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## The effects of a widespread, showy invasive plant (*Rubus armeniacus*) on pollinator visitation rates, pollen deposition, and seed set in a rare native wildflower (*Sidalcea hendersonii*)

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**THE EFFECTS OF A WIDESPREAD, SHOWY INVASIVE PLANT  
(*RUBUS ARMENIACUS*) ON POLLINATOR VISITATION RATES,  
POLLEN DEPOSITION, AND SEED SET IN A RARE  
NATIVE WILDFLOWER (*SIDALCEA HENDERSONII*)**

By Natasha S. Johnson

Accepted in Partial Completion  
of the Requirements for the Degree  
Master of Science

Moheb A. Ghali, Dean of the Graduate School

ADVISORY COMMITTEE

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## **MASTER'S THESIS**

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Natasha S. Johnson  
June 4, 2010

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A thesis presented to the faculty  
of Western Washington University

In partial fulfillment of the requirements for the degree  
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by  
Natasha S. Johnson  
June 2010

## Abstract

The relentless spread of invasive plant species has illuminated their capacity for disrupting essential ecosystem services, including the pollination of native flowers. Invaders that are particularly showy, resource-laden and widespread appear to be the most likely to create pollinator competition. Native plants that are most likely to be impacted by the encroachment of such invaders are those that are sensitive to disturbance, locally rare and obligate-outcrossers. This study examined the effects of a widespread showy invader of the Pacific Northwest, *Rubus armeniacus*, on an imperiled endemic wildflower, *Sidalcea hendersonii*. These species are sympatric, have overlapping flowering phenologies and provide pollinators with similar resources: copious amounts of pollen and nectar. Pollinator observations revealed that 77% of species that visited *S. hendersonii* also visited the invader and that *R. armeniacus* flowers received more than three times as many total visits as *S. hendersonii* flowers. Further, three of the most common insect pollinators in the study system, *Apis mellifera*, *Thymelicus lineola*, (Lepidoptera: Hesperiiidae) and *Nacerdes melanura* (Coleoptera: Oedemeridae) were non-native. Invasive pollen deposition on native stigmas was high, with 67% of the *S. hendersonii* stigmas containing at least one *R. armeniacus* pollen grain. However, there was no relationship between either invasive pollen deposition or *S. hendersonii* seed set and distance from the invader (1m, 15m and 50m). Across all distances, pollen supplementation revealed that *S. hendersonii* was pollen-limited, with an average increase in seed set of 37% in pollen-supplemented flowers. Thus, either *R. armeniacus* does not have a reproductive effect on *S. hendersonii* and the observed pollen limitation was a result of other environmental factors, or *R. armeniacus* creates a pollen-

limiting force that is felt across the entire study area equally. Regardless, the first-year seed set of this rare, self-incompatible species in a highly invaded environment was on par with several wild *S. hendersonii* populations in British Columbia (Marshall 1997) and the high degree of visitor diversity suggests that the plant will be serviced even in highly invaded communities. This result, and the general vigor and health of all the transplanted study plants, sheds a positive light on the restoration potential of a rare endemic. Moreover, a main threat to the species, as reported in British Columbia and Oregon populations, is seed predation by weevils (*Macrorhoptus sidalcea* and *Anthonomus melancholicus*) (Marshall 1997, Marshall and Ganders 2001, Gisler and Love 2005), which were not found at the Ferndale, Wash., study site, suggesting that restored *S. hendersonii* populations may actually have greater reproductive success than remnant endemic populations, at least in the short term.

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## Introduction

Invasive plants are a looming threat to global biodiversity (Vitousek et al. 1997, Pimentel et al. 2005, Levine et al. 2003, Bjercknes et al. 2007, Gundale et al. 2008). Besides out-competing native plants for water, sunlight, space and nutrients (Richardson and Pysek 2000), studies in the last few decades have shown that some alien plants are capable of drastically altering the community structure of a site by impairing essential ecosystem services, such as nutrient cycling, soil renewal, erosion and flood control, water purification, carbon sequestration and the pollination of native flowers (Heinz Center 2008, Pejchar and Mooney 2009, Stout and Morales 2009).

Invasive plants that alter soil moisture can have dramatic impacts, particularly in arid ecosystems. For example, in the southwestern U.S., salt cedars (*Tamarix ramosissima*) invade stream banks, caching an annual 1.4 to 3 billion cubic meters more water than the native riparian vegetation (Zavaleta 2000 in Pejchar and Mooney 2009). Cheatgrass (*Bromus tectorum*), native to the Mediterranean and southwest Asia, has monopolized more than 200,000 km<sup>2</sup> of U.S. soil, primarily in arid regions of the western states (Humphrey and Schupp 2004). Because it forms dense monocultures and sequesters water more efficiently than native pine seedlings and grasses, cheatgrass alters not only community composition, but soil structure, nitrogen availability and erosion patterns (Gundale et al. 2008). Cheatgrass is also responsible for changing fire regimes, as it is among the first plants to colonize areas disturbed by fire, out-competing more fire-resistant species and leading to more frequent burns (Gundale et al. 2008).

Other invaders alter the chemistry of the soil. A classic example is the evergreen firetree, *Morella faya*, introduced to Hawaii from the Canary Islands in the late 1800s. As a nitrogen-fixer, *M. faya* enriches young volcanic soils at an estimated rate of 90 times that of native trees (Vitousek and Walker 1989). This unnatural fertilization makes it easier for other alien species to colonize and harder for native trees to establish (Walker 1990). Invasive plants that are allelopathic leach chemicals out of their roots, stems, leaves or pollen that act as phytotoxins to neighboring plants and seeds (Callaway et al. 2005). One of the most familiar culprits is spotted knapweed (*Centaurea maculosa*), which exudes  $\pm$ catechin. In susceptible species, this phytotoxin promotes reactive oxygen species, eventually inducing cell death from the root tip upward (Fitter 2003).

