $P=0.8324$; 2012- H': $W=364.5$, $n_1=n_2=30$, $P=0.0529$ D: $W=364.5$, $n_1=n_2=30$, $P=0.0529$), but interestingly, diversity was higher in 2011 than in 2012 at both sites (Webster- H': $W=338$, $n_1=16$, $n_2=30$, $P=0.0130$ D: $W=340$, $n_1=16$, $n_2=30$, $P=0.0113$; Johnson- H': $W=355.5$, $n_1=16$, $n_2=30$, $P=0.0004$ D: $W=356.5$, $n_1=16$, $n_2=30$, $P=0.0003$) (Table 3).

Table 3. Results of Evenness, Shannon's Diversity (H') and Simpson's Diversity (D) indices for visiting insect morphotypes at Webster nursery and Johnson prairie.

Plant Species	**Year**	**Site**	**E**	**H'**	**D**
Balsamorhiza deltoidea	2011	Nursery	0.000**a**	0.000**a**	0.0000**a**
Balsamorhiza deltoidea	2011	Prairie	0.167**a**	0.116**a**	0.0833**a**
Balsamorhiza deltoidea	2012	Nursery	0.399**b**	0.285**b**	0.1933**b**
Balsamorhiza deltoidea	2012	Prairie	0.107**a**	0.087**a**	0.0555**a**
Lupinus albicaulis	2011	Nursery	0.363**a**	0.352**a**	0.1980**a**
Lupinus albicaulis	2011	Prairie	0.427**a**	0.369**a**	0.2315**a**
Lupinus albicaulis	2012	Nursery	0.218**a**	0.161**b**	0.1062**b**
Lupinus albicaulis	2012	Prairie	0.058**b**	0.051 **b**	0.0325**b**

Different letters after S, E, H', and D values indicate a significant difference between sites and years based on two-sample Wilcoxon test results.

Insect community composition and total number of visits by each group varied between sites and years (Figures 5 & 6). In 2011 at Webster nursery, the greatest number of visits to *B. deltoidea* was made by honeybees and green metallic bees and bumblebees were absent. In 2012, bumblebees made the greatest number of visits and honeybees and green metallic bees were absent. Bumblebees visited *B. deltoidea* more frequently than any other morphotype at both sites in 2012, but not in 2011. In 2011, bumblebees made the greatest number of visits to *L. albicaulis* at Johnson prairie, but almost no visits were made by bumblebees to *L. albicaulis* at Johnson prairie in 2012.

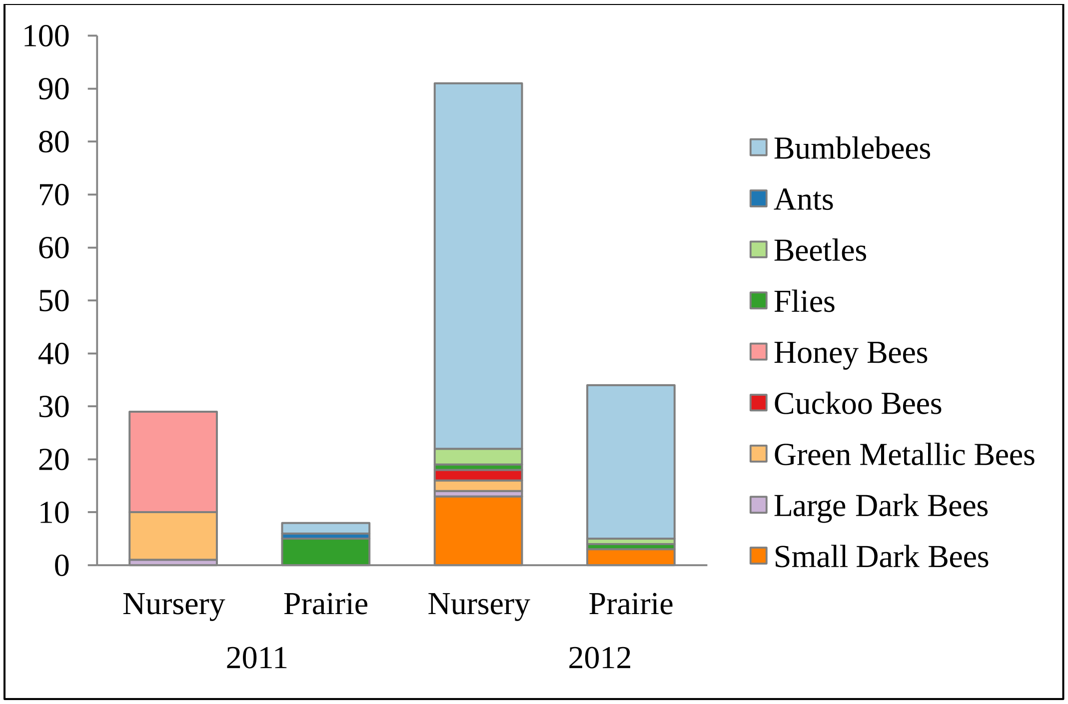


Figure 5. Total number of visits (from all observations summed) made to *Balsamorhiza deltoidea* by each insect morphotype.

