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

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A Thesis
Submitted in partial fulfillment
of the requirements for the degree
Masters of Environmental Studies
The Evergreen State College
December 2012

This Thesis for the Master of Environmental Studies Degree

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has been approved for

The Evergreen State College

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ABSTRACT

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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.

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Acknowledgments

Many thanks to all who helped me with this project. I thank Carri LeRoy for all her support as my thesis reader. I thank the Center for Natural Lands Management and in particular, Cheryl Fimbel for her suggestions, guidance, and support. I thank H. Elizabeth Kirkpatrick, University of Puget Sound, for her suggestions on designing this pollinator experiment. I thank Joint Base Lewis-McChord for permission to conduct research on their lands. I thank Greg Dasso,The Evergreen State College, for helping me get set-up in the lab for tetrazolium testing. I also thank The Evergreen State College Foundation and the Evergreen Sustainability Fellowship committee for their financial support. Finally, a special thank you to my grandma, Polly Robinson, for her help with sewing pollinator exclusion bags.

Chapter 1: Introduction and Literature Review

Introduction

This chapter is a review of the scientific literature on several aspects of pollination ecology: pollinator specialization, diversity influences, behavior, and effectiveness. Each aspect of pollination ecology selected serves as a valuable community-level component for understanding the pollination web, a composition of all the plant-pollinator interactions in an ecosystem. Little is known about the pollination web of Puget lowland prairies, and understanding these components could help land managers make decisions for maintaining prairie floral diversity.

The Puget lowland prairie ecosystem itself is threatened. Only three percent of the original Puget lowland prairie habitat remains in highly fragmented patches (South Sound Prairies Working Group 2012), and represents Washington State's rarest ecosystem. Land surrounding these prairie fragments has been converted by urban development, agriculture, and coniferous forest encroachment. Isolation limits species dispersal in this insular habitat. Numerous stakeholders—the Center for Natural Lands Management (CNLM), Washington Department of Fish and Wildlife, Washington Department of Natural Resources, U.S. Department of Defense, Thurston County, Wolf Haven Sanctuary, and private land owners (among others) attempt to keep advancing forests along prairie edges and invasive species at bay.

Vegetation of the Puget lowland prairies is a regionally unique, Idaho fescue-white-top aster community type (Chappell and Crawford 1997) dominated by mostly perennial forbs (Dunwiddie et al. 2006). The Washington Natural Heritage Program monitors six plant species known to be rare in this landscape. *Castilleja levisecta* Greenm., the hemi-parasitic endangered golden paintbrush with only 12 remaining populations, is most notable. Conservation efforts to protect floral diversity focus on maintaining suitable habitat for plants but managers have only begun to consider giving attention to restoration of historical functional components such as pollination.

Several plant species are grown in nurseries for seed to replant into the prairies to boost native plant populations including Puget balsamroot (*Balsamorhiza deltoidea* Nutt.) and sickle-keel lupine (*Lupinus albicaulis* Douglas). *Balsamorhiza deltoidea* can be found on the west coast of North America along the western slopes of the Cascade and Sierra Nevada mountain ranges (Douglas & Ryan 2001), though only 10 known populations remain in Washington State (Fazzino et al. 2011). The Washington Natural Heritage Program (2012) has listed this species as a potential species of concern. Populations of *B. deltoidea* are threatened by habitat destruction, invasive species (Douglas & Ryan 2001), and habitat fragmentation and isolation (Fazzino et al 2011). Increasing the size of the remaining populations could help *B. deltoidea* resist these threats.

Lupinus albicaulis is not listed as a species of concern, but is nonetheless an important plant species for Puget lowland prairie restoration. The Puget blue butterfly (*Icaricia icarioides blackmorei*) is a Washington State species of concern that uses *L. albicaulis* as a larval host plant (Schultz et al. 2009). *Lupinus* species are also important contributors to soil nitrogen dynamics which can shape plant communities after disturbance (Elliott et al. 2011), such as the Puget lowland prairies after natural fires.

Little is known about pollinators in the Puget lowland prairies. The Nature Conservancy and the CNLM (Fimbel and McKinley 2010) have begun to identify the most abundant insect species. Four of the most common floral visitors at the prairies are bees (of the order Hymenoptera). ‘Sweat bees’ *Halictus tripartitus* and *Lasioglossum nevadense* are tiny and solitary (individual bees that construct their own nests without the help of others; Michener 2006). *Bombus vosnesenskii* (the yellow-faced bumblebee) and *Bombus mixtus* (the brown-tailed bumblebee) are large, social, colony-forming bees most commonly seen pollinating local flowers. Most research attention has been given to studying and conserving rare butterfly species (Hays et al. 2000; Chramiec 2004; Stinson 2005; Hanson et al. 2010; Schultz et al. 2011)**,** including Taylor’s Checkerspot (*Euphydryas editha taylori*), that pollinate prairie plants while feeding on them. Flies, beetles, and wasps comprise most of the other floral visitors. Animals other than insects, such as hummingbirds, have been seen visiting Puget lowland prairie flowers, though they have been observed much less often (Fimbel and McKinley 2010). In this thesis, insects are assumed to be the main pollinators of plants at the prairies.

Why is it important to understand the biology of pollinators when protecting the floral diversity of an ecosystem? Pollination is the process that facilitates sexual reproduction in plants. More than 67% of plant species are estimated to be dependent upon animals to transfer pollen from the anthers of one plant to the stigmas of another (Kearns and Inouye 1997). Some species of plants can self-pollinate, though this results in unmixed genes and often far fewer viable seeds. The ability to evolve and survive in a changing environment requires genetic diversity, which is generally increased due to insect pollination. Insufficient pollination can be a limiting factor for seed production and thus the survival of plant species.

Land managers must take into account many habitat considerations for preserving floral composition, in addition to the protection of pollinators (Tepedino et al.

2011). Natural pollinator limitation has been found to be evident in most plants studied (Buchmann and Nabhan 1996). By understanding the role pollinators play in the Puget lowland prairies, obstacles to pollination may be identified and then managed to promote successful reproduction of target plant species.

Pollinator Specialization

Plant-pollinator relationships can vary from site to site at the community level. Understanding the types of relationships that can occur and the frequencies at which they typically occur can help focus research attention on keystone pollinator species. Modeling the relationships at a site is one way to direct conservation efforts, though there are many factors that can complicate this method.

Specialization refers to the number of degrees of connectivity existing between each pollinator species and each plant species. A pollinator is a generalist if the insect visits and forms mutualistic relationships with many different flower species. If an insect only uses resources from one plant species, it is considered a specialist. The same “generalist” and “specialist” terms may be applied to plants depending on whether many species of animals or only one pollinates the flowers.

Combinations of pollinator and plant relationships tend to occur at different frequencies. Generalist pollinators commonly visit either generalist or specialist plants creating redundancy in the food web (Jordano et al. 2006). For example, honey bees collect pollen from more plant species than any other animal pollinator. Honey bees can easily find another resource to use if one of these plants becomes extinct, to continue their own survival (Buchmann and Nabhan 1996). Specialist-specialist relationships are rarer. *Ficus carica* L. (fig trees) are only pollinated by *Blastophaga spp.* wasps and *Blastophaga spp.* only pollinate *Ficus carica*. If either the fig trees or the wasps became extinct, so would the other (Buchmann and Nabhan 1996). It is unknown if specialist-specialist relationships exist in the Puget lowland prairies. Knowledge of which mutualistic relationships are more vulnerable could provide a criterion for determining which plants and pollinators are the most important to protect when designing conservation plans.