Invaders can also disrupt important reproductive mutualisms by infiltrating existing pollination webs (Stout and Morales 2009) and creating pollinator competition and/or pollinator sharing with native plants (Parker 1997, Brown et al. 2002, Ashman et al. 2004). Pollinator competition is when flowers vie for visits from the same suite of pollinators, while pollinator sharing occurs when the same individual pollinator visits two or more different flower species. Insufficient pollen deposition or contaminated stigmas can lead to reproductive decline in native plants. Because an estimated 48.7% of all angiosperms are obligate out-crossers (Igic and Kohn 2006), reproductive decline due to pollen competition is of particular concern for plants that are threatened or rare (Brown and Kephart 1999).

The successful integration of invasive plants into native pollination webs is well-documented (Stout and Morales 2009). For example, in the Mediterranean native bees and

beetles visit invasive *Carpobrotus* (Bartomeus et al. 2008a); in the United States domesticated honeybees and native bumblebees visit invasive purple loosestrife (*Lythrum salicaria*) (Brown and Mitchell 2001); in Chile native flies, bees and butterflies visit invasive dandelion (*Taraxacum officinale*) (Muñoz and Cavieres 2008); and in Ireland bees, native bumblebees, beetles and butterflies visit an invasive *Rhododendron* (Stout et al. 2006). Not all invaders are so successful at integrating, however. Studies of French broom (*Genista monspessulana*) suggest that it may suffer from a lack of pollinators in the U.S., whereas the closely-related sympatric invader, Scotch broom (*Cytisus scoparius*) does not (Parker and Haubensak 2002). Reduced pollination of invasives does not necessarily equate to reduced spread, of course. Kudzu (*Pueraria montana*) has been highly successful in monopolizing hundreds of acres in the southeastern U.S. even though it does not benefit from the high diversity of pollinating ants it is accustomed to in Japan and China (Harvey 2009).

Potential pollen limitation created by invasive species is critical to investigate because changes in pollinator behavior may affect communities at the landscape scale – even before competition for other resources ensues (Chittka and Schürkens 2001). In the case of invasive plants that become dominant floral resources, there are bound to be interactions with native flora in the form of shifts in pollinator abundance and/or pollinator behavior (Memmott and Waser 2002). Generally, there is great flexibility in plant-pollinator relationships, with most plants capable of being pollinated by a wide variety of pollinator types and most pollinators able to visit many floral forms (Proctor et al. 1996, Waser et al. 1996). However, recent declines in bee populations, widespread habitat loss, climate change

and the continual encroachment of invasive species (Kearns et al. 1998, Memmott and Waser 2002, Wilcock and Neiland 2002, Aizen et al. 2008b) are cause for more detailed examination of these interactions. Long-term declines of native pollinators in invaded systems, for example, may lead to more intense competition, to the detriment of less showy or less abundant endemic plants (Waser 1978, Waser et al. 1996).

#### *Changes to pollen quantity*

Competition for pollinators is common among co-occurring plant species (Robertson 1895, Knight et al. 2005) mostly because, contrary to historic assumptions, the majority of plants and pollinators are generalists (Waser 1978, Waser et al. 1996). The inherent structure of pollination webs, with a core of generalist plants and pollinators, encourages stability, as few species are completely dependent on one particular host or pollinator (Adler and Irwin 2006, Bartomeus et al. 2008b). In fact, most temperate plants are capable of being pollinated by a wide range of insects (Proctor et al. 1996). However, the strength of pollinator preference has likely played a key role in shaping the current structure of plant communities (Knight et al. 2005). In contrast, the rapid colonization of invasive plants causes shifts in plant communities in an extremely short period of time (Fleishman et al. 2005; Aizen et al. 2008b). The most extreme turnovers are invasive-plant monocultures, but even patches of highly attractive invasive plants have been shown to have effects on the local plant community when the invader has qualities that make it more desirable to pollinators than plants in the existing pollination web (Bjerknes et al. 2007). These qualities include richer

nectaries, greater pollen loads (Muñoz and Cavieres 2008), and a high density of floral resources (Kirchner et al. 2005).

The incorporation of invasive plants into native pollination webs is ubiquitous. Because few plant invaders are transported with the co-evolved pollinators from their homelands, most must rely on integrating into existing pollination webs composed of native or naturalized pollinators. The direct and indirect effects of these modifications to pollination webs are a newly emerging area of pollination research (Wilcock and Neiland 2002, Tylienakis 2008, Tscheulin et al. 2009, Vila et al. 2009). In their multi-study review, Bjerknes et al. (2007) suggest that invasion by a showy generalist plant has two potential results. First, the increased resources could boost pollinator abundance in the immediate vicinity and facilitate visitation to neighboring native plants. This “magnet-species” concept is generally accepted (Thomson 1978, Ghazoul 2006) and often applied in horticultural settings to lure visitors to less attractive flowers. For example, in Chile the alien weed *Carduus pycnocephalus* received increased pollinator visitation and had 32% higher seed set when grown in the presence of the showy invasive species *Lupinus arboreus* (Molina-Montenegro et al. 2008), presumably because the presence of *L. arboreus* brought in more pollinators than *C. pycnocephalus* could attract on its own (Molina-Montenegro et al. 2008).