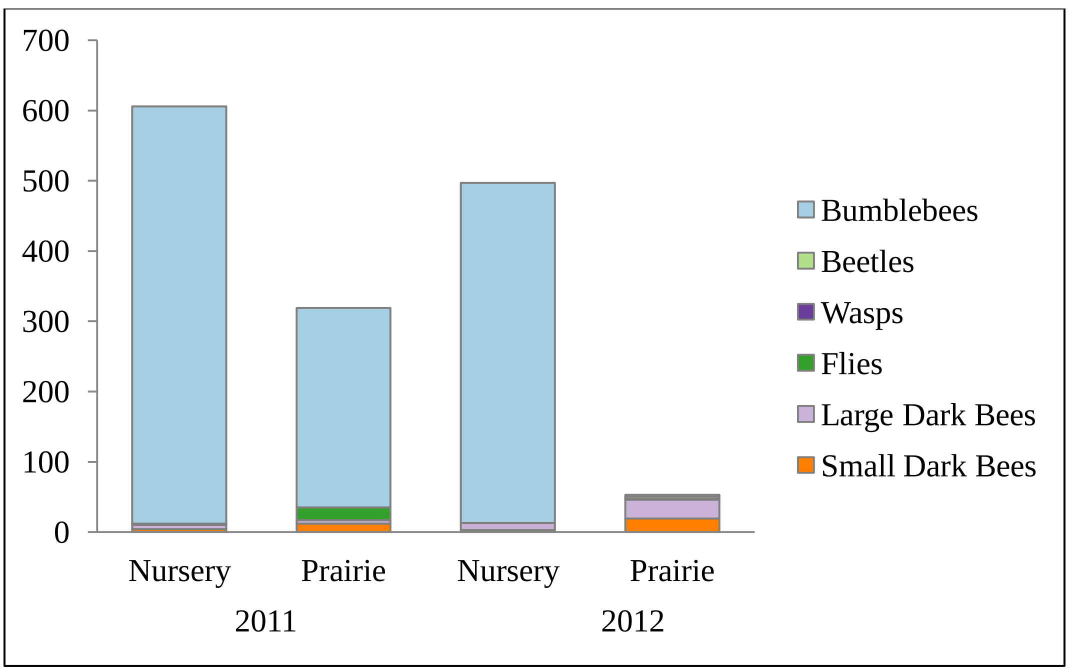


Figure 6. Total number of visits (from all observations summed) made to *Lupinus albicaulis* by each insect morphotype.

In 2011 and 2012 significantly different insect communities visited plants between Webster nursery and Johnson prairie. Specifically, in 2011 differences in community structure existed only for insects visiting *L. albicaulis* (Table 4); however, in 2012 differences in community structure existed for insects visiting both *B. deltoidea* and *L. albicaulis* (Figure 7; Table 4).

Table 4. perMANOVA Results for Influence of Location (Websterv nursery and Johnson prairie) on Community Structure of Visiting Insects

Plant Species	**Year**	**F**	**d.f.**	**P**
Balsamorhiza deltoidea	2011	1.3607	11	0.3538
Balsamorhiza deltoidea	**2012**	**9.7535**	**59**	**0.0002**
Lupinus albicaulis	**2011**	**4.4255**	**31**	**0.0006**
Lupinus albicaulis	**2012**	**4.6195**	**59**	**0.0006**

Significant results are in bold.

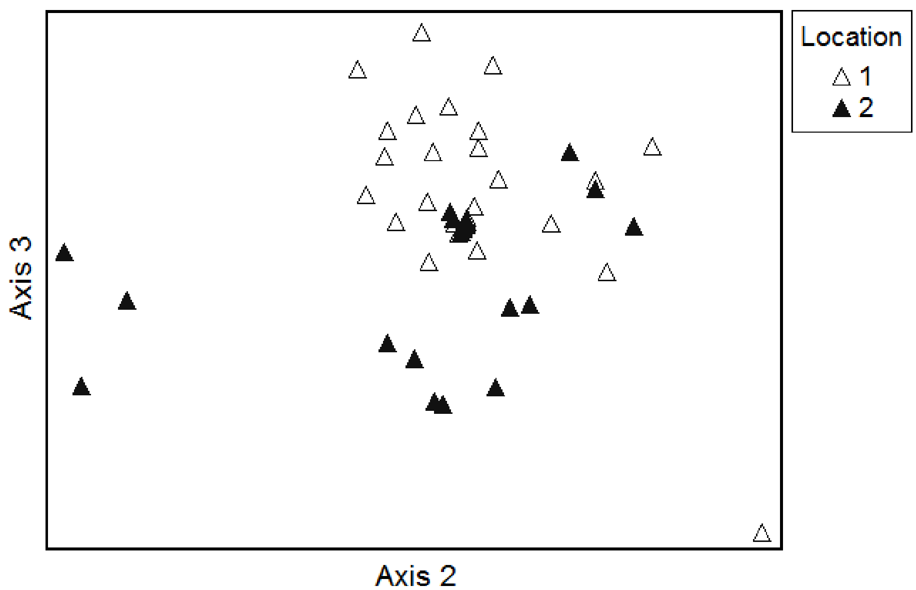


Figure 7. A representative NMDS ordination plot of influence of site differences on visiting insect community structure for *Lupinus albicaulis* in 2012. Location 1= Webster nursery. Location 2= Johnson prairie.

Indicator species analysis provides evidence for the preferences of certain insects for certain environmental conditions. Data were pooled across the nursery and prairie for these analyses. *Bombus mixtus* and *B. vosnesenskii* were significant indicator species for *L. albicaulis* at Webster nursery in both years (Table 5).