Several studies have been conducted to determine which type of pollinators, if removed from an ecosystem, would cause a cascade of plant species extinctions. Anderson et al. (2011) found reduced seed production and plant density in a population of New Zealand gloxinia (*Rhabdothamnus solandri* A. Cunn.) where this specialist plant species had lost its bird pollinators, but no change in other populations of *R. solandri* where there had not been a functional extinction of the bird species this plant depended on. When plant species react to habitat destruction, asymmetric networks appear to be ideal for specialist plants. When specialist plants have generalist pollinators, the specialist plants have been found to preserve more connections to their pollinators, and therefore resist disturbance better than specialist plants with specialist pollinators (Abramson et al. 2011).

Pauw (2007) researched an orchid-pollinating generalist bee (*Rediviva peringueyi*) in South Africa. He found a decline in *R. peringueyi* populations correlated with a decline in viable seeds of the specialist orchid species that depended on this bee for pollination. However, the number of viable seeds did not decline in a generalist orchid pollinated by many insect species other than *R. peringueyi* (Pauw 2007). If the goal of a restoration project is to conserve plant biodiversity, these experiments provide evidence that protection of generalist pollinators with a higher degree of connectivity may be decided to be more critical than conserving specialist pollinators, particularly when a plant community is dominated by specialist plant species depending on pollination from a single generalist pollinator.

In Puget lowland prairies, bees appear to be the most generalized pollinators, visiting the largest variety of flowers. This indicates that they should be protected to conserve rare plants. Research has not yet been performed to determine the critical animal species pollinators for the endangered *C. levisecta* though researchers have observed bumblebees visiting these flowers (Wentworth 1997). If *C. levisecta* turns out to be a specialist depending on only bumblebees for maintaining genetic diversity in the twelve remaining populations, bumblebee conservation strategies will be important to include in any *C. levisecta* conservation plan. If many other plant species also depend on bumblebees, then their protection becomes even more critical.

Memmott et al. (2004) used a computer model of simulated pollinator extinctions to test the robustness of floral communities. Simplifying the real world ecosystem down to types of mutualistic relationships between the plant species and their respective pollinators allowed these scientists to identify the insects whose extinctions would precipitate the fastest decline in floral diversity. When researchers removed the generalist pollinators first, plant diversity declined most rapidly and the opposite became true when the modelers removed the specialist pollinator species first (Memmott et al. 2004). The results of these models can help land managers make general guidelines for conserving pollinators with the goal of protecting the greatest number of flower species; however managers must be aware that real life pollination webs are much more complex than their models.

Plant extinction rates may vary due to other pollination web processes not accounted for in the Memmott et al. (2004) models. The number of pollinator species present typically outnumbers plant species and their interactions are nested so the pollinator that is the second most generalized visits a subset of plant species that the most generalized pollinator uses, the third most generalized pollinator visits a subset that the second most generalized visits, and so on (Memmott et al. 2004; Fang & Huang 2012). This overlap allows for more redundancy in the system and therefore, slower declines in plant diversity (Memmott et al. 2004). Nevertheless, James et al. (2012) argue that nestedness is a less important indicator of food web stability than simply the general connectivity of the network (i.e. the number of partners a species has).

Extinction of a pollinator species may not precipitate a collapse of the food web if the other pollinator species in the web adapt their foraging strategies (Kaiser-Bunbury 2010). If a plant becomes extinct, a pollinator may choose to forage on a different plant species. If a plant species' pollinator becomes extinct, other pollinator species may begin foraging on the plant. Fang and Huang (2012) observed in a four-year study that highly diverse pollinator networks were relatively stable even while the pollinator assemblage varied year to year. Kaiser-Bunbury et al. (2010) found that random extinctions do not affect the stability of a plant-pollinator network due to foraging adaptability, but removal of the strongest interactors may cause a sudden collapse of the pollination web. Anthropogenic disturbance more often leads to the extinction of keystones in a food web, such as bumblebees, than natural disturbance (Kaiser-Bunbury et al. 2010).

Plant extinction rates may also be faster if a floral community includes rare plants at a high risk for extinction due to other factors than pollinator limitation such as population fragmentation, climate change, nutrient limitation, small population size, etc. Roberts et al. (2011) modeled the effects of predicted climate change on generalist and specialist bee species. All species were found be at risk, though the most specialized bee species was at the highest risk (Roberts et al. 2011).The plant-pollinator relationships that exist at a site depend on the diversity of plants and animals present.

Pollinator Diversity Influences

The species of pollinators that can be present at a site depends on what floral resources exist. Floral diversity therefore influences pollinator diversity, and spatial arrangement and range of floral resources also matter.

Plants often depend on the services of animal pollinators and pollinators, in turn, depend on floral resources. Because animals more or less co-evolved with certain plants (becoming specialists or generalists), the distinctive morphology and energy requirements of the different taxa require varied flower types to forage from. For example, Potts et al. (2003) found increased bee diversity to be strongly related to an increase in variety of floral resources available to support the nutrition and feeding requirements of the different species. Hines and Hendrix (2005) developed a landscape resource index for floral resource diversity, and abundance of flowering ramets for their study sites, and found this tool to be a useful predictor of bumblebee diversity. Competition for nectar between different taxa, such as bees and butterflies, may also influence the pollinator diversity at a site (Davis et al. 2008). Finally, nesting resources also often indicate whether or not a high diversity of pollinating insects will be found in a region.

Animals require special places to rear their young. Solitary bees need suitable soils to build ground nests and social bees often build homes under dry, dense vegetation (Hines and Hendrix 2005, Davis et al. 2008). Butterflies look for certain plant species to be larval hosts. Davis et al. (2008) discovered pollinator conservation strategies may need to vary by taxa due to dissimilar habitat needs and arrangements.

For pollinator diversity, spatial arrangement of floral resources and habitat substrate matter. Hines and Hendrix (2005) studied bumblebees and found the abundance of these animals to be correlated with the size of the area of prairie habitat around their nesting sites. Narrow grassy areas may be important for providing connectivity between habitat patches (Hines and Hendrix 2005). Some pollinators that are critical for a certain plant population may migrate, so habitat protection is therefore essential all along the route the migratory animal takes during its lifecycle (Buchmann and Nabhan 1996). The more different kinds of microhabitats existing at a site, the more pollinator diversity there can be.

Pollinator habitat range is wider than the area of the protected Puget lowland prairies. Management of the surrounding land may be necessary, making landowners also a part of the greater pollination web. Managers may need to plant more early, late, and long-blooming flowering plants as food resources to sustain pollinators when their main food sources wilt. Fimbel and McKinley (2010) experimented with putting artificial nesting blocks out on some of the prairie sites and had success with attracting insects, though more research is needed to determine if the insects inhabiting the blocks benefit the pollination web or not. Many solutions can be found for altering and conserving pollinator diversity at the prairies as the pollination web becomes understood. Diversity is an important consideration because each insect species behaves uniquely.

Pollinator Behavior

Animal behavior can be a critical component to the pollinator web. The spatial pattern a pollinator chooses when moving from flower to flower can determine if genetic diversity is spread in floral reproduction or not. If an insect too often jumps from one species to a different species, pollen may be lost on the wrong plant and not make the journey to a conspecific for fertilization. By staying too long on one plant, a pollinator may help the plant to self-fertilize but hinder the genetic mixing that promotes genetic diversity.

Certain insect species are attracted to dense patches rather than isolated flowers (Garcia-Meneses & Ramsay 2012). When plants are grouped close together, they tend to be visited by more pollinators looking for higher reward for less effort (Garcia-Meneses & Ramsay 2012). This could either be beneficial to the plant species or detrimental. The presence of more flowers decreases the probability that an individual flower will be pollinated due to increased floral competition (Sih and Baltus 1987). Patches of plants often consist of closely related plants and pollinators have been found to lower reproductive output by concentrating visits to a single large patch (Garcia-Meneses & Ramsay 2012). White-top aster (*Aster curtus* Cronq.), a clonal Puget lowland prairie plant, forms dense patches that may attract many pollinators. If the insects only visit flowers within one patch among clones though, genetic diversity will not increase because all clones within a patch produce pollen with identical genetic material. Wirth et al. (2011) discovered higher seed set in arctic alpine forget-me-not (*Eritrichium nanum* (Vill.) Schrid.) plants growing at low conspecific density than high conspecific density likely due to both reduced floral competition for pollinators and more effective outbreeding.

