Pollinators may not limit native seed set at Puget lowland prairie restoration nurseries

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Abstract—Land managers often rely on large-scale production of native seeds in nurseries for replanting into natural environments as part of restoration strategies. Nursery managers question if unmanaged insects can be sufficient to pollinate large increases in native forbs planted into young nurseries in non-native landscapes. This study investigated pollination of deltoid balsamroot (*Balsamorhiza deltoidea* Nutt.) and sicklekeel lupine (*Lupinus albicaulis* Douglas) at a native seed nursery compared to dense patches of native plants at a natural Puget lowland prairie to determine if insect visitation affected viable seed production for those two species. In 2011 and 2012, insect visitation rates were recorded for each plant species at more than 62 plots within two study areas. 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. However, pollen limitation was not evident for either plant species at either site. Natural pollination by insects and supplemental hand-cross-pollination treatments did not yield different quantities of viable seed. Factors other than pollinator visitation, such as soil nutrients and seed handling practices, may be influencing seed viability, but increasing insect visitation will not likely significantly increase seed viability for these two species at this restoration nursery. Planting dense rows of native plant species may be enough to attract a sufficient amount of unmanaged insects to provide adequate pollination for seed production for some species at even young nurseries.

Keywords: restoration nursery, pollen limitation, insect visitation, prairie restoration, *Balsamorhiza deltoidea*, *Lupinus albicaulis*

Introduction

Pollinators play a key role in the reproduction of wild plants as they are linked to viable seed production and native plant population growth. Pollinators and their activities thus provide an ecosystem-wide service, especially for landscapes with many insect-dependent forb species like prairies (Kremen et al. 2007). Even self-compatible plant species may rely on pollinators to provide conspecific pollen to mix genes, preventing inbreeding depression (Heschel & Page 1995; Price et al. 2008). 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 reproductive 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 meet restoration demands.

When plants produce fewer viable seeds than maternal resources allow because of insufficient quantity or quality of pollen, they are pollen-limited (Wagenius & Lyon 2010).

Several aspects of pollination can influence the production of viable seeds. Insect visitation rates can positively affect pollen receipt (Engel & Irwin 2003). Differences in pollinator community structure can affect overall pollination effectiveness (Perfectti et al. 2009) and pollinator behaviour, such as order in which a pollinator visits flowers, can affect whether a flower is self-pollinated or cross-pollinated (Kunin 1993). When plants are pollen-limited due to insufficient pollinator activity, they are pollinator-limited (Dieringer 1992).

The Puget lowland prairie ecosystem has been fragmented by coniferous forest encroachment and urban and agricultural development so that now only 2-3% of the original habitat remains (Dunwiddie & Bakker 2011). Re-establishing native flora has been a priority of Puget lowland prairie conservation partners (Stanley et al. 2008), and these partners rely on large-scale production of native seeds in their nurseries for replanting into the Puget lowland prairies as part of their restoration strategies. Some years restoration practitioners have struggled to produce large quantities of viable seeds for certain plant species at their largest nursery, Webster Nursery. These managers have questioned whether or not two critical restoration species, *Balsamorhiza deltoidea* Nutt. (Asteraceae) (deltoid balsamroot) and *Lupinus albicaulis* Douglas (Fabaceae) (sicklekeel lupine), are producing the highest proportion of viable seeds possible at this restoration nursery and if there is a way to increase the proportion. Results of testing by the nursery in 2013

revealed that Webster Nursery produced 1,424 g of *B. deltoidea* seed with 38.8% viability, and 10,299 g of *L. albicaulis* seed with 35.3% viability (S. Smith, Center for Natural Lands Management, pers. comm.). The cause of this problem may be due to seed handling or storage techniques, inadequate environmental conditions where the plants are grown (such as soil nutrients, weather, etc.), seed predation, or pollen limitation. This study addressed the latter by investigating the status of pollination at Webster Nursery and comparing it to dense stands of native plants at a natural Puget lowland prairie to determine if inadequate pollination restricted viable seed production at the nursery.