Table 5. Indicator Species and Morphotype P Values for Location (Webster nursery or Johnson prairie)

Visiting Insect Species/Morphotype	***Balsamorhiza deltoidea*** **2011**	**2012**	***Lupinus albicaulis*** **2011**	**2012**
Small Dark Bees	-----	0.0562	0.1826	0.1166
Large Dark Bees	1.0000	1.0000	1.0000	0.4433
Green Metallic Bees	1.0000	0.4881	-----	-----
Honey Bees	1.0000	-----	-----	-----
Cuckoo Bees	-----	0.4819	-----	-----
Bombus mixtus	1.0000	1.0000	**0.0002***	**0.0002***
Bombus vosnesenkii	-----	0.0904	**0.0196***	**0.0044***
Bombus sitkensis	-----	0.0788	0.2703	0.7491
Bombus melanopygus	-----	0.2466	1.0000	1.0000
Bombus flavifrons	-----	1.0000	-----	-----
Bombus californicus	-----	-----	-----	1.0000
Wasps	-----	-----	1.0000	-----
Beetles	-----	0.7441	1.0000	0.2334
Flies	0.1840	1.0000	0.2270	1.0000
Ants	1.0000	-----	-----	-----

Significant results are in bold. *Significant indicator for Webster nursery **Significant indicator for Johnson prairie

Bombus mixtus and *B. vosnesenskii* were also significant indicator species for a light breeze and clear skies for *L. albicaulis* in 2011 (Tables 6 & 7). When conditions were partly cloudy, more often than not, no insect visitors were present (Table 7). In 2012, *B. melanopygus* was a significant indicator of temperatures around 13 ^{0}C and an absence of visiting insect species was a significant indicator of high wind speeds for *B. deltoidea* (Tables 7 & 8). For insects visiting *L. albicaulis* in 2012, *B. mixtus* was a significant indicator species for temperatures around 16 ^{0}C; *B. sitkensis* and *B. mixtus* were significant indicator species for calm wind speeds; small dark bees and large dark bees were significant indicators of clear skies; and *B. mixtus* was a significant indicator species for mostly cloudy skies (Tables 6, 7, & 8).

Table 6. Indicator Species and Morphotype P Values for Wind Speed

Visiting Insect Species/Morphotype	***Balsamorhiza deltoidea*** **2011**	**2012**	***Lupinus albicaulis*** **2011**	**2012**
Small Dark Bees	-----	0.3891	0.2747	0.6469
Large Dark Bees	1.0000	0.4293	0.6293	0.6263
Green Metallic Bees	1.0000	0.3415	-----	-----
Honey Bees	1.0000	-----	-----	-----
Cuckoo Bees	-----	0.9196	-----	-----
Bombus mixtus	1.0000	0.8026	**0.0148****	**0.0034***
Bombus vosnesenkii	-----	0.4015	**0.0004****	0.1104
Bombus sitkensis	-----	0.7027	0.6519	**0.0122***
Bombus melanopygus	-----	0.3935	1.0000	0.8006
Bombus flavifrons	-----	0.6707	-----	-----
Bombus californicus	-----	-----	-----	0.2118
Wasps	-----	-----	0.3851	-----
Beetles	-----	0.7898	0.3695	0.2547
Flies	0.1810	0.1814	0.4485	0.6133
Ants	1.0000	-----	-----	-----
No Species	1.0000	**0.03708*****	0.6283	0.2659

Significant results are in bold. * Significant indicator for calm wind conditions **Significant indicator for light breeze ***Significant indicator for windy conditions

Table 7. Indicator Species and Morphotype P Values for Cloud Cover

Visiting Insect Species/Morphotype	***Balsamorhiza deltoidea*** **2011**	**2012**	***Lupinus albicaulis*** **2011**	**2012**
Small Dark Bees	-----	0.4649	0.3873	**0.0014***
Large Dark Bees	0.1676	1.0000	0.5083	**0.0006***
Green Metallic Bees	0.4915	0.3243	-----	-----
Honey Bees	0.4959	-----	-----	-----
Cuckoo Bees	-----	0.2983	-----	-----
Bombus mixtus	1.0000	1.0000	**0.0154***	**0.0252*****
Bombus vosnesenkii	-----	0.3563	**0.0250***	0.0856
Bombus sitkensis	-----	0.6415	0.1252	0.0676
Bombus melanopygus	-----	1.0000	0.2585	1.0000
Bombus flavifrons	-----	0.4937	-----	-----
Bombus californicus	-----	-----	-----	1.0000
Wasps	-----	-----	1.0000	-----
Beetles	-----	0.5631	1.0000	0.2507
Flies	0.1532	1.0000	0.0568	0.4237
Ants	1.0000	-----	-----	-----
No Species	1.0000	0.1658	**0.0070****	0.2943

Significant results are in bold. *Significant indicator of clear skies **Significant indicator of partly cloudy skies *Significant indicator of mostly cloudy skies

Table 8. Indicator Species and Morphotype P Values for Temperature

Visiting Insect Species/Morphotype	***Balsamorhiza deltoidea*** **2011**	**2012**	***Lupinus albicaulis*** **2012**
Small Dark Bees	-----	0.4265	0.9860
Large Dark Bees	0.1716	0.5827	0.2076
Green Metallic Bees	1.0000	0.3003	-----
Honey Bees	1.0000	-----	-----
Cuckoo Bees	-----	0.1356	-----
Bombus mixtus	1.0000	1.0000	**0.0330****
Bombus vosnesenkii	-----	0.2711	0.9362
Bombus sitkensis	-----	0.8544	0.5093
Bombus melanopygus	-----	**0.0428***	0.4191
Bombus flavifrons	-----	0.5811	-----
Bombus californicus	-----	-----	0.7123
Beetles	-----	0.2216	0.9526
Flies	0.8620	0.1198	1.0000
Ants	1.0000	-----	-----
No Species	1.0000	0.3071	0.5105

Significant results are in bold. *Significant indicator of temperatures around 13 ^{0}C
**Significant indicator of temperatures around 16 ^{0}C

Environmental conditions influenced visiting insect community structure. Wind speed and cloud cover significantly influenced visiting insect community structure for *L. albicaulis* in 2011 (Table 9); and temperature, wind speed, and cloud cover significantly influenced community structure of insects visiting *L. albicaulis* in 2012 (Table 9).