Some pollinator species have been found to be attracted to sparer patches of flowers rather than denser. Nielsen et al. (2012) found that honeybees had higher visitation rates to more dense patches of flowers, but bumblebees and hoverflies visited sparser flower patches more frequently possibly due to reduced competition.

Pollinator competition for flowers can influence visiting insect diversity and behavior. Predation by larger insects on small ones may deter small pollinators from visiting a plant. Keys et al. (1995) found that by excluding large pollinators from visiting a flower using a coarse mesh bag, they could test the effectiveness of small insects only. However, Keys et al. (1995) noted that the abundance of small pollinators then found on the flower may be an unnatural result if this method results in a refuge from typical predation.

Different types of pollinators may forage at different times of the day or year. Early visiting animals may drink all the nectar and pollinators coming to visit later will avoid those flowers that are empty of a reward. Some pollinator species forage earlier or later in the season than others, thus competition for floral resources may vary throughout the year or with the number of flower species in bloom. Competition may be lessened at times as some animals are hardier to warmer or cooler temperatures or to wind and air moisture.

Introduced, non-native insect species can compete with native pollinators. In Nielsen et al.'s (2012) research, the presence of honeybees affected visitation rates of other pollinator species and pollinator community composition variously depending on the plant species studied. A correlation was found between decreased coffee (*Coffea Arabica* L.) fruit production and a decrease in native pollinator diversity due to competition with honey bees (Badano & Vergara 2011). The amount of time that different pollinator species interact with a flower can shift depending on competition, and because different pollinators have different levels of effectiveness this can influence plant reproductive success.

Pollinator Effectiveness and Efficiency

The basic principles of how generalist and specialist pollinators structure the pollination web can be disrupted when examining the effectiveness or efficiency of the visiting species. A pollinator is "effective" if a plant produces a greater number of viable seeds after being pollinated by the animal minus the number of viable seeds the plant would produce without any animal interaction (i.e. seeds produced by self-pollination). Pollinator "efficiency" refers to the animal's effectiveness divided by a measurement of space or time (Keys et al. 1995, Spears 1983). Ecologists have used many different methods to measure these variables.

Pollinator effectiveness can be measured indirectly or directly. Indirect methods assume pollination success by observing floral features such as: pollination-mediated floral color changes, the number of mechanically tripped flowers, or the number of pollen grains on a stigma (Engel and Irwin 2003). Direct measurements investigate the number of viable seeds produced. Spears (1983) published a paper offering an equation for calculating the pollinator effectiveness (PE) of a single animal species, which is still used by many pollination ecologists in current research (Keys et al. 1995; Perfectti et al. 2009), and often called “Spear’s PE”:

$$PE = (Pi - Z)/ (U - Z)$$

Pi= mean number of seeds set / flower by a plant population receiving a single visit from species i
Z= mean number of seeds set / flower by a population receiving no visitation.
U= mean number of seeds set / flower by a population receiving unrestrained visitation

In a standard experiment to determine the effectiveness of all pollinators on a given plant, a researcher hand pollinates some of a plant’s flowers to ensure complete pollination and allows other flowers to be freely pollinated by the usual animals (Kearns and Inouye 1993; Cane 2005; Fazzino et al. 2011). By counting and comparing the viable seeds produced by each experimental set of flowers, pollinator efficiency can be directly inferred (Kearns and Inouye 1993; Cane 2005; Fazzino et al. 2011). When conducting any experiment involving seed measurements, researchers must keep in mind that seed viability can be affected by other variables aside from pollinator efficiency such as soil nutrients and moisture content (Engel and Irwin 2003, Fimbel and McKinley 2010).

This direct method of measuring effectiveness has already been applied to one species of plant in the Puget lowland prairies, *B. deltoidea* (Puget balsamroot), in a study by Fazzino et al. (2011). Seed set was compared between naturally-pollinated inflorescences and hand-pollinated inflorescences. Pollinators were not effective and *B. deltoidea* was found to be pollinator-limited as hand-pollinated inflorescences produced more potential germinants than naturally-pollinated inflorescences. In 2012, I adapted methods from Fazzino et al. (2011) to also test *B. deltoidea* for pollinator limitation (see Chapter 2). Seed-set from hand-pollinated inflorescences were again compared to naturally-pollinated inflorescences, but seed viability was measured using a tetrazolium assay. Seeds were soaked in a 1% solution of 2,3,5-triphenyltetrazolium; a dye that indicates living tissue if the seed embryo stains red. Surprisingly, no evidence of pollinator limitation was found (see chapter 2).

Computer modeling performed by Perfectti et al. (2009) illustrates the importance of understanding the effectiveness and efficiency of the animal species in addition to whether the pollinators are generalists or specialists. One theory about why plants evolved to become specialists speculates they did so to attract only the most efficient pollinator. A generalist plant may have the advantage of being able to survive if one of its pollinators becomes extinct, but may be at a disadvantage if many of its visitors do not effectively pollinate. Perfectti et al. (2009) put several plants and their respective pollinators of various efficiencies into this model and simulated scenarios where the diversity of the pollinator assemblage differed. They discovered that low diversity in pollinators resulted in most successful plant reproduction when the most abundant pollinators were the most effective. Higher pollinator diversity may be better though, according to optimal plant fecundity, when the most effective pollinators are not the most abundant (Perfectti et al. 2009).

Along with viable seeds produced, an additional variable must be monitored to study pollinator efficiency. Keys et al. (1995) determined the efficiency of specific pollinators by monitoring the length of time a pollinator spent on a spike and the distance the pollinator traveled along the inflorescence per number of pods developed in addition to PE. Engel and Irwin (2003) recorded the rate of visitation by hummingbirds per number of pollen grains found on the stigmas of scarlet gilia (*Ipomopsis aggregata*

(Pursh) V.E. Grant) flowers. Jauker et al. (2012) measured the effect of density of visiting insects by putting a known number of insects in a cage with a known volume containing flowering plants. Both red mason bees (*Osmia rufa*) and hoverflies (*Eristalis tenax* and *Bpisyrphus balteatus*) will pollinate oilseed rape (*Brassica napus* L.), but it takes about five times the density of hoverflies to pollinate an oilseed rape plant as red mason bees for the plant to produce the same number of seeds (Jauker et al. 2012). Calculating an efficiency rate allows scientists to determine the necessary abundance of a pollinator species to be effective at pollinating the plants in a region.

Pollinator efficiency and effectiveness can vary year to year or within a single flowering season. Native pollinator assemblages can vary significantly from one year to the next resulting in fluctuations in plant fecundity (Rader et al. 2012). Kudo et al. (2011) found that queen bees were more efficient pollinators of rhododendron (*Rhododendron aureum* Georgi) than worker bees. Because queen bees forage earlier in the season than worker bees, pollinator effectiveness changed during the season (Kudo et al. 2011).

For many plants, rate of insect visitation is a critical factor for reproductive success. Engel and Irwin (2003) found a positive relationship between insect visitation rates and pollen receipt. Vazquez et al. (2005) compiled pollination data from the literature and used a mathematical model to determine that frequency of visitation is a factor usually contributing more to seed production than the effectiveness of the visitor. Arroyo et al. (1982) counted visits to a known number of flowers during 10-minute intervals to calculate visitation rates for an experiment that determined the effects of altitude on pollination. In 1985, the same method was used in a second part of the study to find the effects of temperature on visitation rates (Arroyo et al. 1985). Several more researchers (Inouye and Pyke 1988; Berry and Calvo 1989; Kearns 1990; Mcall and Primack 1992; Boyd 2004; Grindeland et al. 2005) followed suite in the next years, using similar visitation rate methods for comparison of their studies (Kearns and Inouye 1993).