More specifically, to better understand the role of pollination at a native seed production facility, we investigated the following research questions: (1) Do insect visitation rates to dense floral patches differ between a nursery site and a natural prairie site for two prairie forbs? (2) Is there evidence of pollen limitation for either plant species at either the nursery or prairie sites?

MATERIALS AND METHODS

Study Plant Species

We focused this study on two native prairie plants, *B. deltoidea* and *L. albicaulis*. Both plant species grow along the west coast of the United States and into Canada (USDA Natural Resources Conservation Service 2012). These plants are both found at natural prairie sites and are being produced from seed at Webster Nursery, Tumwater, WA, USA.

Deltoid balsamroot (*B. deltoidea*) is a species of potential concern in Washington State (Washington Natural Heritage Program 2012), its flowers are popular with insect visitors, and it is a valued restoration plant. The federally endangered butterfly, Taylor's checkerspot (*Euphydryas editha taylori*), frequently uses this plant as a nectar resource. *Balsamorhiza deltoidea* 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. Fazzino et al. (2011) documented that this plant species is self-incompatible and does not reallocate resources among flower heads.

Sickle-keel lupine (*L. albicaulis*) provides food for caterpillars and adults of 'blue' butterflies, such as the Puget blue (*Plebejus icarioides blackmorei*), a species of concern in Washington State, and occasionally the federally endangered Fender's blue butterfly (*Icaricia icarioides fenderi* Macy) (Wilson et al. 1997). This plant is a popular floral resource for several species of native bees and provides vertical vegetative structure on the low stature Puget prairies. *Lupinus albicaulis* 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 simple carpel with several ovules (Hitchcock & Cronquist 1998). An average of five ovules was presumed to be in each carpel of the flowers in this study, which was calculated by counting the number of cells in the collected

pods, including those that were empty (likely due to ovule abortion). The *L. albicaulis* inflorescences tested in this study had an average of 48.1 flowers per inflorescence. Little is known about the pollination system of *L. albicaulis*. *Lupinus* species in general are typically self-compatible, though some require a pollinator to trigger autogamy and have increased seed set when cross-pollinated (Kaye 1999, Kittelson & Maron 2000).

Study Areas

Washington Department of Natural Resources (DNR) owns Webster Nursery, a portion of which is leased and managed by conservation partners to produce seed from native plants at a large scale for restoring Puget lowland prairies. The plants are grown outdoors in dense rows. The native seed nursery was first established in 2008 with partial rows (~100 m) of 10 species. The rows planted with *B. deltoidea* and *L. albicaulis* were last fertilized during their installation in 2008, are watered only by rain, and were not sprayed with pesticides or herbicides in 2011 or 2012 (Angela Winter, nursery manager, pers. comm. 2012). Other species grown at Webster Nursery include: hookedspur violet (*Viola adunca*), spring gold (*Lomatium utriculatum*), nine-leaf biscuitroot (*Lomatium triternatum*), shortspur seablush (*Plectritis congesta*), slender cinquefoil (*Potentilla gracilis*), harsh Indian paintbrush (*Castilleja hispida*), golden paintbrush (*Castilleja levisecta*), sea pink (*Armeria maritima*), Nuttall's Larkspur (*Delphinium nuttallii*), woolley sunflower (*Eriophyllum lanatum*), buttercup (*Ranunculus occidentalis*), Pacific lupine (*Lupinus lepidus*), bicolor lupine (*Lupinus bicolor*), farewell to spring (*Clarkia amoena*), and camas (*Camassia quamash*). Farmland, open grassland, residential properties, and forested areas surround the 5.3 km² nursery (Tab. 1, Fig. 1).