Conversely, pollinators could prefer the alien plant, spending less time visiting native flowers. To date, the latter, competition-driven hypothesis has stronger support than the magnet-species hypothesis in invaded systems. In the Bjerknes et al. (2007) review, only two of 15 native species experienced increased visitation when the invader was present, while six

native species had decreased visitation. The remaining seven species experienced no significant change in number of visits. Furthermore, in all species in the review, the seed set of co-occurring natives (where quantified) was either reduced or unchanged in the presence of the invader.

The impact of an invasive plant on the pollination of sympatric natives may also be tightly coupled to the magnitude of the invasion. For example, in the Chilean Andes, Muñoz and Cavieres (2008) manipulated the abundance of the widespread invasive weed *Taraxacum officinale*, revealing a nonlinear relationship between invader density and both visitation and seed set in the native flora. At low densities, the showy invader boosted the length of pollinator visitation to a native aster by 25% and increased its seed set by 15%. When *T. officinale* density increased, however, the visitation to natives decreased by 26% and seed set dropped by 10%. Similarly, Rathcke (1988) found that the reduction in visitation rates to natives was most pronounced when the invader was present at high densities. Such results are particularly noteworthy because many invasive plants become dominant in their new environments (e.g., *Pueraria lobata* (Harvey 2009), *Lythrum salicaria* (Brown and Mitchell 2001), *Rubus armeniacas* (Caplan and Yeakley 2006), *Bromus tectorum* (Humphrey and Schupp 2004), and *Phragmites australis* (Lelong et al. 2007)).

Real and Rathcke (1988) posited that resource availability is at the heart of pollinator shifts from native to invasive plants, reasoning that pollinators may selectively visit flowers that are more closely aggregated, making foraging more efficient. The most common floral pollinators, bees, are adept at making educated foraging choices (Proctor et al. 1996), and

individuals are known to remember and return to plants with resource-rich flowers (Amaya-Marquez 2009). If a large number of pollinators switch hosts to these nectar-rich invasive plants, it could lead to altered foraging behavior in entire pollinator populations. The most dramatic result of a pollinator behavioral shift would be exclusion of certain native plants, in which case two results are possible: Reduced seed set and therefore diminished population size of the less attractive species, or, where possible, an increased reliance on selfing (Proctor et al. 1996). Reduced seed set due to pollinator host-switching has been documented in several uninvaded communities, including the meadows of North America where *Mimulus ringens* loses pollinators to *Lobelia siphilitica* and suffers an average seed set loss of 34% (Bell et al. 2005). An increased reliance on selfing is seen in northeastern U.S. forests where *Kalmia latifolia* is almost completely ignored by bees when it grows in the vicinity of a sympatric native *Vaccinium erythrocarpum*. *K. latifolia* in these communities was found to rely almost entirely on self-fertilization (Rathcke and Real 1993).

#### *Changes to pollen quality*

Invasive species may also impair normal pollination if native flowers receive mixed pollen loads. This is not unlikely, as it is common for one pollinator to visit many species in a single foraging trip (Adler and Irwin 2006, Bartomeus et al. 2008b), a behavior referred to as inconstant foraging (Brown and Mitchell 2001). Many bee and bumblebee species are known to work more than one flower species at a time, particularly if they are scouting for new food sources. However, relatively little is known about the foraging behaviors of solitary bees (Proctor et al. 1996). If a pollinator is able to pick up and carry an invasive plant's pollen, it

may deposit that interspecific pollen on native flowers' stigmas (Bjerknes et al. 2007). For example, Lopezaraiza-Mikel et al. (2007) compared experimental plots infested with *Impatiens* with those where the invader had been removed and found that natives in invaded study plots received significantly more visits. However, about 96% of the total pollen being transferred in the invaded plots belonged to *Impatiens*, setting up the potential for mass heterospecific deposition. Indeed, some 71% of native stigmas had *Impatiens* pollen.

The consequences of receiving interspecific pollen can be dramatic. In a series of experimental studies with the widespread invader, purple loosestrife (*Lythrum salicaria*), Brown and Mitchell (2001) found that when pollinators moved between the invasive plant and native winged loosestrife (*L. alatum*) during a single foraging outing, the transfer of invasive pollen to native stigmas caused a suite of competitive interactions, including stigma clogging, stylar clogging and pollen allelopathy. Observations of *L. alatum* stigmas following interspecific-pollen deposition revealed corkscrew-shaped pollen tubes characteristic of inhibition on both conspecific and heterospecific pollen. In a separate study examining the effects of allelopathy by wind-pollinated species, Murphy and Aarssen (1995) found that deposition as low as 0.1 pollen grains/ $\mu\text{L}$  of *Phleum pratense* (Poaceae) was sufficient to inhibit germination in several of the grass species studied. Few invasive species have been tested for allelopathic capabilities, so the extent of pollen allelopathy and other inhibition strategies is unknown (Murphy and Aarssen 1995).

Waser (1978) refers to all effects due to interspecific pollen transfer (IPT) as impacts on the “quality” of pollination, in contrast to the aforementioned visitation frequency, which

relates to the “quantity” of pollination. Each of these forces can create pollen limitation, which is typically measured by comparing pollen-supplemented seed set to natural seed set (Ashman et al. 2004, Jakobsson et al. 2009). The two effects are often related, as reduced pollen quality due to inconstant foraging can also reduce pollen quantity if pollinators lose conspecific pollen during visits to other flowers (Wilcock and Neiland 2002, Lopezaraiza-Mikel et al. 2007).