In the next chapter, I use research methods explored in this literature review to investigate the current state of pollination at the Puget lowland prairies and at a nursery that supplies native seeds for restoration projects at the prairies. At each study site, I observed and recorded rates of insect visitation to selected plant species using methods adapted from Arroyo et al. (1982). I also related visitation rate to the effectiveness of the pollinators and explored pollinator limitation by collecting seeds from inflorescences that had been hand-pollinated or naturally-pollinated and tested their viability. To do this, I adapted methods from Fazzino et al. (2011) and tetrazolium testing procedures outlined in the *International Rules for Seed Testing* manual (International Seed Testing Association 2012).

Chapter 2: Manuscript formatted for Journal of Pollination Ecology.

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).

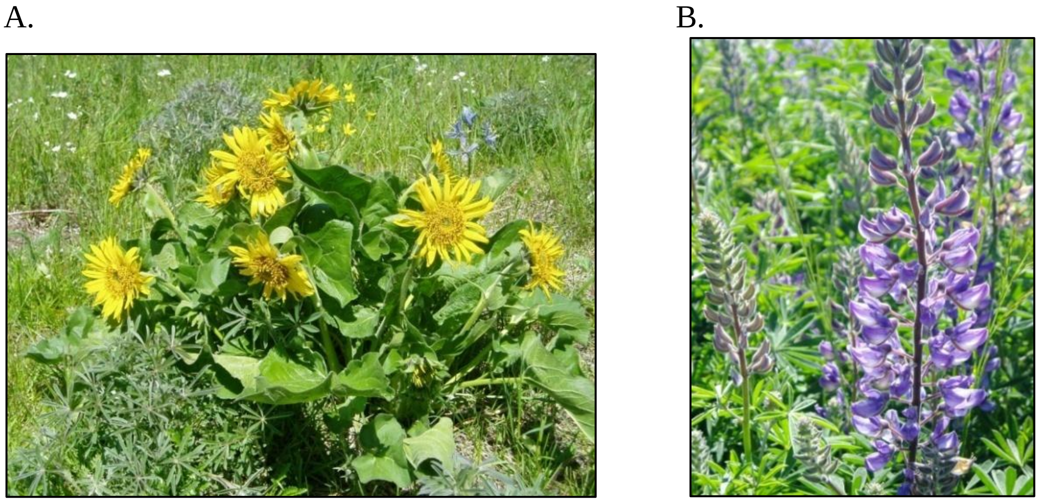


Figure 1. A= *Balsamorhiza deltoidea*, B= *Lupinus albicaulis*. The study plants found on Puget lowland prairies, Thurston Co., 2011.

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*

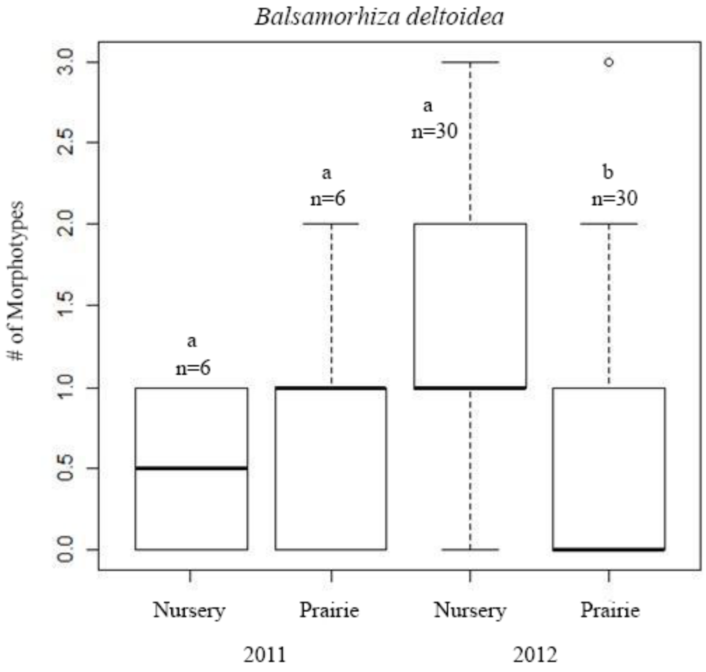
	2011 (n_1=n_2=16)		**2012 (n_1=n_2=10)**	
Insect Morphotype/Species	**W**	**P**	**W**	**P**
Small Dark Bees	164.5	0.0988	72.0	0.0666
Large Dark Bees	126.0	0.9216	46.0	0.7280
Bumblebees (total)	**54.0**	**0.0054**	**2.0**	**0.0002**
Bombus sitkensis	147.5	0.4102	45.0	0.5842
Bombus mixtus	**29.5**	**0.0001**	**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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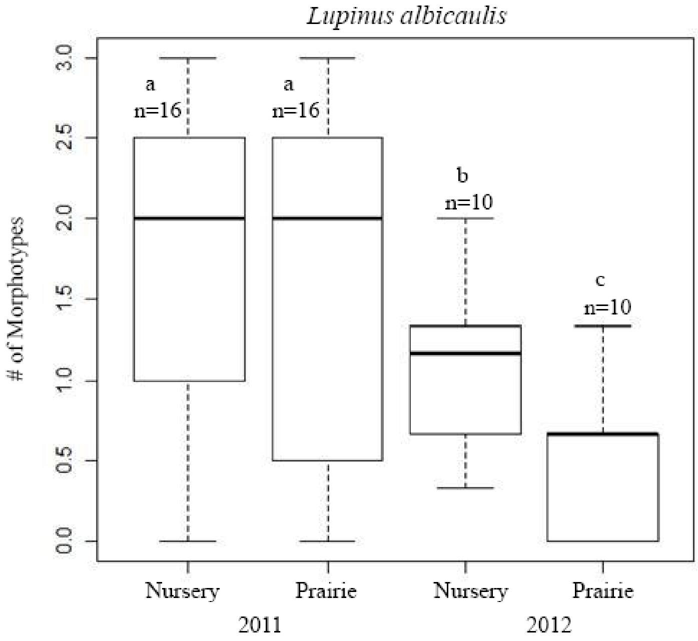


Figure 4. Visiting insect morphotype richness for A) *Balsamorhiza deltoidea* and B) *Lupinus albicaulis* at Webster nursery and Johnson prairie in 2011 and 2012. Different letters above bars indicate a significant difference between sites and years.

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.