The US Department of Defense manages Johnson Prairie, a natural prairie site on Joint Base Lewis-McChord. Johnson is one of the few remaining Puget lowland prairies dominated by semi-native vegetation, and is located near Rainier, WA. *Camassia quamash* is a dominant flowering species and the site includes similar plant species as grown at Webster Nursery in clumped patches throughout the fescue-dominated grassland. This prairie is subject to some recreational activity, though less military training activity than other prairie sites located on the base (Stinson 2005). A portion of this site, including the study area, was burned in August, 2011 for restoration purposes. Coniferous forest and open non-native grasslands border this 7.5 km² prairie site (Tab. 1, Fig. 1).

TABLE 1.   Percent land-use in a 1 km buffer around the study areas in Thurston County, WA.

Land-use	Webster Nursery	Johnson Prairie
Open Grassland	7.0%	7.2%
Forestland	55.4%	92.8%
Agriculture	14.1%	-----
Residential	23.5%	-----

(A) Webster Nursery

(B) Johnson Prairie

Legend:
- Study Area
- Webster Nursery
- Johnson Prairie
- Agriculture
- Residential
- Forestland
- Open Grassland
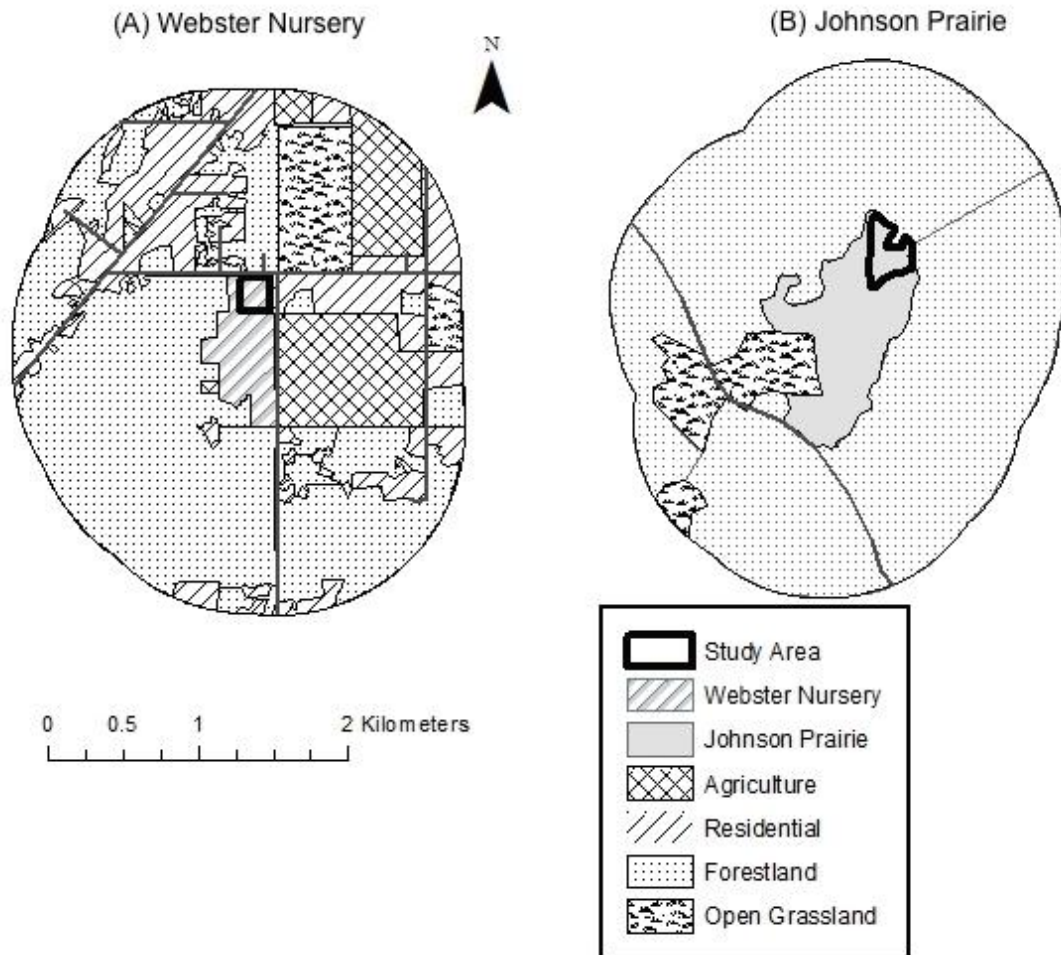
Scale: 0   0.5   1   2 Kilometers