### *Ecological consequences*

Discerning the importance of pollination quantity and quality on seed set by natives requires disentangling these effects from the multitude of other factors that might lead to correlations between the presence of invaders and reduced seed set in natives. Invaders can compete with natives for many resources other than pollinators, and the habitat disturbances that facilitated the establishment of the invader may be directly detrimental to native plants (Elton 1958). Such impacts may cause native plants to reallocate resources away from reproduction, thereby lowering seed set even when pollen is not the limiting factor (Knight et al. 2005, Bjerknes et al. 2007). Isolating pollinator-mediated effects from these other factors requires an experimental design that includes treatments that compare natural seed set to that of pollen-supplemented plants. Without this comparison, differences in seed set could be attributed to myriad factors other than pollen limitation.

Using such methods, a suite of studies show that the impacts of invasive plants on pollination quality and quantity can vary substantially among systems. In some cases, both types of impacts have been shown to be important. Continuing their native v. invasive

loosestrife studies in Ohio, Brown et al. (2002) calculated that seed set in the native loosestrife species decreased 22% in 1997 and 34% in 1998 in the presence of the invader. This was attributed to both fewer visits (reduced quantity) and IPT (reduced quality). IPT may be a particularly important force in *Lythrum salicaria*-invaded plots because this alien plant produces four times more flowers per plant and twice as much pollen per flower than its native counterpart (Brown and Mitchell 2001). Combining this copious resource with a high degree of pollinator sharing (pollinator movement between native and invasive species accounted for between one-third and two-thirds of all pollinator movements), there was considerable opportunity for deposition of interspecific pollen on native stigmas.

In contrast, frequent pollinator sharing between native and invasive plants does not cause decreased pollination success for native plants in other systems. Bartomeus et al. (2008a) found that, although native Mediterranean pollinators move invasive *Carpobrotus* pollen around extensively, and 73% of pollinators collected on native species had invasive pollen on their bodies, the interspecific pollen count on native stigmas was low and seed set was not significantly affected. The authors hypothesized that this could be due to differences in flower morphology (i.e., *Carpobrotus* pollen is carried on a part of the insects' bodies that does not facilitate transfer to native stigmas). Similarly, in a study by Flanagan et al. (2009), native bees co-visited invasive *Lythrum salicaria* and native *Mimulus ringens*, picking up copious amounts of pollen from each. However, unlike the findings with *L. salicaria* and the native loosestrife (Brown and Mitchell 2001, Brown et al. 2002), very little *L. salicaria* pollen was deposited on *M. ringens* stigmas. This suggests that the degree to which IPT is a

factor may be linked to the floral structure of the plants involved, with close relatives and/or plants with similar floral structures being more susceptible to problems associated with IPT. However, this study also involved experimental hand-pollination of *M. ringens* with varying ratios of conspecific and heterospecific pollen and found that, even in treatments with high ratios of invasive pollen, there was no significant difference in *M. ringens* seed set.

The inconsistent effects of invaders on pollen limitation suggest that the effects of invasive plants on the pollination of natives are species- or system-specific (Bjerknes et al. 2007). Although it is likely that the invaders with the greatest impact are showy and rich in resources (Chittka and Schürkens 2001), there are too few studies to date to test this hypothesis using a meta-analytic approach. Similarly, it stands to reason that the native plants that would be most affected by the pollinator-mediated effects of invasives would be obligate out-crossers (Wilcock and Neiland 2002). Angiosperms that are capable of selfing are known to benefit from some level of cross-pollination (Proctor et al. 1996), but obligate out-crossers rely on cross-pollination for seed set and may be most affected by new competitors. Because a majority of studies have examined the effects of invader-caused pollen limitation and seed set in facultatively autogamous native plants (Bjerknes et al. 2007, but see Rathcke 1988 and Larson and Barrett 2000), this hypothesis cannot yet be rigorously tested. Moreover, because pollinator communities behave differently across time or space (Proctor et al. 1996), the most important factor to investigate in pollen-limitation studies is seed viability.

If an invasive plant can influence the fitness of natives through pollen limitation, the magnitude of that effect on the native-plant community should be examined from a landscape

perspective. Because most pollinators are highly mobile, management plans must take into account the projected foraging range of the pollinator species involved. Honeybees have been known to forage at distances of up to 12km (Beekman and Ratnieks 2000), while some bumblebees forage up to 2km from the nest (Walther-Hellwig and Frankl 2000). Thus, reproductive consequences for native flora may extend considerably farther than the immediate vicinity of the invader, and interactive effects may begin to manifest long before the invader is in proximity.

### *Hypotheses*

To test the hypothesis that a showy invasive plant can influence pollination service to an obligately out-crossing native plant, I focused on interactions between a widespread noxious invader and an imperiled out-crossing native plant. My primary goal was to establish whether invasive *Rubus armeniacus* (Rosaceae) poses a reproductive threat to the imperiled native wildflower *Sidalcea hendersonii* (Malvaceae) in the Pacific Northwest. To accomplish this goal, I tested the following suite of hypotheses:

Hypothesis 1: Proximity to *R. armeniacus* influences the rate of pollinator visitation to *S. hendersonii*.

Hypothesis 2: Proximity to *R. armeniacus* influences the composition of pollen on *S. hendersonii* stigmas.

Hypothesis 3: Proximity to *R. armeniacus* influences the seed set of *S. hendersonii*.

Hypothesis 4: Proximity to *R. armeniacus* influences the degree to which *S. hendersonii* is pollen-limited.