Table 9. MRPP Results for Influence of Temperature, Wind Speed, and Cloud Cover on Community Structure of Visiting Insects

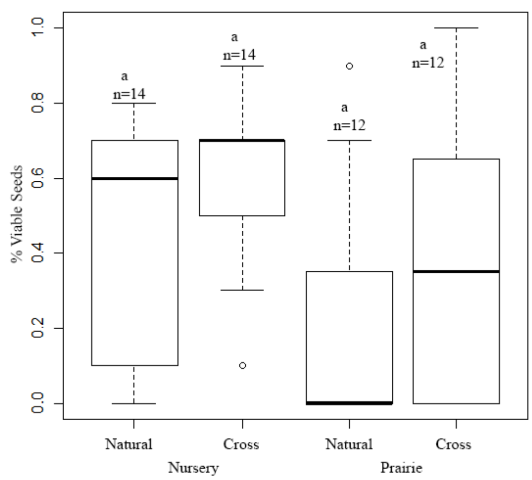
		Temperature		**Wind Speed**		**Cloud Cover**	
Plant Species	**Year**	**A**	**P**	**A**	**P**	**A**	**P**
Balsamorhiza deltoidea	2011	-0.042	0.732	0.016	0.261	-0.038	0.642
Balsamorhiza deltoidea	2012	0.052	0.126	0.012	0.328	0.068	0.352
Lupinus albicaulis	2011	-----	-----*	**0.042**	**0.008**	**0.068**	**0.001**
Lupinus albicaulis	2012	**0.071**	**0.033**	**0.085**	**0.006**	**0.027**	**0.018**

Significant results are in bold. *In 2011, temperatures were all in the same range for all observations taken for *L. albicaulis*.

Pollinator Limitation

Pollinator limitation was not evident for either plant species at either site. No significant difference was found between number or percentage of viable seeds produced by naturally-pollinated inflorescences and hand-cross-pollinated inflorescences for *B. deltoidea* at either site in 2012 (Figure 8).

A.



B.

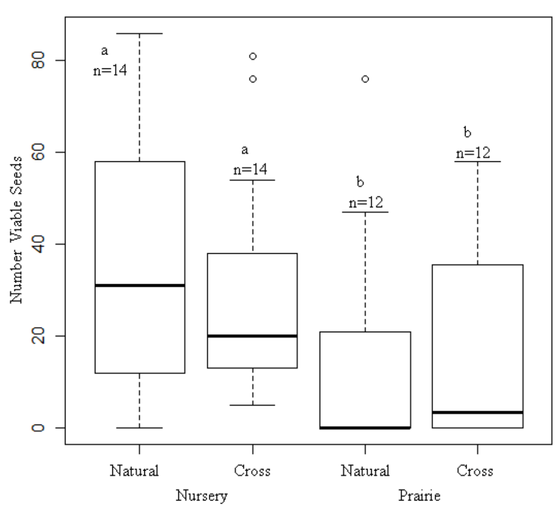


Figure 8. A) Percent and B) number of viable seeds produced by naturally pollinated and hand-cross pollinated *Balsamorhiza deltoidea* inflorescences at Webster nursery and Johnson prairie in 2012.

Although no pollinator limitation was observed for either plant species, some of the *L. albicaulis* treatments did produce different numbers of viable seed. Naturally-pollinated inflorescences produced a significantly greater number of viable seeds than the hand-self-pollinated inflorescences ($W=102.5$, $n_1=n_2=20$, $P=0.0016$) and the unassisted self-pollinated inflorescences ($W=293.5$, $n_1=n_2=20$, $P=0.0025$) (Figure 9). Hand-cross-pollinated inflorescences produced a significantly greater number of viable seeds than the hand-self-pollinated inflorescences ($W=288$, $n_1=n_2=20$, $P=0.0035$) and the unassisted self-pollinated inflorescences ($W=284.5$, $n_1=n_2=20$, $P=0.0051$) (Figure 0). No significant difference was found between numbers of viable seeds produced by naturally-pollinated and control inflorescences (Figure 9).