FIGURE I.   Land-use in 1km buffer around: A) Webster Nursery (46.951817 latitude, -122.962126 longitude) and B) Johnson Prairie (46.927746 latitude, -122.732272 longitude). Spatial Reference: NAD27 UTM Zone 10N. Digitized using ESRI® ArcGIS® 10.2 World Imagery basemap Source: ESRI, DigitalGlobe, GeoEye, i-cubed, Earthstar, Geographics, CNES/Airbus DS, USDA USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community, 2014.

Visitation Rates

The methods used for this study were adapted from Arroyo et al. (1982) who recorded the number of visits to a known 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). For this study, plots were selected to collect visitation rate data for both study plant species in 2011 and 2012. At Webster nursery, the target plant species were planted in dense rows. Plot locations were selected at Webster Nursery by breaking each row of the target plant species into two-meter segments. Rows were 1.5 m deep. This plot size was chosen, as it was the largest area that could be observed by one person without missing visits and entirely encompassed most patches of the target species at Johnson prairie. Plots were then randomly selected. If a plot selected was directly adjacent to a plot previously selected, it was thrown-out and a new random number was generated to ensure at least two-meters of distance between plots.

At Johnson prairie, both plant species have a clumped distribution, spread across the site in patches. To reduce the chance of potential confounding factors differentially contributing to visitation and seed set rates, only patches of plants with similar densities to the planted rows at Webster nursery were selected at Johnson prairie for plot locations. The selected patches contained few other flowering species to reduce the chance of small scale floral competition or facilitation happening at the Johnson prairie plots and not at the monoculture plots at Webster nursery. A 7,937 m² macroplot was chosen at Johnson prairie in the Northeast corner where the majority of patches of the target species were. Patches of plants with similar floral densities as found at the nursery were identified within the macroplot, and plot locations were selected randomly from those patches. All plots selected were spaced at least two-meters apart.

At both sites, 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 (2 m × 1.5 m). Plot densities varied from 0.3-9.3 inflorescences per square meter for *B. deltoidea*, and from 4.6-82.0 inflorescences per square meter for *L. albicaulis*, but

variation in plot density was similar between sites (Appendix 1 and 2). In 2011, six plots were selected for *B. deltoidea* and 16 plots for *L. albicaulis* at each location and observed once. In 2012, 30 plots were selected at each site for *B. deltoidea*, and each observed once (*N* = 30). Ten plots were selected at each site and each sampled three times for *L. albicaulis* in 2012 (*N* = 10). After sampling *B. deltoidea,* we noted that visitation rates varied more than anticipated during the bloom period, likely due to different insect species being more active at certain times of the year. On average, visits on the first day of observations were 2.68 visits per flower per hour higher at Webster nursery and 0.63 visits per flower per hour higher at Johnson prairie, than on the last day of observation. Although *B. deltoidea* plots were sampled relatively evenly throughout the bloom period, the experimental design was changed for *L. albicaulis* in 2012 based on a recent study (Tscheulin & Petanidou 2011), to reduce the influence of variation in visitation rates. Instead of recording each plot only once, we thus recorded visits to plots for three rounds of timed intervals and calculated a mean number of visits per flower per hour. In each of the three rounds, the order in which the ten plots were observed was randomized.

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 per plot, and 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 °C, clear to cloudy skies with shadows present, and still air to light breeze). We assumed all flowers in bloom were receptive to pollen.