#### *Rubus armeniacus*

Himalayan blackberry (*Rubus armeniacus*, Focke) is a widespread, noxious weed that spread quickly through the Pacific Northwest after its alleged introduction by Luther Burbank in 1885 (Ceska 1999). It is native to southwest Asia but so completely naturalized in Western Europe that for many years its origins were mistakenly traced there. Botanical historians have now determined the plant invaded Europe in 1835 and came to North America in 1885 as an agricultural crop (Ceska 1999). The confusion surrounding its origin is further testament to this plant's ability to quickly and successfully naturalize in a wide variety of habitats. Indeed, this cosmopolitan species seems to have less critical requirements for water, nutrients, light, and soil pH and texture than other *Rubus* species (Amor 1973). There has also been considerable debate over the nomenclature of *R. armeniacus*, likely due to the species' ability to produce viable fruit without pollination, resulting in distinct lineages with different characteristics (Boersma et al. 2006). *R. armeniacus* is now determined to be synonymous with *R. discolor*, *R. fruticosus* and *R. procerus* (Ceska 1999).

*R. armeniacus* produces profuse displays of white to pale-pink perfect flowers consisting of five large petals, 50-200 stamens and 50-100 pistils (Nybom 1989). In lowland western Washington, it flowers from early June to August (N. Johnson, personal observation). Its drupelet berries are attractive to mammals and birds, which disperse the seeds great distances (Tirmenstein 1989, Lockwood et al. 2007). The species also spreads

vegetatively when the stems, called canes, touch the ground and the tips take root, a process called “tiprooting” (Boersma et al. 2006). In this way, a patch can widen at a rate of 3 meters/year (Boersma et al. 2006). A long-lived perennial, *R. armeniacus* can form dense thickets 5 meters tall (Tirmenstein 1989).

*R. armeniacus* is considered a serious pest in the Pacific Northwest, especially west of the Cascades from California to British Columbia (Hitchcock and Cronquist 1973) from sea level to 1500 meters (Boersma et al. 2006). The invader is so prolific that it is only listed on Washington state’s Class C noxious weed list, as its complete eradication is improbable at this point (Boersma et al. 2006). Residents may be partially to blame. In western Washington *R. armeniacus* is the most commonly harvested wild berry and many people cultivate it in their gardens (Tirmenstein 1989).

As in many other regions it has invaded, *R. armeniacus* persists in a multitude of diverse habitats, including roadsides, clearings, oak woodlands and riparian areas, where it displaces native species (Amor 1973, Boersma et al. 2006, Fierke and Kauffman 2006). Its superlative ability to alter community structure via competition for resources such as light, space, nutrients and water may belie its effects on native plants’ reproductive success through competition for pollinator services. Because *R. armeniacus* is often the dominant floral resource in invaded areas, it is probable that its presence exerts an effect on resident pollinators’ behavior. The showy, densely packed flowers, long flowering season and the fact that it has native relatives in the region (including *R. ursinus*, *R. spectabilis*, and *R. parviflorus*), make it a good choice for a pollen-limitation study, as these features have been

shown to be particularly desirable to pollinators (Bjerknes et al. 2007). Its popularity with native pollinators has not been fully explored in the literature (but see Cane 2005 and Jacobs et al. 2009), however the invader is known to be an important resource for the agriculturally important European honeybee (*Apis mellifera*) (Boersma et al. 2006).

### *Sidalcea hendersonii*

Henderson's checkermallow (*Sidalcea hendersonii*, Watson) is an herbaceous perennial native to the Pacific Northwest coast, primarily distributed from Vancouver Island to southern Oregon (Hitchcock and Cronquist 1973), a range within which it grows in moist, sunny areas, particularly on or near tide flats, coastal prairies, islands and moist meadows. It is the most northerly species of the genus (Roush 1931), which has its center of diversity in California and extends southward to Mexico. The known range of *S. hendersonii* was recently extended 750 miles northwest in 2003 to southeastern Alaska when a ranger at Tongass National Forest discovered a small population of the wildflowers (U.S. Forest Service 2009).

Historically, *S. hendersonii* was well distributed along the Pacific Northwest coast (Roush 1931) and its prevalence in the pollen record of southern British Columbia, dating back some 3600 years (Mathewes and Clague 1994), suggests *S. hendersonii* was abundant prior to European colonization. However, the species has gradually declined in modern times to only about 100 known populations (Gisler and Love 2005). *S. hendersonii* is a Class 1 species "endangered or threatened throughout its range" in Oregon and studies are currently being undertaken to identify reintroduction areas (Gisler and Love 2005). After its discovery

near Juneau, Alaska, the species was identified as “critically imperiled” in that state (Goldstein et al. 2009). In British Columbia, it carries the rank R2 (rare) and in 2004 it was globally designated G3 (species that are rare or uncommon globally). A petition to the U.S. Department of the Interior to list *S. hendersonii* as endangered or threatened was denied (U.S. Dept. of the Interior 2006).

*S. hendersonii* has dark pink to purple flowers that begin blooming in early to mid-June and continue to the end of August in coastal western Washington. The inflorescence is a compound raceme that blooms in a staggered, overlapping sequence with anywhere from one to more than a dozen flowers blooming on a single inflorescence at a time (Hitchcock and Cronquist 1973). The fruit is a schizocarp with one seed per carpel. The plant has a hardy, woody taproot with erect stems and can be up to 1.5m tall (Roush 1931). In her guide to the genus in 1931, Roush describes the species as well defined and not closely related to any others in its native region.

Most members of the genus *Sidalcea* exhibit sexual dimorphism (Roush 1931, Marshall 1997, Schultz 2003a) and *S. hendersonii* plants are gynodioecous, meaning separate plants consist of either perfect (hermaphrodite) flowers or pistillate (female) flowers with sterile, rudimentary anthers (Roush 1931, Schultz 2003b). The female plants have smaller, darker colored flowers (Schultz 2003a,b) and are easy to differentiate from hermaphrodites. Although most females in gynodioecious species make up only 10% to 35% of the individuals in a population and have much higher seed set (Proctor et al. 1996), the two sex morphs of *S. hendersonii* are often equally abundant and seed production of females and

hermaphrodites appears to be about equal (Marshall 1997). However, some wild *S. hendersonii* populations have a female-biased population because hermaphrodites are preferentially selected by seed-eating weevils (Marshall 1997, Marshall and Ganders 2001, Gisler and Love 2005).