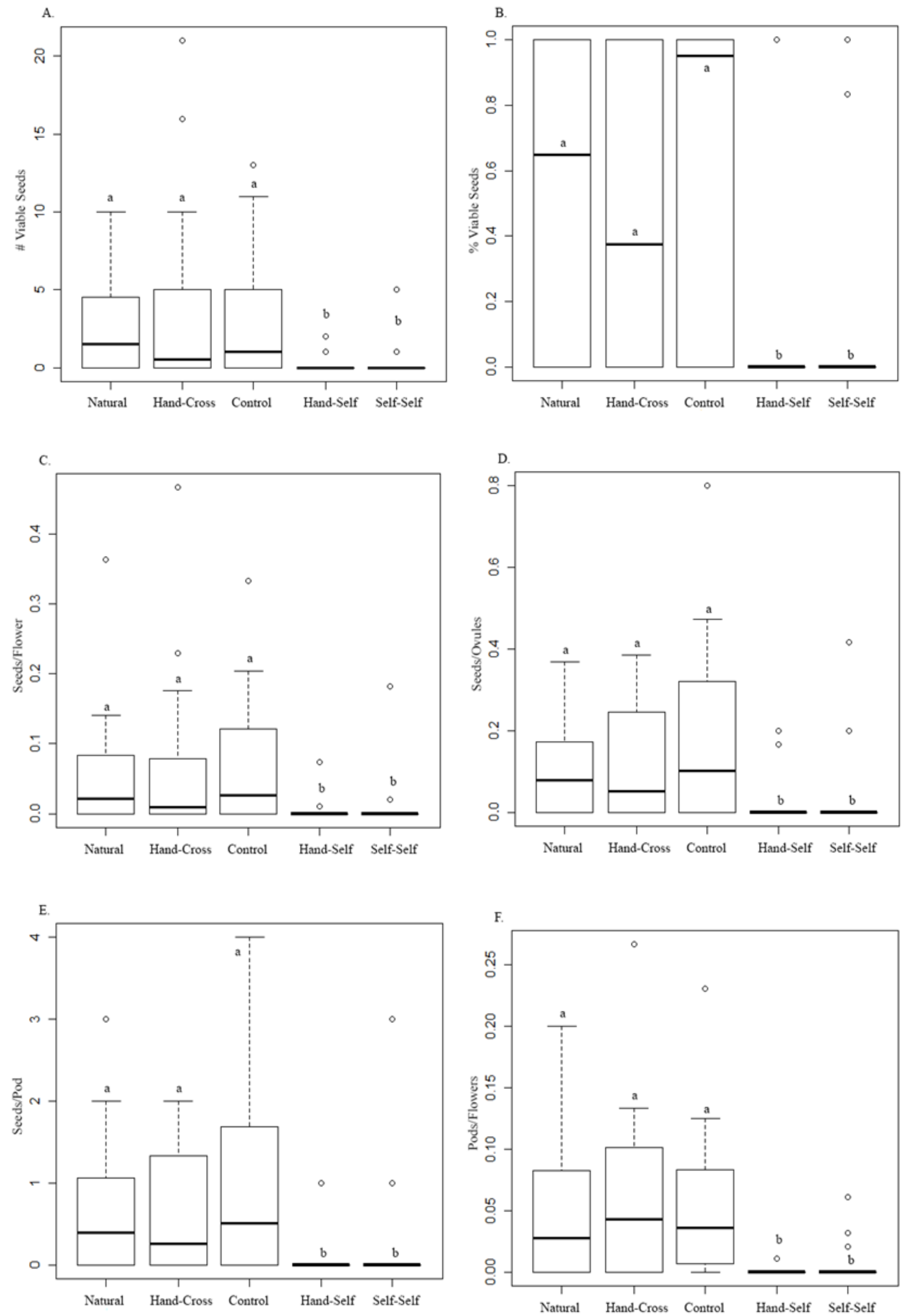


Figure 9. A) Number and B) percentage of viable seeds, C) number of seeds per flower, D) seeds per ovule, E) seeds per pod, and F) pods per flower produced by *Lupinus albicaulis* for each treatment. n_1=n_2=20 for all treatments.

Visitation Rate vs. Viable Seed Production

Visitation rate was not found to significantly influence the number or percentage of viable seeds produced by either plant species (Table 10). Seeds per flower, seeds per ovule, seeds per pod, and pods per flower of *L. albicaulis* were not found to be significantly related to insect visitation rates (Table 10).

Table 10. Results of Linear Regressions Comparing Insect Visitation Rates (# Visits per Inflorescence per Hour) to Various Measures of Reproduction

Plant Species	**Reproductive Measures**	**F**	**d.f.**	**P**
Balsamorhiza deltoidea	% Viable Seeds Produced	1.12	1,25	0.3000
Balsamorhiza deltoidea	# Viable Seeds Produced	1.12	1.25	0.3000
Lupinus albicaulis	% Viable Seeds Produced	1.74	1,18	0.2038
Lupinus albicaulis	# Viable Seeds Produced	0.17	1,18	0.6860
Lupinus albicaulis	Seeds per Flower	0.01	1,18	0.9100
Lupinus albicaulis	Seeds per Ovule	0.02	1,18	0.8959
Lupinus albicaulis	Seeds per Pod	0.00	1,18	0.9445
Lupinus albicaulis	Pods per Flowers	0.59	1,18	0.4515

Balsamorhiza deltoidea plant size was compared to seed production to determine the influence of overall productivity on fecundity. Diameter of the inflorescence was not found to significantly affect the percentage or number of seeds produced by *B. deltoidea* (Table 11) although there was a non-significant positive trend (Figure 10). Plant volume was also not found to significantly affect seed production (Table 11), but results showed a non-significant negative trend (Figure 11).