The number of visits made by insects was recorded during each 10 min period. A visit was recorded only if the insect landed on the reproductive parts of a flower. 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. All visiting insects were identified in flight. Because identification could not accurately be made to a species level and no local guide for pollinator identification for this area was available, the observed 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), syrphids (Syrphidae), ants (Formicidae), wasps (Hymenoptera: Apocrita), and beetles (Coleoptera) based on Donovall & vanEngelsdorp (2008). It could not be determined whether visiting honeybees came from feral populations or nearby managed hives. Because cuckoo bees, flies, ants, wasps, and beetles were either absent or rare, and potentially did not facilitate pollination, these groups were removed from the statistical analysis. If no insects from a particular morphotype visited, a value of zero was recorded and the trial was not discounted.

Permutative two-way ANOVAs (Manly 2007) were used to compare mean visitation rates at the nursery to the prairie for each plant species in each year, and to detect if there was a year by site interaction. Two tests compared overall visitation rates (one per plant species). For *B. deltoidea* we ran an additional six permutative ANOVAs for the insect morphotypes to determine if there were effects of location, year or their interaction on individual morphotypes. For *L. albicaulis* we ran an additional three permutative ANOVAs for selected insect morphotypes. All analyses were conducted using Resampling Stats for Excel 2007. Alphas were adjusted using Bonferroni corrections to address multiple comparisons. We used an alpha = 0.003 for the two overall visitation rate tests, an alpha = 0.008 for six separate individual insect visitation rate tests for visitors of *B. deltoidea,* and an alpha = 0.017 for three separate individual insect visitation rate tests for visitors of *L. albicaulis.*

Pollen Limitation

Procedures for the pollen 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 pollen limitation on Puget prairies. 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 pollinator limitation experiment. Two similar inflorescences were chosen within each plot and marked with thread before the styles had matured. One inflorescence was left to be naturally-pollinated, and the second inflorescence was hand-pollinated as well as naturally-pollinated.

Hand-pollination treatments were applied every other day to all flowers of the selected inflorescences until the stigmas shriveled, then all inflorescences in both treatments were covered with a coarse mesh bag to prevent seed predation. When the fruits matured, the inflorescences were collected, and the seeds were extracted and counted.

A tetrazolium assay was used to test the seeds for viability using procedures adapted from the International Seed Testing Association (2012). Ten plump seeds were randomly selected from each inflorescence for *B. deltoidea*, and all seeds from each of 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 adjusted to 6.8. All seed coats were pierced before soaking the seeds in the tetrazolium solution. After four hours, the embryos were examined for the red staining that indicates viability.

A permutative two-way ANOVA was also used to compare the percentage of viable seeds produced by the inflorescences of each treatment group for each plant species, and to detect if there was a treatment by site interaction. To determine if there was pollen limitation for either plant species at Webster Nursery or Johnson Prairie, we compared
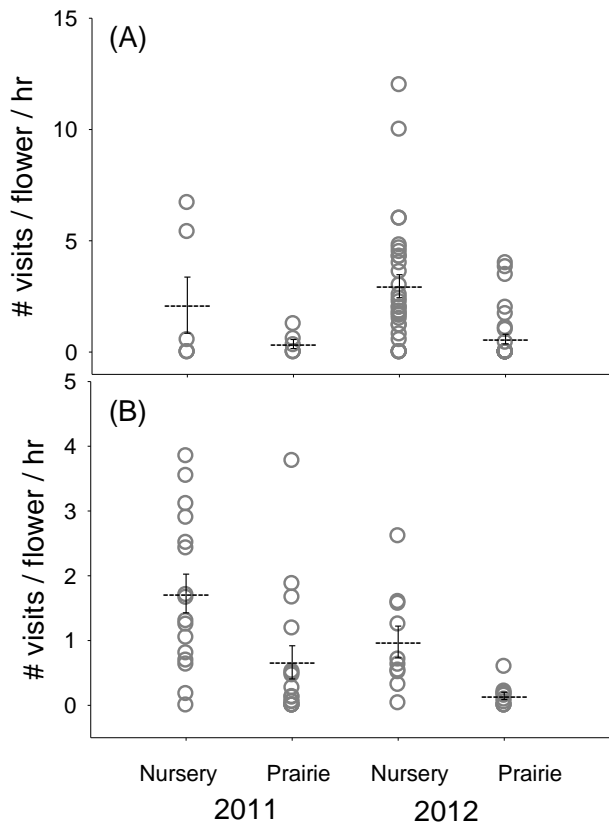
FIGURE 2. Overall insect visitation rates (# visits / plant / hr) at Webster Nursery and Johnson Prairie for: A) *Balsamorhiza deltoidea*, and B) *Lupinus albicaulis* in 2011 and 2012. Open gray circles represent each visitation rate and black horizontal lines represent means +/- 1 standard error. Bonferroni correction for two tests would necessitate an alpha = 0.025.