*S. hendersonii* is an obligate out-crosser, with hermaphroditic flowers exhibiting protandry, in this case achieved via delayed extension of the style (Roush 1931). In hermaphrodites, the stigma remains sequestered inside the staminal column for a day or two while the anthers mature and pollen is released. By the second or third day, the styles of hermaphrodites rapidly elongate and then flare out in a whorl, exposing the sticky stigmatic surface. In female plants, for which protandry is not necessary, stigmas may emerge before the bud opens (N. Johnson, personal observation). Like *R. armeniacus*, *S. hendersonii* flowers have rich nectaries and hermaphrodites produce copious pollen, suggesting there may be overlap in the types of pollinators visiting the two species. Marshall (1997) reported visits by *Apis* (honeybee), *Bombus* (bumblebee) and *Vanessa* (butterfly) species.

The pollen of all *Sidalcea* species is large, globose and spinescent (Roush 1931), making it easy to identify. *S. hendersonii* pollen in the study plants ranged from 55 $\mu$ m to 65 $\mu$ m in diameter, in contrast to the much smaller (25 $\mu$ m to 30 $\mu$ m) pollen of *R. armeniacus*. (See Appendix A for pollen images of *S. hendersonii*, *R. armeniacus*, and other plants that were common at the study sites.) The fruit of *Sidalcea* is a small, dry capsule that typically contains 5-9 seeds (Roush 1931) clustered around a central axis. A viable seed is approximately 2mm in length (Schultz 2003a) and when the capsule matures, about a month

after flower, the papery capsule deteriorates and the seeds are released (N. Johnson, personal observation).

*S. hendersonii* is a species of conservation concern. In the 2006 petition to the U.S. Department of the Interior to list *S. hendersonii* as threatened or endangered, petitioners stated that the wildflower is threatened by habitat loss due to development, weevil predation and encroachment by invasive species, including reed canary grass (*Phalaris arundinacea*), Scotch broom (*Cytisus scoparius*) and purple loosestrife (*Lythrum salicaria*) (U.S. Dept. of the Interior 2006). As reintroduction efforts are undertaken in Oregon, managers should consider the threat of invasive species, not only as colonizers, but as potential sources of pollen limitation for this rare native wildflower. A study of an invaded *S. hendersonii* population in British Columbia (Denoth and Myers 2007) suggested no significant effect of *L. salicaria* on *S. hendersonii* abundance or vegetative growth, however the work did not involve pollination or seed set. The broad flowering overlap of *R. armeniacus* and this imperiled, obligate out-crosser, when considered along with the potential for pollinator-mediated effects of invasives to be felt over large spatial scales, suggests that the presence of *R. armeniacus* could create pollen limitation in areas being restored for *S. hendersonii*. Thus, the results of this study not only inform our understanding of the effects of invasive plants on the fitness of out-crossing natives, but also have direct conservation implications.

## Methods

### *Study site*

Research was conducted at Lake Terrell Wildlife Area, managed by the Washington Department of Fish and Wildlife, and adjacent Intalco-owned fields in Ferndale, Wash., between June and August 2009. The sites were located at approximately 48.84° N, 122.69° W, and 2km from the Georgia Strait. The surrounding area is rural, composed mostly of undeveloped land and early successional forest on private property. I chose the area for its minimal use for recreation and management, its similarity to one of the preferred habitats of *S. hendersonii* (marsh meadows), and the high level of invasion by *R. armeniacus*, which is found in large, discrete patches throughout the properties.

Few native wildflowers bloomed at the sites during the study period. Those that were present within 100 meters of the plots included a couple patches of fireweed (*Chamerion angustifolium*), a large bush of rosy spiraea (*Spiraea splendens*), scattered hound's-tongue hawkweed (*Hieracium cynoglossoides*) and half a dozen single Monterey centaury (*Centaurium muehlenbergii*). *S. hendersonii* was not present on the property prior to the study, enabling me to manipulate its spatial arrangement for the purpose of assessing how interactions with *R. armeniacus* vary with distance from the nearest patch of the invader.

Invasive plant species that were abundant at the site included reed canary grass (*Phalaris arundinacea*), woolly vetch (*Vicia villosa*), white clover (*Trifolium repens*), yellow and purple wild radish (*Raphanus sativus*), common dandelion (*Taraxacum officinale*),

common tansy (*Tanacetum vulgare*), tansy ragwort (*Senecio jacobaea*), oxeye daisy (*Leucanthemum vulgare*), lady's thumb knotweed (*Polygonum persicaria*), broadleaf plantain (*Plantago major*), and purple Canada thistle (*Cirsium arvense*). Forested areas adjacent to the study fields contained several flowering tree species – big-leaf maple (*Acer macrophyllum*), red alder (*Alnus rubra*), black cottonwood (*Populus trichocarpa*), and mountain ash (*Sorbus aucuparia*) – none of which shared bloom time with the study plants. All plants were keyed using Hitchcock and Cronquist's "Flora of the Pacific Northwest" (1973).