Table 11. Results of Linear Regressions Comparing Inflorescence Diameter or Plant Volume to Seed Production for *Balsamorhiza deltoidea*

Comparison	**F**	**d.f.**	**P**
Inflorescence Diameter to % Viable Seeds Produced	1.84	1,24	0.1877
Inflorescence Diameter to # Viable Seeds Produced	1.84	1,24	0.1877
Plant Volume (cm^3) to % Viable Seeds Produced	0.42	1,24	0.5253
Plant Volume (cm^3) to # Viable Seeds Produced	0.42	1,24	0.5253

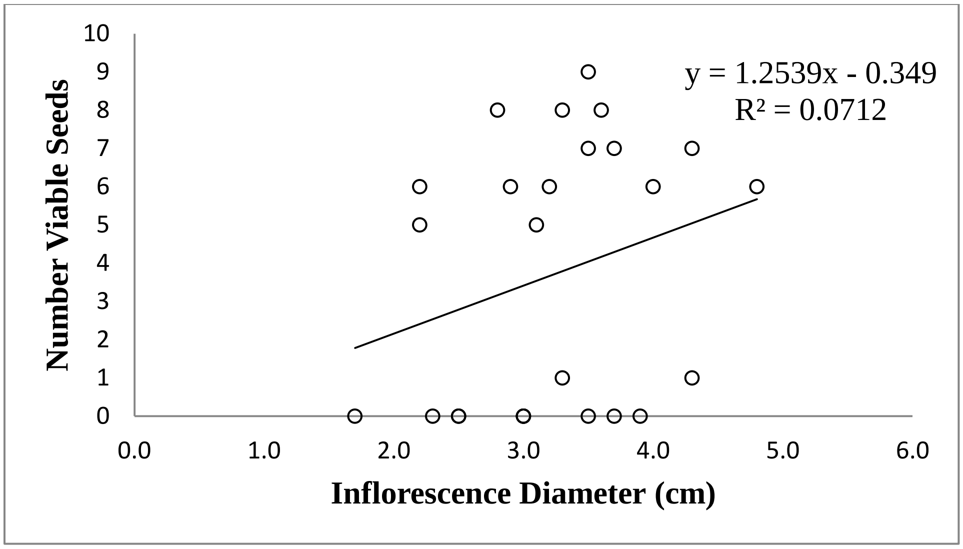


Figure 10. Inflorescence diameter vs. number of viable seeds produced by *Balsamorhiza deltoidea*

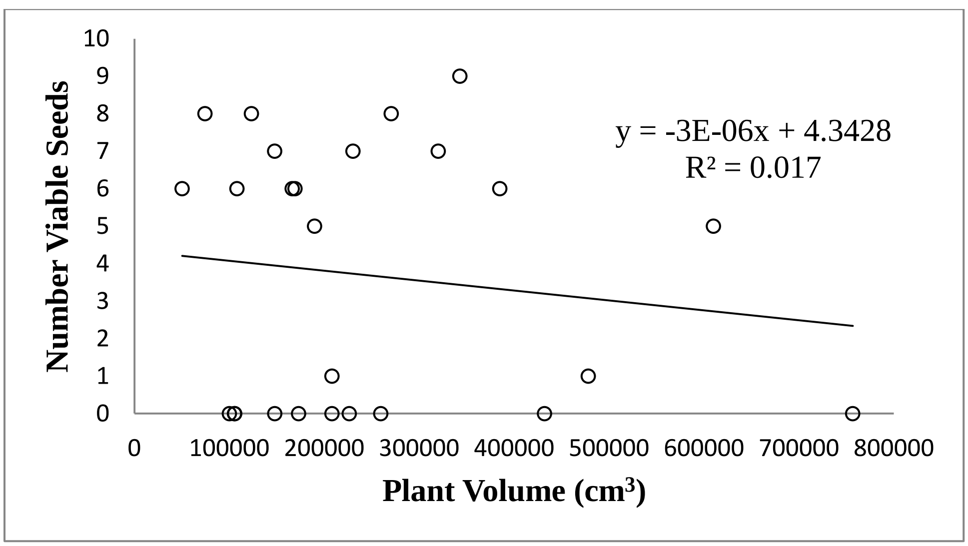


Figure 11. Plant volume vs. number of viable seeds produced by *Balsamorhiza deltoidea*

DISCUSSION

Characteristics of Webster nursery appear to be attracting higher insect visitation than at Johnson prairie. Insect visitation rates at the nursery exceeded rates at the prairie unexpectedly given that the nursery is located in an area with assumed fewer resources for pollinators. Sample size may have been too low to detect a difference in visitation rates between sites for *B. deltoidea* in 2011. Plants for native seed production have been grown at Webster nursery only in the last three years, so there has not been much time for these resources to attract pollinator populations. Matteson et al. (2012) found it inappropriate to generalize about landscapes created by humans as land-use types can vary greatly in suitability for pollinators. Some researchers found that bee abundance increases in human-constructed landscapes developed with a superabundance of floral resources, and that a combination of natural and developed landscapes can provide a greater diversity of habitat resources (Frankie et al. 2009). Also, some bees can rapidly increase offspring production in response to an increase in floral resources because less foraging time means less time they are exposed to predators and parasites (Goodell 2003). I recommend considering characteristics at Webster that may be attracting more insects, and then investigating ways to enhance these at Johnson. Since some insect group visitation rates differed between the sites, this creates an opportunity to design restoration strategies geared toward specific insect types to increase visitation at Johnson prairie. For example, nesting habitat and floral resources that attract *B. mixtus* could be enhanced at Johnson to encourage more activity from this particular species that is known to visit *L. albicaulis* frequently, given the evidence from Webster nursery.

Environmental conditions can influence visiting insect community composition at both nursery and prairie sites. Insect types can have different levels of effectiveness at pollinating flowers so a change in the visiting insect community can affect plant reproduction differently. Visiting insect community composition, proportion of visits made by each insect morphotype, and insect morphotype richness, diversity, and evenness varied between years. Certain insect morphotypes preferred certain weather conditions. Weather conditions during sampling times may have affected the visitation rate results and community composition data for comparisons between sites for *L. albicaulis*. There was no evidence that temperature, wind speed, or cloud cover influenced observations for *B. deltoidea* as they were the same during observation times at Webster nursery and Johnson prairie, but other factors such as time of year or weather conditions earlier in the year may have been influential. Sampling dates and bloom times were several weeks earlier in 2012 than 2011, and this could have affected the insect community present between years.