the percentages of viable seed produced by the hand-pollinated inflorescences to the naturally pollinated inflorescences. All analyses were conducted using Resampling Stats for Excel 2007. Alphas were adjusted using Bonferroni corrections for two comparisons (alpha = 0.025).

RESULTS

Insect visitation rates differed between Webster Nursery and Johnson Prairie, both overall and for many of the insect groups. Overall visitation rates were significantly higher at Webster Nursery than at Johnson Prairie for both *B. deltoidea* (Fig. 2; SSsite: 92.77, $P < 0.001$; SSyear: 7.46, $P = 0.496$; SSsite*year: 2.58, $P = 0.691$) (Appendix I) and *L. albicaulis* (SSsite: 12.32, $P < 0.001$; SSyear; 5.49, $P = 0.039$; SSsite*year: 0.18, $P = 0.715$) (Appendix II).

Webster Nursery also had significantly higher visitation rates than Johnson Prairie for several insect morphotypes visiting each of the plant species, specifically bumblebees and small dark bees (Tab. 2, Appendix I & II). In 2012, there were significantly higher rates of bumblebee visits at both sites than in 2011. In addition, there were higher visitation rates for small dark bees and bumblebees to *L. albicaulis* at Webster Nursery.

Pollen limitation was not evident for either plant species at either site. No significant difference was found between percentage of viable seeds produced by naturally-pollinated inflorescences or hand-cross-pollinated inflorescences for *B. deltoidea* (SSpollen: 3232.69, $P = 0.100$; SSsite: 8566.90, $P = 0.005$; SSpollen*site: 74.13, $P = 0.8021$) (Appendix III) or *L. albicaulis* (SSpollen: 2.35, $P = 0.985$; SSsite: 51212.67, $P < 0.001$; SSpollen*site: 286.09, $P = 0.724$) (Fig. 3, Appendix IV). For both species, the trend in seed viability was significantly higher for seeds from the Nursery site (Fig. 3).

TABLE 2. Results of permutative two-way ANOVAs (SS and *P*-values) comparing insect visitation rates at Webster nursery and Johnson prairie for both *B. deltoidea* and *L. albicaulis* in 2011 and 2012. Significant results are in bold. All significant site effect results indicate higher visitation rates at Webster Nursery than at Johnson Prairie. Bonferroni corrections for six tests (*B. deltoidea*) would necessitate an alpha = 0.008, and for three tests (*L. albicaulis*) would necessitate an alpha = 0.017.

Insect Morphotype	Source	SS	P
B. deltoidea			
Small Dark Bees	Site	1.76	0.010[1]
	Year	1.36	0.206
	Site × Year	0.92	0.284
Large Dark Bees	Site	0.17	0.499
	Year	<0.01	1.000
	Site × Year	<0.01	1.000
Green Metallic Bees	Site	0.60	0.235
	Year	4.83	0.165
	Site × Year	4.83	0.141
Honey Bees	Site	0.62	1.000
	Year	8.12	0.170
	Site × Year	8.12	0.170
Bumblebees	**Site**	**43.00**	**0.002**
	Year	51.96	0.041[1]
	Site × Year	25.51	0.172
Syrphids	Site	0.05	0.117
	Year	**0.39**	**0.006**
	Site × Year	**0.39**	**0.002**
L. albicaulis			
Small Dark Bees	**Site**	**0.01**	**0.013**
	Year	<0.01	0.501
	Site × Year	<0.01	0.698
Large Dark Bees	Site	<0.01	0.757
	Year	0.03	0.136
	Site × Year	<0.01	0.601
Bumblebees	**Site**	**13.09**	**0.001**
	Year	6.50	0.024[2]
	Site × Year	0.12	0.780