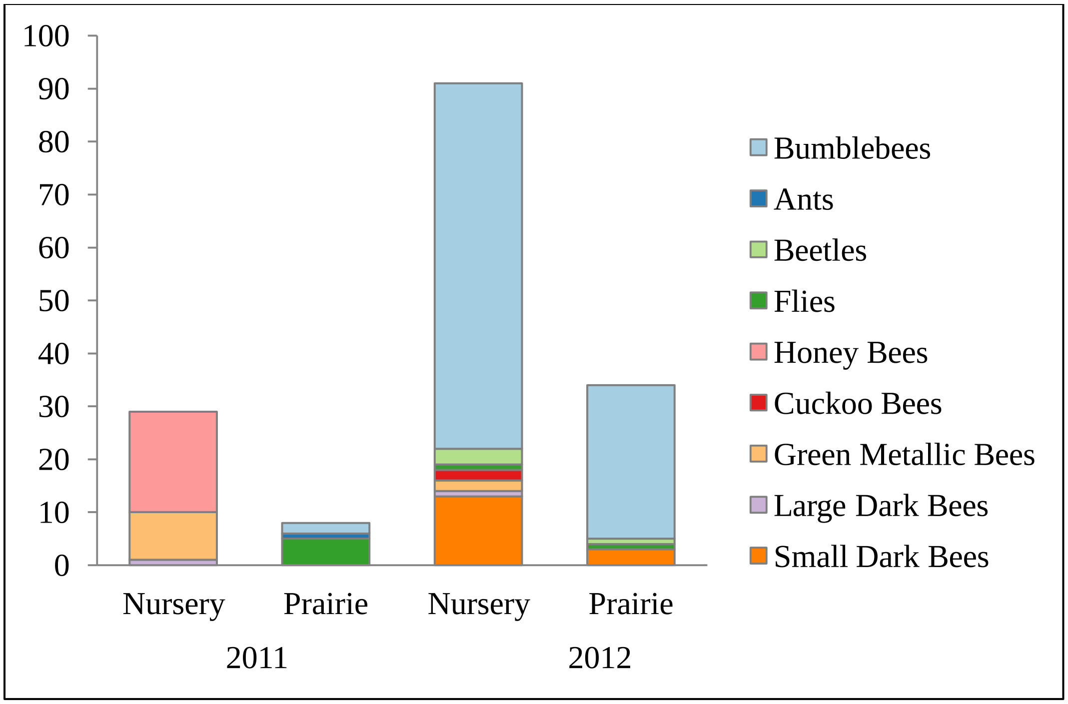


Figure 5. Total number of visits (from all observations summed) made to *Balsamorhiza deltoidea* by each insect morphotype.

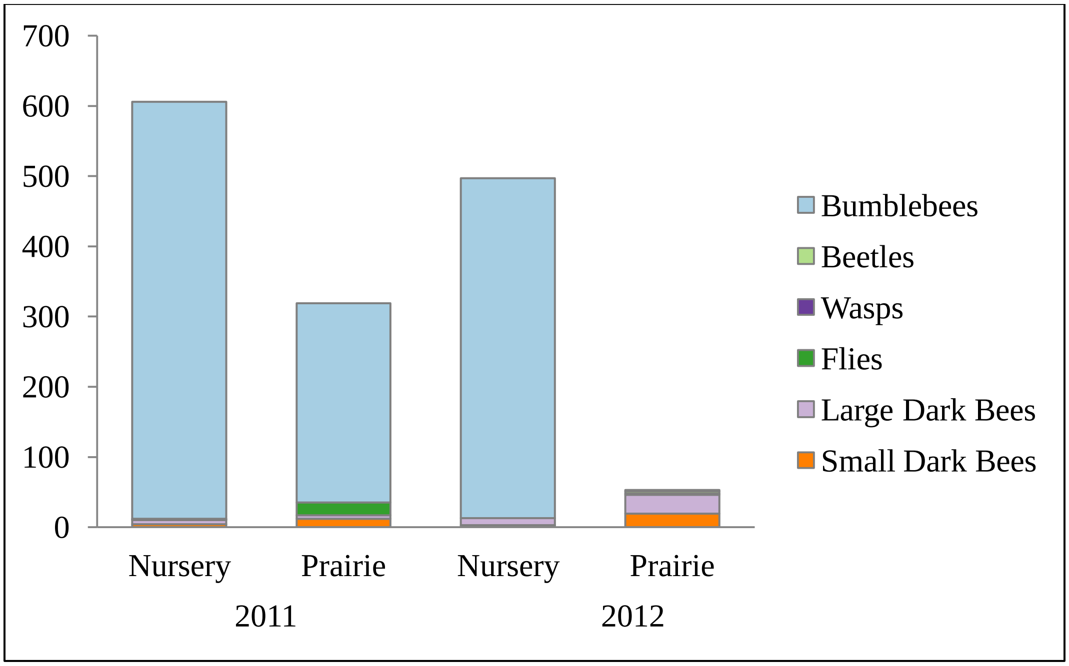


Figure 6. Total number of visits (from all observations summed) made to *Lupinus albicaulis* by each insect morphotype.

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 (Webstery 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.

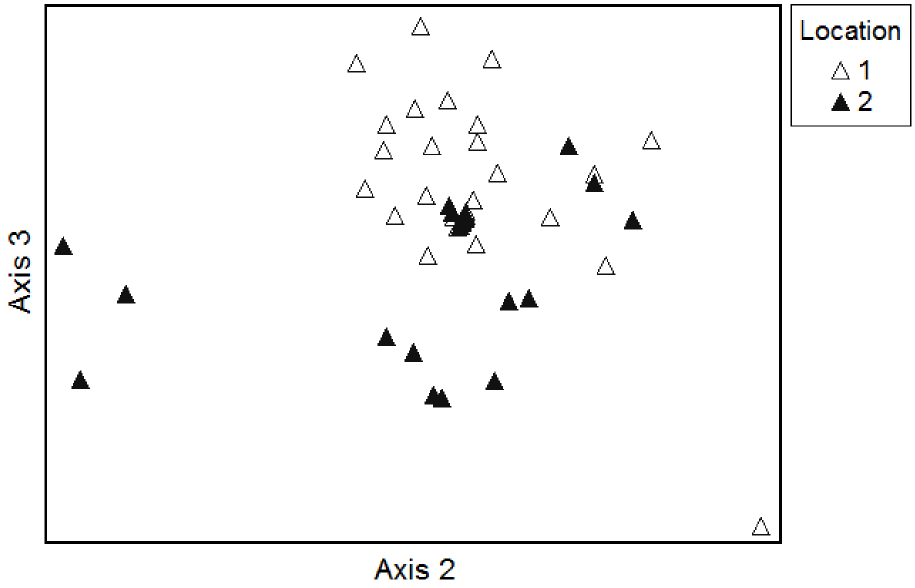


Figure 7. A representative NMDS ordination plot of influence of site differences on visiting insect community structure for *Lupinus albicaulis* in 2012. Location 1= Webster nursery. Location 2= Johnson prairie.

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

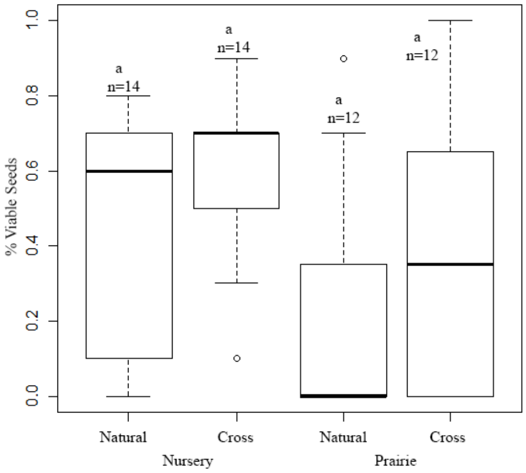
		Temperature		**Wind Speed**		**Cloud Cover**	
Plant Species	**Year**	**A**	**P**	**A**	**P**	**A**	**P**
Balsamorhiza deltoidea	2011	-0.042	0.732	0.016	0.261	-0.038	0.642
Balsamorhiza deltoidea	2012	0.052	0.126	0.012	0.328	0.068	0.352
Lupinus albicaulis	2011	-----	-----*	**0.042**	**0.008**	**0.068**	**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.