The variation in visiting insect community composition seen in this study is not surprising given that other researchers have found weather conditions and seasonal fluctuations to be significant factors influencing community composition and insect visitation rates. Different insect species have been found to have different preferential weather conditions for foraging (Arroyo et al. 1982). Temperature and cloud cover have been found to influence insect visitation rates more than humidity, wind speed, season, and time of day in another study, although all factors had some influence depending on the study site (McCall & Primack 1992). Lower temperatures have been found to coincide with lower levels of insect activity in general (Arroyo et al. 1985). Weather conditions and bloom times can vary from year to year and site to site, so visiting insect communities and rates of visitation can vary as well. Both study years occurred during La Niña weather conditions characterized by lower temperatures and more cloud cover than most years (National Weather Service 2012). My results highlight that long term data collection is needed to make more accurate generalizations of visitation to a site.

Increasing insect visitation at Webster nursery may not be a conservation priority given a lack of evidence for pollinator limitation for either study plant species at either site. In addition, no evidence was found that supplemental pollen increases viable seed production for the plants in this study. Fazzino et al. (2011) found that hand-pollinated inflorescences produced more sprouting seeds than naturally-pollinated inflorescences for *B. deltoidea* in the Puget lowland prairies. In contrast, the *B. deltoidea* plants in this study were either not pollinator-limited or the hand-pollinated inflorescences did not receive enough supplemental pollen by hand to show a difference. Increasing the number of replicates in a repeated study may yield different results for both plant species. Although cross-pollination is necessary for maintaining genetic diversity, autogamous plants may still produce viable seeds in the absence of pollinators (Arathi et al. 2002). *Lupinus albicaulis* did not show evidence for autogamy, although the self-pollinated inflorescences may have produced fewer viable seeds than the cross-pollinated inflorescences if the pollinator exclusion bags covering them caused a treatment effect.

In this experiment, I assumed more pollen would increase viable seed production. Ashman et al. (2004) state that when maximum seed production is reached there are no longer unfertilized ovules for additional pollen to be of benefit. Cane & Schiffhauer (2003) discovered a point of pollen saturation on stigmas. Supplemental pollen negatively affected seed weight in Hegland &Totland's (2008) study on pollinator limitation at the community level. I did not find evidence that insect visitation influenced viable seed production for the study plants; however, visitation rate is only one of many factors that may influence the number of viable seeds a plant produces. Availability of resources such as soil nutrients, water, and light can also affect plant reproduction (Stephenson 1981;

Corbet 1998; Bos et al. 2007), and seed handling and storage practices can affect seed viability. In addition, changes in light and temperature during germination can affect *L. albicaulis* seed viability (Morey & Bakker 2011). I recommend that land managers turn efforts towards investigating the influence of the above factors on native seed production in future studies.

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LITERATURE CITED

Arathi HS, Rasch A, Cox C, Kelly JK (2002) Autogamy and floral longevity in *Mimulus guttatus*. International Journal of Plant Sciences 163:567-573.

Arroyo MTK, Armesto JJ, Primack RB (1985) Community studies in pollination ecology in the high temperate Andes of central Chile II. Effect of temperature on visitation rates and pollination possibilities. Plant Systematics and Evolution 149:187-203.

Arroyo MT, Primack R, Armesto J (1982) Community studies in pollination ecology in the high temperate Andies of central Chile I. Pollination Mechanisms and Altitudinal Variation. American Journal of Botany 69:82-97.

Ashman T, Knight TM, Steets JA, Amarasekare P, Burd M, Campbell DR, Dudash MR, Johnston MO, Mazer SJ, Mitchell RJ, Morgan MT, Wilson WG (2004) Pollen limitation of plant reproduction: Ecological and evolutionary causes and consequences. Ecology 85:2408-2421.

Berry PE, Calvo RN (1989) Wind pollination, self-incompatibility, and altitudinal shifts in pollination systems in the high Andean genus *Espeletia* (Asteraceae). American Journal of Botany 76:1602-1614.

Bos MM, Veddeler D, Bogdanski AK, Klein A, Tscharntke T, Steffan-Dewenter I, Tylainakis JM (2007) Caveats to quantifying ecosystem services: Fruit abortion blurs benefits from crop pollination. Ecological Applications 17:1841-1849.

Boyd AE (2004) Breeding system of *Macromeria viridiflora* (Boraginaceae) and geographic variation in pollinator assemblages. American Journal of Botany 91:1809-1813.

Cane JH, Schiffhauer D (2003) Dose-response relationships between pollination and fruiting refine pollinator comparisons for cranberry (*Vaccinium macrocarpon* [Ericaceae]). American Journal of Botany 90:1425-1432.

Corbet SA (1998) Fruit and seed production in relation to pollination and resources in Bluebell, *Hyacinthoides non-scripta*. Oecologia 114:349-360.

Dieringer G (1992) Pollinator limitation in populations of *Agalinis strictifolia* (Scrophulariaceae). Bulletin of the Torrey Botanical Club 119:131-136.

Elliott CW, Fischer DG, LeRoy CJ (2011) Germination of three native *Lupinus* species in response to temperature. Northwest Science 85:403-410.