[1]No longer significant at Bonferroni-corrected alpha = 0.008
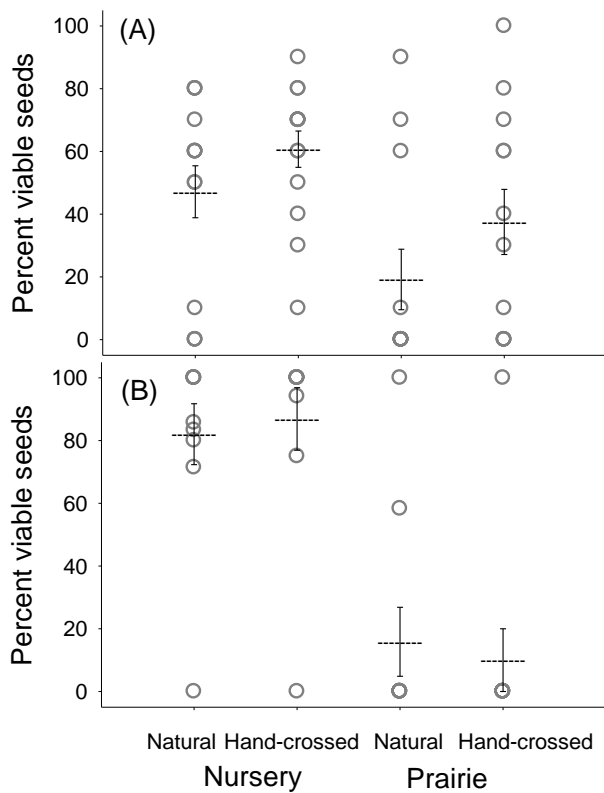[2]No longer significant at Bonferroni-corrected alpha = 0.017

FIGURE 3. Percentage of viable seeds produced by hand-cross pollination and natural pollination at either Webster Nursery or Johnson Prairie: A) *Balsamorhiza deltoidea* inflorescences, and B) *Lupinus albicaulis* inflorescences. Open gray circles represent percent viability and black horizontal lines represent means +/- 1 standard error. Bonferroni correction for two tests would necessitate an alpha = 0.025.

DISCUSSION

Characteristics of Webster Nursery appear to be facilitating higher rates of insect visitation to these two plant species than at the natural prairie site, Johnson Prairie. The nursery is located in an area with fewer assumed floral resources for native pollinators, though insects appear to be responding to the large influx of floral resources at the relatively new nursery that started in 2008. Although visitation was higher at the nursery site, we found no evidence of pollen limitation and no differences in viability for seeds from either location. Restoration managers at the nursery site were hoping to determine methods for increasing native plant yield and this study suggests that neither increased pollinator populations nor hand pollination would increase seed viability for these two species.

Several studies suggest that human developed landscapes are not necessarily pollinator deprived. Matteson et al. (2012) found it inappropriate to generalize about landscapes created by humans due to high variability in habitat suitability for pollinators within land-use categories. 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). Some insects, such as

larger bumblebees, have relatively large foraging areas (Greenleaf et al. 2007). Hagen et al. (2011) found the foraging area for some *Bombus sp.* to be between 0.25-43.53 hectares in one to four days. It is most probable that the high densities of flowers and the many blooming species found in a small area are attracting pollinators to this native plant nursery. Some bees can rapidly produce more offspring in response to an increase in floral resources because greater foraging efficiency means less time they are exposed to predators and parasites (Goodell 2003). For some plant species, rapid accumulation of dense native plant resources at a native plant nursery may attract sufficient unmanaged insects to provide pollination services similar or higher than those found in native landscapes. Restoration nurseries are human-altered landscapes with surpluses of native floral resources, which recent studies have found to be ideal factors in drawing diverse and abundant pollinator responses (Winfree et al. 2011). The visitation rate results of this study support the conclusion that restoration nurseries that are several years old may have sufficient unmanaged insects to pollinate many of their forbs.