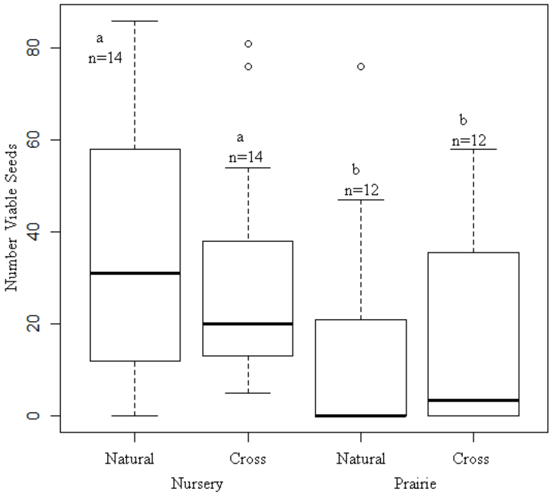


Figure 8. A) Percent and B) number of viable seeds produced by naturally pollinated and hand-cross pollinated *Balsamorhiza deltoidea* inflorescences at Webster nursery and Johnson prairie in 2012.

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).

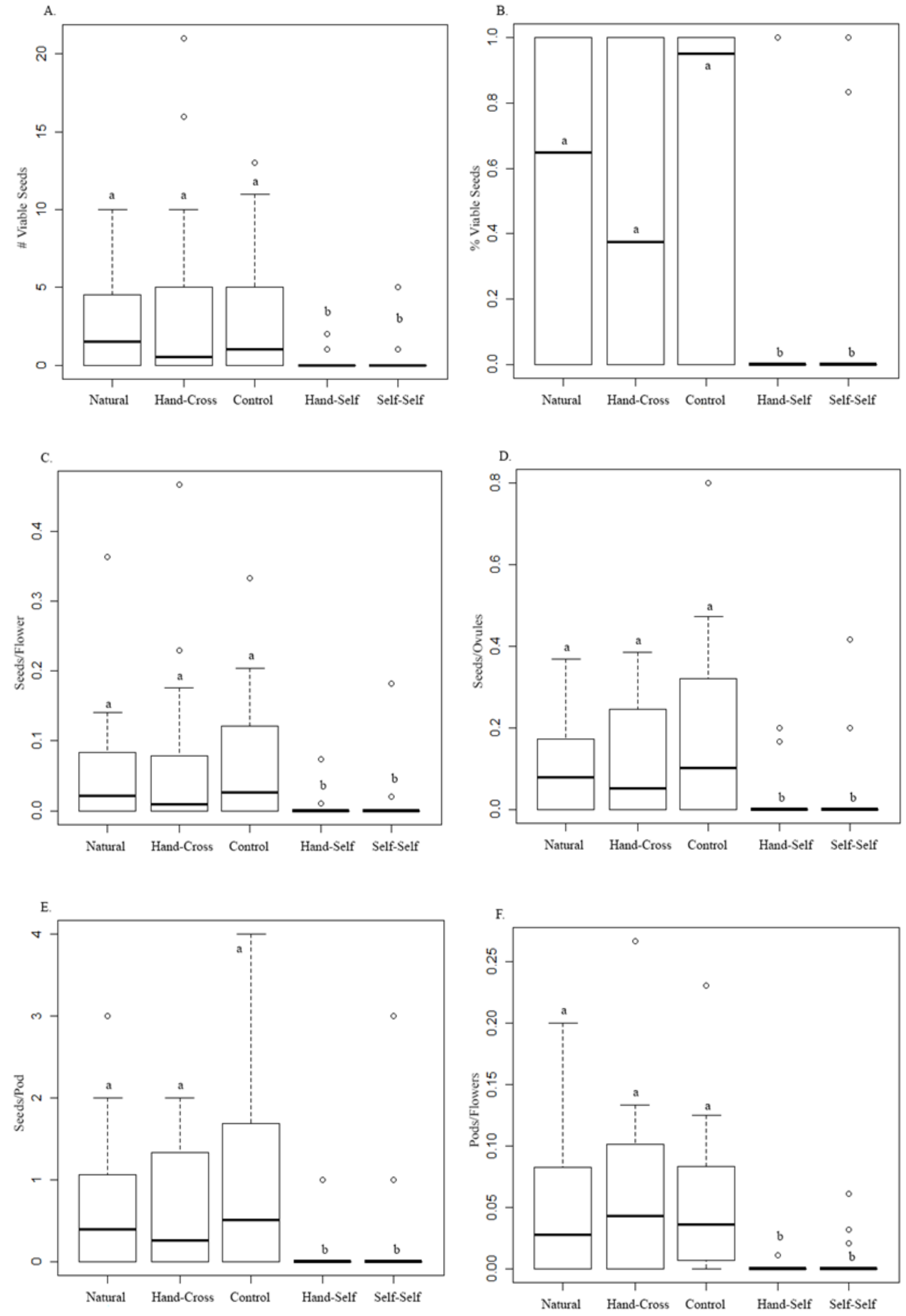


Figure 9. A) Number and B) percentage of viable seeds, C) number of seeds per flower, D) seeds per ovule, E) seeds per pod, and F) pods per flower produced by *Lupinus albicaulis* for each treatment. $n_1 = n_2 = 20$ for all treatments.

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

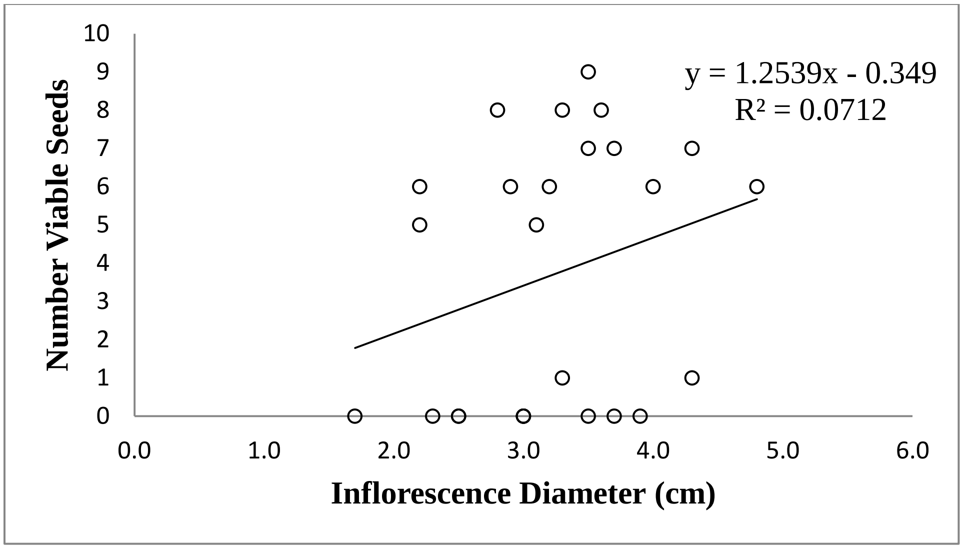


Figure 10. Inflorescence diameter vs. number of viable seeds produced by *Balsamorhiza deltoidea*

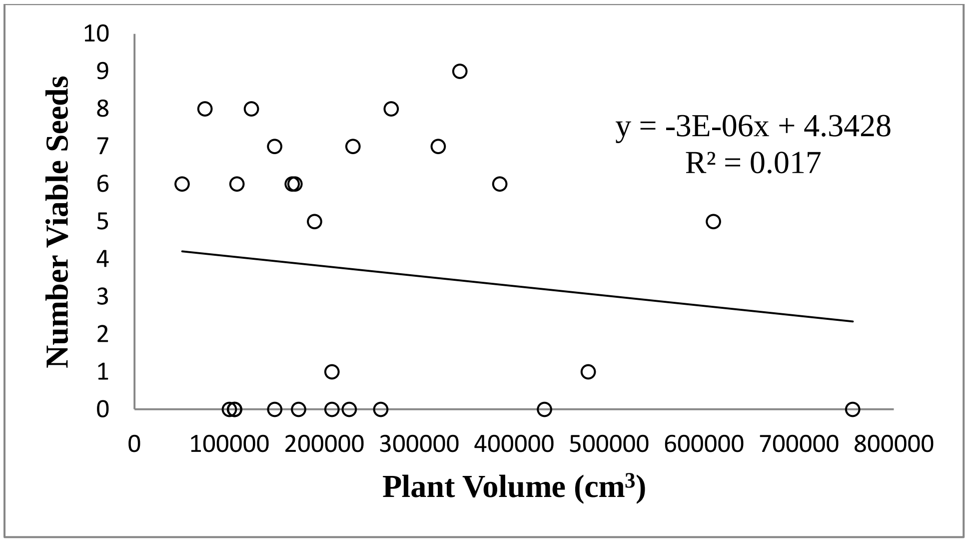


Figure 11. Plant volume vs. number of viable seeds produced by *Balsamorhiza deltoidea*

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.