### *Experimental design*

This study examined the effect of *S. hendersonii* visitation, pollen deposition and seed set as a function of proximity from *R. armeniacus*. I set up three distance plots 1m, 15m, and 50m from the nearest *R. armeniacus* patch at each of the five sites. Each site was located at least 500m away from the next nearest site (Figure 1). I wanted each site to encompass a variety of environmental conditions, including wind exposure, surrounding vegetation, soil type, moisture and cardinal direction from *R. armeniacus* patches, in order to minimize the noise of site-specific environmental conditions and allow me to focus solely on the role of distance from the invader on pollen limitation.

None of the research in this study was done in the complete absence of *R. armeniacus*. Even the farthest distance treatment (50m from *R. armeniacus*) was well within foraging range of most flying pollinators (Larson and Barrett 2000). Thus, the focus of this study was on the effect of a native plant's proximity to an invader, rather than on the effect of

the presence/absence of the invader. I arranged the three distance treatments in a triangle, with each treatment about 50m from the other two distance treatments (Figure 2). This arrangement was selected to ensure that the treatments for distance from blackberry were not confounded by distance from the nearest *S. hendersonii* patch.

Three weeks prior to commencing data collection, I planted 16 *S. hendersonii* plants at each distance at each site. Plants were purchased as bare-root stock obtained from a wholesale native-plant nursery in Whatcom County, Wash. (Fourth Corner Nurseries), which grows all their plants from local seed sources. This ensured that individuals were genetically distinct from one another, and that they were of locally-adapted genotypes. Prior to planting experimental individuals, 60 plants were purchased early, grown in a greenhouse for one month and hardened off for two weeks in a sheltered location before transplanting. These plants, four at each distance location, flowered earlier than the others and were used to practice pollen-supplementation technique as well as test the appropriateness of the site conditions before the rest of the study plants were transplanted. The remaining 180 plants were transplanted on the day of purchase.

To deter mammalian herbivory of the experiment plants, the 16 plants in each distance treatment were divided into two cages that each consisted of a 1.5m-high wire fence (5.08cm by 10.16cm mesh) lined at the base with chicken wire buried to a depth of 0.3m. Within a cage, plants were spaced about 30cm apart to avoid crowding. Density at each distance cage was 8 plants in a 7m<sup>2</sup> area and the two cages were placed about 2m apart for ease of access. Prior to the start of the experiment, I removed all above-ground plant biomass

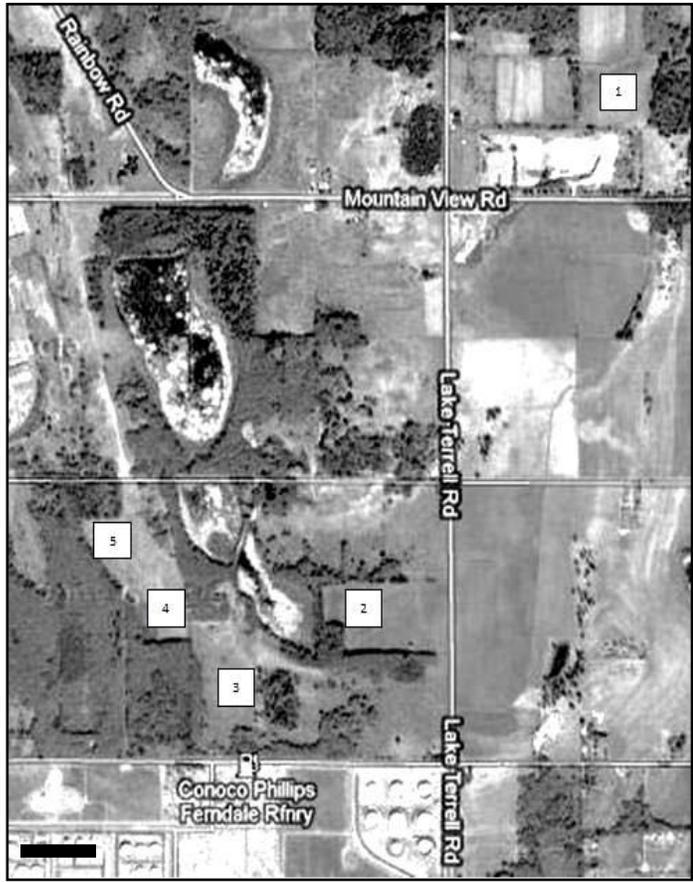


Figure 1. Aerial map of study sites, numbered 1-5, in Ferndale, Wash. Scale bar represents 200m. *Image courtesy GoogleMaps.com*