Engel EC, Irwin RE (2003) Linking pollinator visitation rate and pollen receipt. American Journal of Botany 90:1612-1618.

Fazzino L, Kirkpatrick HE, Fimbel C (2011) Comparison of hand-pollinated and naturally-pollinated Puget balsamroot (*Balsamorhiza deltoidea* Nutt.) to determine pollinator limitations on South Puget Sound lowland prairies. Northwest Science 85: 352-360.

Fimbel C (2010) Center for Natural Lands Management. Personal Communication.

Fontaine C, Dajoz I, Meriguet J, Loreau M (2006) Functional diversity of plant-pollinator interaction webs enhances the persistence of plant communities. PLoS Biology 4:0129-0135. [online] URL: www.plosbiology.org (accessed June 2011).

Frankie GW, Thorp RW, Hernandez J, Rizzardi M, Ertter B, Pawelek JC, Witt SL, Schindler M, Coville R, Wojcik VA (2009) Native bees are a rich natural resource in urban California gardens. University of California. California Agriculture [online] URL: http://californiaagriculture.ucanr.edu (accessed Oct 2012).

Goodell K (2003) Food availability affects *Osmia pumila* (Hymenoptera: Megachilidae) foraging, reproduction, and brood parasitism. Oecologia 134:518-527.

Hegland SJ, Totland O (2008) Is the magnitude of pollen limitation in a plant community affected by pollinator visitation and plant species specialization levels? Oikos 117:883-891.

Inouye DW, Pyke GH (1988) Pollination in the snowy mountains of Australia: Comparisons with montane Colorado, USA. Australian Journal of Ecology 13:191-210.

International Seed Testing Association (2012) International Rules for Seed Testing (2012ed.). Bassersdorf: Switzerland.

Kearns CA, Inouye DW (1993) Techniques for Pollination Biologists. University Press of Colorado, Niwot.

Kremen C, Williams NM, Aizen MA, Gemmill-Herren B, LeBuhn G, Minckley R, Packer L, Potts SG, Roulston T, Steffan-Dewenter I, Vazquez DP, Winfree R, Adams L, Crone EE, Greenleaf SS, Keitt TH, Klein A, Regetz J, Ricketts TH (2007) Pollination and other ecosystem services produced by mobile organisms: A conceptual framework for effects of land-use change. Ecology Letters 10:299-314.

Matteson KC, Grace JB, Minor ES (2012) Direct and indirect effects of land use on floral resources and flower-visiting insects across an urban landscape. Oikos doi: 10.1111/j.1600-0706.2012.20229.x [online] URL: onlinelibrary.wiley.com (accessed Nov. 2012).

Mayer C, Adler L, Armbruster S, Dafni A, Eardley C, Huang S, Kevan PG, Ollerton J, Packer L, Ssymank A, Stout JC, Potts SG (2011) Pollination ecology in the 21st century: Key questions for future research. Journal of Pollination Ecology 3:8-23.

McCall C, Primack RB (1992) Influence of flower characteristics, weather, time of day, and season on insect visitation rates in three plant communities. American Journal of Botany 79:434-442.

McCarty JP (2001) Ecological consequences of recent climate change. Conservation Biology 15:320-331.

Morey M and Bakker J (2011) Effects of light and temperature regimes on germination and viability of native WA prairie species. Undergraduate senior project. School of Forest Resources, University of Washington, Seattle, WA.

National Weather Service (2012) Climate Prediction Center. National Oceanic and Atmospheric Administration [online] URL: www.nws.noaa.gov (accessed Oct 2012).

USDA Natural Resources Conservation Service (2012) Plants Database. [online] URL: www.plants.usda.gov (accessed Oct 2012).

Perfectti F, Gomez JM, Bosch J (2009) The functional consequences of diversity in plant-pollinator interactions. Oikos 118:1430-1440.

Price MV, Campbell DR, Waser NM, Brody AK (2008) Bridging the generation gap in plants: Pollination, parental fecundity, and offspring demography. Ecology 89:1596-1604.

South Sound Prairies Working Group (2011) Historic prairie landscape. South Sound Prairies [online] URL: http://www.southsoundprairies.org/historic-prairie-landscapes/ (accessed Feb 2012).

Stanley AG, Kaye TN, Dunwiddie PW (2008) Regional strategies for restoring invaded prairies: Observations from a multisite, collaborative research project. Native Plants Journal 9:247-254.

Stephenson AG (1981) Flower and fruit abortion: Proximate causes and ultimate functions. Annual Review of Ecology and Systematics 12:253-279.

Stinson DW (2005) Washington State Department of Fish and Wildlife. Washington State status report for the Mazama pocket gopher, streaked horned lark, and Taylor's checker spot. Olympia.

Tscheulin T, Petanidou T (2011) Does spatial population structure affect seed set in pollen-limited *Thymus capitatus*? Apidologie 42:67-77.

Vila M, Weiner J (2004) Are invasive plant species better competitors than native plant species? – Evidence from pair-wise experiments. Oikos 105:229-238.

Washington Natural Heritage Program (2012) Washington Department of Natural Resources [online] URL: http://www1.dnr.wa.gov/nhp/refdesk/lists/plantrnk.html (accessed August 2012).

Wilson MV, Hammond PC, Schultz CB (1997) The interdependence of native plants and Fender's blue butterfly. In: Kaye TN, Liston A, Love RM, Luomo D, Meinke RJ, Wilson MV (eds) Conservation management of native flora and fungi. Native Plant Society of Oregon, Corvallis, pp 83-87.