ACKNOWLEDEMENTS

I thank H. Elizabeth Kirkpatrick, University of Puget Sound, for her suggestions on designing this pollinator experiment. I thank the Center for Natural Lands Management for their support and Joint Base Lewis-McChord for permission to conduct research on their lands. I thank Greg Dasso, The Evergreen State College, for helping me get set-up in the lab. I also thank The Evergreen State College Foundation and the Evergreen Sustainability Fellowship committee for their financial support. Finally, a special thank you to my grandma, Polly Robinson, for her help with sewing pollinator exclusion bags.

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Chapter 3: Interdisciplinary Connections

Pollination ecology is inherently interdisciplinary. Throughout the process of completing this thesis, I crossed back-and-forth over lines of several disciplines within the realm of natural science. My personal interest in this topic stemmed from a background in botany, but this thesis required that I learn entomology, ecology, multivariate statistics, and even a bit of chemistry.

Nevertheless, pollination ecology is not just about natural science. This thesis has both political and economic connections. Through the process of selecting this topic, I found that many different stakeholders are involved in Puget lowland prairie conservation due to policies written for the protection of biodiversity. Investigating ways to improve restoration practices and to prevent further losses to this ecosystem has potential benefits for human society at large. Pollinators provide an essential ecosystem service that affects the local economy, agriculture, and homeowners. A better understanding of the pollination web could influence conservation policy at many levels.

PUGET LOWLAND PRAIRIE STAKEHOLDER VIEWS

Numerous stakeholders are invested in protecting the Puget lowland prairies for various reasons and a better understanding of pollination could influence conservation policies. Concerned stakeholders exist at all levels, including: federal, state, and county agencies, private non-profit organizations, farmers, and private citizens.

Federal Agencies

Federal agencies have the duty to uphold the Endangered Species Act of 1973 mandating that endangered species and their habitat be protected. This act, among other conservation policies, gives a voice to many plants and animals that are a part of the threatened Puget lowland prairie ecosystem. At the federal level, the Endangered Species Act involves the U.S. Fish and Wildlife Service, the U.S. Department of Defense, the U.S. Natural Resources Conservation Service, and the U.S. Environmental Protection Agency when it comes to considering Puget lowland prairie protection.

The U.S. Fish and Wildlife Service is responsible for administering the Endangered Species Act to recover listed species to levels at which their protection is no longer necessary (U.S. Fish and Wildlife Service 2012). Three species found in the Puget lowland prairies are considered for listing under this act —Taylor’s checkerspot butterfly (*Euphydryas editha taylori*) has been proposed as endangered, Streaked Horn Lark (*Eremophila alpestris strigata*) has been proposed as threatened, and the Mazama pocket gopher (*Thomomys mazama*) is a candidate (U.S. Fish and Wildlife Service 2012). One plant species, golden paintbrush (*C. levisecta*) is already listed as threatened (U.S. Fish and Wildlife Service 2012). If pollinator species are found to be keystone species that are critical for endangered plant survival or for survival of plants that are critical habitat for endangered animals, they would need to be considered in habitat conservation plans for species listed under the Endangered Species Act.

Two thirds of the remaining 20,000 acres of Puget lowland prairies are located on Joint-Base Lewis McChord (JBLM), owned by the U.S. Department of Defense (USAEC 2012). Some of the healthiest examples of Puget lowland prairie habitat exist on these lands (Cheryl Fimbel, CNLM, pers. comm. 2010). Historically, the Puget lowland prairies had been maintained by Native American burning (South Sound Prairies 2012). Fire is an important part of the ecosystem that prevents conifer encroachment (South Sound Prairies 2012). Often training activity on Joint-Base Lewis McChord has had the unintended consequence of setting fire to the Puget lowland prairies on base and has unexpectedly benefited the ecosystem. Not all training activity is beneficial to this ecosystem and training restrictions could still be imposed on this large area of land because it is critical habitat for all three Puget lowland prairie candidate species. To avoid these restrictions, the Army Compatible Use Buffer program was formed to create land conservation partnerships with JBLM and other organizations to restore prairies on and around training lands (USAEC 2012).

USDA Natural Resources Conservation Services (NRCS) goal is to maintain a sustainable food supply as well as healthy ecosystems (U.S. NRCS 2012).Under the Food, Conservation, and Energy Act of 2008, otherwise known as the Farm Bill, the NRCS is authorized to create conservation programs for agricultural lands (U.S. NRCS 2012). One of the NRCS programs, the Wildlife Habitat Incentive Program, has been used in Thurston County to restore Puget lowland prairies by providing financial incentives to develop habitat for fish and wildlife on private lands (U.S. NRCS 2012). The Farm Bill has already established conserving wild pollinator habitat as a priority goal (U.S. Natural Resources Conservation Service 2008).

Tasked with regulating any activity that may harm the environment, the U.S. Environmental Protection Agency (EPA) has provided funding for conservation and restoration projects for a unique subset of Puget lowland wet prairies (U.S. Environmental Protection Agency 2012). The EPA partners with other agencies and non-profit organizations to protect the Puget lowland prairies. Under the Federal Environmental Pesticide Control Act of 1972, the EPA regulates pesticides that can harm pollinators and they may make adjustments to this policy as new information on the effects of chemicals on insects becomes available (Burlew 2010).

State Agencies

State agencies have the responsibility of maintaining natural resources sustainably while promoting economic use of those resources to enhance the state's economy. Washington State Department of Natural Resources, Department of Transportation, Department of Fish and Wildlife, and even Department of Corrections all participate in Puget lowland prairie restoration.

Washington Department of Natural Resources manages two Puget lowland prairie sites, Mima Mounds and Rocky Prairie, as Natural Area Preserves. The Natural Areas Preserve Act of 1972 designates these areas to be protected as high quality examples of Washington State's native ecosystems to be used for education, scientific research, and to maintain biological diversity (Washington State Department of Natural Resources 2012). Native Puget lowland prairie pollinators are necessary components of this rare Washington State ecosystem, and if they are threatened, these insects may fall under the Natural Areas Preserve Act as a component in need of protection.

In 1981, the Washington Natural Heritage Program (WNHP) was established within DNR by the Washington legislature to identify, manage, and share information on priority species and ecosystems for environmental assessments and conservation planning (Washington State Department of Natural Resources 2012). Several plant species found in the Puget lowland prairies are on WNHP's rare plant species list including *B. deltoidea* (Washington Natural Heritage Program 2012).

Washington Department of Transportation's Environmental Services Office is dedicated to protecting critical habitat for the prairie species listed under the Endangered Species Act (Washington State Department of Transportation 2012). Considerations of these habitats are a part of the Agency's environmental impact assessments for all building projects (Washington State Department of Transportation 2012). Roadside right-of-ways have been considered valuable pollinator corridors (Wojcik & Buchmann 2012) which may change WSDOT best management practices for how they maintain their land along state highways.

Washington State Department of Fish and Wildlife owns another piece of the remaining Puget lowland prairies, Scatter Creek Wildlife Area. This site is managed for conservation as well as recreation such as hunting, bird-watching, and horseback riding (Washington State Department of Fish and Wildlife 2012).