Manipulatively increasing insect visitation at Webster nursery may not be a conservation priority given a lack of evidence for pollen limitation for either plant species. In addition, no evidence was found that supplemental pollen increases viable seed production for the plants in this study. An earlier study by Fazzino et al. (2011) found that hand-pollinated inflorescences produced more sprouting seeds than naturally-pollinated inflorescences for *B. deltoidea* in nearby Puget lowland prairies. In contrast, the *B. deltoidea* plants in this study were either not pollen-limited or the hand-pollinated inflorescences did not receive enough supplemental pollen by hand to show a significant difference.

The discrepancy between this earlier study and the results presented here could also be due to differences in weather between study years, as poor flight weather can dampen insect visitation (Vicens & Bosch 2000), or differences in methodology. Weather data were not collected in either study throughout the bloom periods at the study sites, though past local weather reports indicate similar fair weather temperature ranges and precipitation levels in 2009 and 2012 when *B. deltoidea* was in bloom (U.S. Climate Data 2014). Fazzino et al. (2011) examined seed germination and we measured seed viability using lab methods. Due to various factors that can affect germination, and the destructive properties of tetrazolium testing, these two studies are not directly comparable. Future studies could involve both viability and germination testing and should increase the number of replicates to further test for pollen-limitation in both plant species. Though not statistically significant, our results did indicate a trend that hand-pollinated inflorescences produced a higher percentage of viable seeds. Managers may still consider providing supplemental pollen treatments to plants or placing managed bee colonies on site if planning to collect seed during a year with poor weather for insect visitation.

In this experiment, we tested whether or not more pollen would increase viable seed production, though supplemental pollen does not always benefit plant reproduction. When

maximum seed production is reached, there are no longer unfertilized ovules for additional pollen to be of benefit (Ashman et al. 2004). There can be a point of pollen saturation on stigmas (Cane & Schiffhauer 2003); too much pollen added too quickly could lead to an underestimation of pollen-limitation (Ashman et al. 2004). In fact, supplemental pollen negatively affected seed weight in a study on pollen limitation at the community level, as plants may reallocate energy and resources in response (Hegland &Totland 2008). Pollen in this study was collected from separate plants on the opposite side of the study area as the plant that was hand-pollinated to ensure cross-pollination. Visiting insects, however, often transfer a combination of pollen from separate plants and pollen that may not be compatible from flowers of the same plant (Wagenius & Lyon 2010), so the effect of increasing insect visitation may not be directly proportional to hand-pollination treatments.

Finally, the percentage of viable seeds was lower at Johnson Prairie compared to Webster nursery for both species, but more dramatically so for *L. albicaulis* (see Fig. 3). Plants at Johnson Prairie appeared to be smaller than at Webster nursery and may have been affected by seed predators, a pathogen, or limited by lack of irrigation. There may have been differences in plant population age, soil nutrients, or microclimate that influenced these differences.

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 and germination. In addition, changes in light and temperature during germination can affect *L. albicaulis* seed viability (Morey & Bakker 2011). We recommend that land managers turn efforts towards investigating the influence of the above factors on native seed production for these two critical species in future studies. Pollinator visitation and pollen-limitation may not be primary concerns for restoration managers working with these two species in the Puget lowland prairies of western Washington.

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APPENDICES

Additional supporting information may be found in the online version of this article:

Appendix I. Number of insect visits to *Balsamorhiza deltoidea*.

Appendix II. Number of insect visits to *Lupinus albicaulis*.

Appendix III. Number of seeds produced by *Balsamorhiza deltoidea* inflorescences.

Appendix IV. Number of seeds produced by *Lupinus albicaulis* inflorescences.

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