Prisoners are also involved in Puget lowland prairie conservation. The Sustainability in Prisons Project (SPP) provides inmates with job experience while raising Taylor's checkerspot butterflies for release into the Puget lowland prairies, and propagating over 50 species of native prairie plants (TESC and WSDOC 2012). The project was created through a partnership between The Evergreen State College and Washington State Department of Corrections.

Regional Agencies

County agencies hold responsibility for sustainable land-use planning for residential and business communities around the Puget lowland prairies. The remaining Puget lowland prairie fragments are located in both Thurston and Pierce counties. Thurston Parks and Recreation manages Glacial Heritage Preserve which is only open for Thurston County sponsored environmental and educational activities (Thurston County Parks and Recreation 2012). Streaked-horned larks nest at the Olympia airport and because they are a sensitive Puget lowland prairie species, the Port of Olympia has partnered with WDFW to create best management practices to protect Puget lowland prairie species as required by the State Environmental Policy Act (Port of Olympia 2012). Prairies in Pierce County are located on and managed by Joint-Base Lewis McChord. Local agencies may be mandated under the State Environmental Policy Act to create best management practices to protect Puget lowland prairie pollinators if they become limited.

Non-profit organizations aim to protect wildlife and habitat. In 2011, the Nature Conservancy passed on its 17 year legacy of being a primary land manager for the Puget lowland prairies to the CNLM. The CNLM partners with other organizations, including Joint Base Lewis-McChord, to assist with restoration on their lands (South Puget Sound Prairies Working Group 2012). CNLM staff, SPP staff, and prison crews are joined by a dedicated group of volunteers from the community who do everything from pulling invasive weeds to collecting native seed at both the prairies and the native seed nurseries. School groups also participate in Puget lowland prairie restoration with the CNLM and join in educational events hosted by the organization such as Prairie Appreciation Day.

Other non-profit conservation organizations are also active in Puget lowland prairie restoration and education. Wolf Haven International is a Puget lowland prairie landowner. The focus of Wolf Haven is on wolf conservation, though the prairies on their land also attract visitors (Wolf Haven International 2012). The Audubon Society is not only interested in protecting birds, but the ecosystems on which they depend and they have formed partnerships with other organizations to conserve Puget lowland prairie ecosystems as well (National Audubon Society 2012). Capitol Land Trust is a local, community-based organization that buys or accepts donations of land or conservation easements to protect natural areas including several Puget lowland prairies (Capitol Land Trust 2012).

Private citizens are also invested in Puget lowland prairie conservation. The diverse farming sector of this region wants productive crops and native insects often substantially contribute to crop pollination along with introduced honey bees (U.S. Natural Resources Conservation Service 2008). Local, private, land-owning citizens value the aesthetic quality of prairie landscapes, recreational opportunities, and would like to develop their land as they see fit. Federal and private grants are available to land-owners with Puget lowland prairie habitat to provide incentives to conserve this ecosystem (South Puget Sound Prairies Working Group 2012).

WHY CONSERVE BIODIVERSITY?

Today we live in a world of human-dominated ecosystems facing faster anthropogenic extinction rates than ever before (Primack 2010). Biodiversity provides a variety of ecological services that benefit humans. All species play an important role in providing these services. This is why it is essential to understand the role pollinators play in protecting plant biodiversity.

Losing a diversity of species means more than the loss of fun discoveries on a weekend hike, it also means the loss of the ecological functions each individual species provides. Healthy ecosystems composed of complex networks of interactions provide humans with food, shelter, oxygen, medicine, recreation, waste removal, etc. These functions are considered ecosystem services, another is pollination. Sixty to ninety percent of plant species require an animal pollinator (Kremen et al. 2007), and humans would have to figure out how to replace them if they disappear, which would be both costly and time-consuming.

Individual species all play a part in ecosystems. Keystone species have a dominate role to play in structuring ecosystems, but keystone species cannot hold a complex ecosystem and all of its functions together alone. Other species provide, at the very least, redundancy and genetic variation within communities (Primack 2010). Genetic diversity allows species to evolve and survive in a changing environment.

In the threatened Puget lowland prairie ecosystem, pollinators are a potential limiting factor in maintaining floral diversity. Pollinators provide an essential ecosystem service by facilitating sexual reproduction in plants, thereby mixing genes.

POLLINATION AS AN ECOSYSTEM SERVICE

Economically, there are incentives to better understand how pollination affects floral diversity at the Puget lowland prairies. Estimates of the global annual value of pollinator services range from $112 to 200 billion, and no studies have yet attempted to estimate the value of ecosystem services provided by native plants that are due to animal pollination (Kremen et al. 2007). Protecting pollination now, prevents having to figure out what to do if this ecosystem service is lost, decreases the need for supplemental seed and replanting, and supports local agriculture.

Land managers would be left with limited options if pollinators are lost. Replacing the services of native insects with honey bees or hand-pollination may be the only options left, and neither option is ideal. Honey bees are already disappearing from the Northern hemisphere and have not been found to be significant pollinators of wild plant populations in most regions (Ollerton et al. 2012). In fact, some studies have shown that honey bees decrease biodiversity in ecosystems through competition with native insects (Ollerton et al. 2012; Badano & Vergara 2011). Hand-pollination by humans can be costly, time-consuming, and ineffective (Partap & Ya 2012).

If natural pollination can be enhanced, there may be less of a need for supplemental seed and replanting. When pollinators are lost or limited, some plant species cannot maintain their populations (Mayer et al. 2011). When plant species in the Puget lowland prairies do not sustain themselves, the CNLM bolsters the populations by growing plants and seed in nurseries and then replants them into the prairies (Cheryl Fimbel, CNLM, pers. comm. 2010). No evidence for pollinator limitation was found for *B. deltoidea* or *L. albicaulis* in this thesis, but pollinator limitation may be one reason why other plant species struggle to maintain their populations. So, enhancing pollination may be a solution for some land managers who wish to save time and money by decreasing their reliance on native plant nurseries.

Native bees can substantially contribute to crop pollination (Stubbs & Drummond 2001; Greenleaf & Kremen 2006; U.S. Natural Resources Conservation Service 2008) of farms and private gardens in the surrounding area. By using sustainable agriculture practices that strategically use or eliminate pesticide use and promote plant diversity around crop fields, farmers can benefit from this free ecosystem service (Nicholls & Altieri 2012).

Pollination research and conservation may take time and resources, but the benefits clearly outweigh the potential costs of doing nothing. Understanding Puget lowland prairie pollinator systems enables land managers to be alert to changes and declines in pollinator populations that provide this economically beneficial service.

CONCLUSION

If stakeholders decide that action must be taken to protect pollinators, then both the ecology and the social aspects must be considered as an integrated system. An interdisciplinary viewpoint is essential for addressing environmental problems of this nature. Ideologies of many people have shaped this landscape. Land-managers may someday unmask an imbalance in the Puget lowland prairie pollination web using scientific research, but because more power exists in the voices of the human stakeholders, policy may need to be created to serve justice to and balance out the voices of the varied human players with the collective need to preserve biodiversity.

Compromises will need to be made when finding a solution for protecting pollinators. Managers of the preserves can work with agencies and local landowners to encourage pollination conservation. Much of the Puget lowland prairie ecosystem lies on private and agricultural land and though it may be costly in the short term to save patches of native vegetation instead of converting them to crops, farmers could save money in the long run by being active in conservation. Opportunity lies in creating public awareness of the benefits of maintaining pollination systems to rally volunteers to help out with restoration and monitoring. Pollinator protection in the Puget Lowland Prairies is a potential issue where solutions can be found cooperatively by looking at the bigger picture.

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