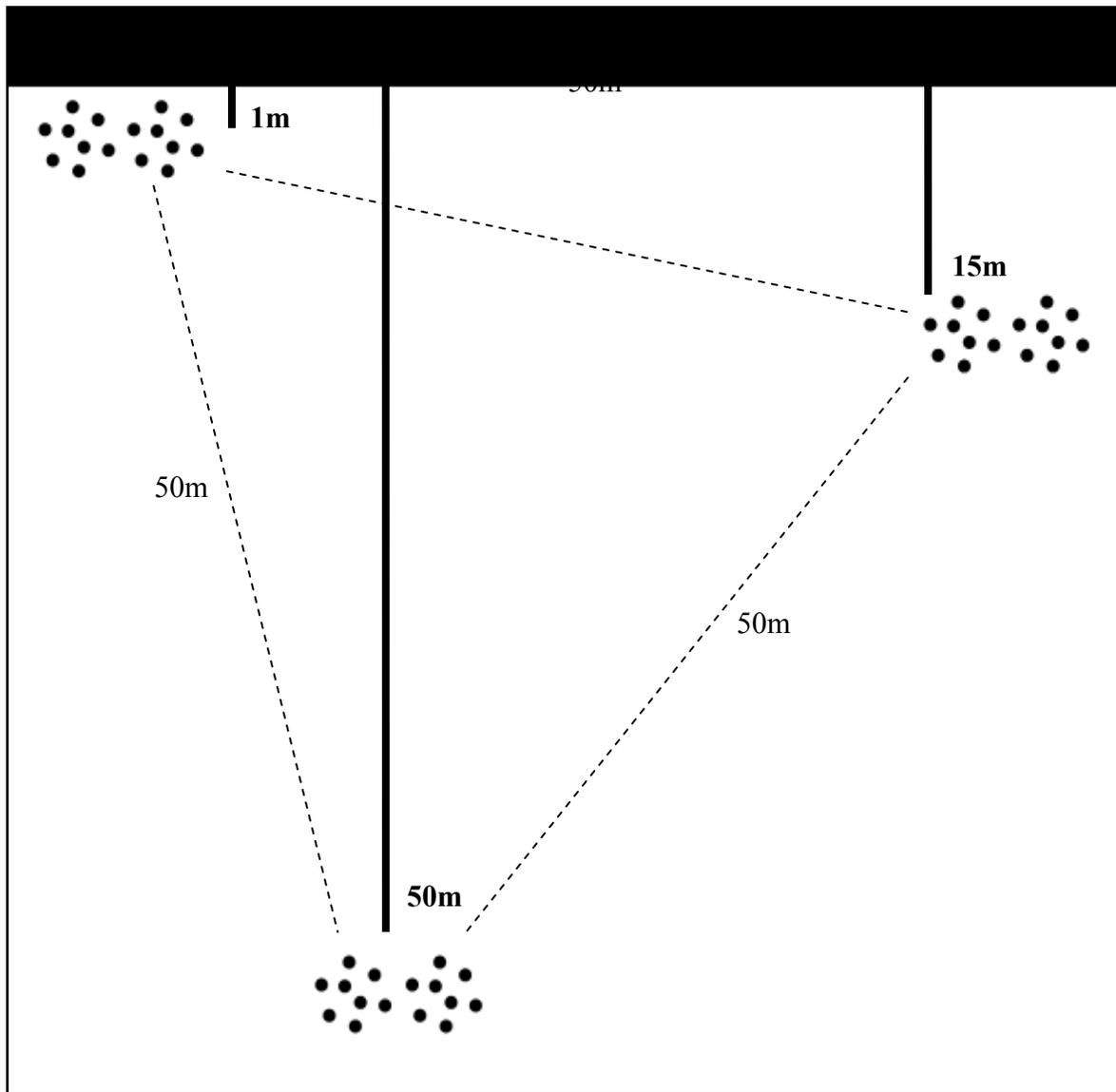


Figure 2. Schematic layout of one study site showing 16 *Sidalcea hendersonii* plants in each of three distance treatments, 1m, 15m and 50m (indicated by solid lines), as measured from the dominant *Rubus armeniacus* patch (black bar) at the site. Each distance plot was at least 50m from the other two plots (signified by the dashed lines) to ensure treatments for distance from *R. armeniacus* were not confounded by distance to the nearest *S. hendersonii* patch.

between the *S. hendersonii* plants and in a 0.5m buffer around the cages. Weeding inside the cages was maintained throughout the experiment.

### *Protocol*

#### **Pollinator observations**

To test the hypotheses that there is a relationship between distance from *R. armeniacus* and total visitation to *S. hendersonii*, I conducted pollinator observations at each site on three dates: July 10, July 16 and July 22-23 (observations on July 22 were interrupted by site maintenance workers and were completed the following afternoon). All observations were completed on sunny, calm days with temperatures between 22-32°C. On each date, the order in which I observed each site and each distance within a site was chosen at random. For each observation at a given distance at a site, one hermaphroditic *S. hendersonii* plant was selected at random to be observed for 20 minutes. I selected an inflorescence on that plant that had 3-5 flowers in bloom and recorded all pollinator visits during the 20-minute observation. I observed inflorescences for observation rather than single flowers because of the low number of *S. hendersonii* visitors in trial runs. Also, few pollinators visited more than one flower on an inflorescence during a visit.

A pollinator's visit was only recorded if it touched the interior of at least one of the flowers of that inflorescence. For each visitor, I recorded its species, length of visit (total time spent on the inside of any flower on the inflorescence) and the total number of flowers visited. To ensure correct pollinator identification, I collected specimens and/or took

photographs of pollinators. I also selected a single *R. armeniacus* flower at the dominant patch at each of the five sites for a 20-minute pollinator observation on each date, following the protocol used for pollinator observations on *S. hendersonii*. Preliminary observations indicated that visitor frequencies were high enough that observing a single *R. armeniacus* flower at a time would produce ample data.

### **Pollen delivery**

To investigate the hypothesis that there is a relationship between distance from *R. armeniacus* and the composition of pollen on *S. hendersonii* stigmas, I collected one stigma at each plot on July 4, July 10, July 16 and July 22/23 (54 stigmas, collectively). The only exception to this regular stigma collection was that six different distance plots lacked a fourth replicate due to the absence of flowers on one of the four collection days (July 4: Site 1 at 1m; July 16: Site 4 at 1m and 15m; July 22/23: Site 2 at 50m; and Sites 3 and 5 at 15m).

On each collection day, I randomly selected a plant at each distance and then selected a fully closed (i.e., no longer receptive) flower to harvest. I placed each whole flower in an Eppendorf tube and stored them in a -20°C freezer.

To quantify pollen deposition and determine the species composition of pollen deposited on *S. hendersonii* stigmas, I examined each of the collected stigmas with a Vega Tescan 5136MM Scanning Electron Microscope (Tescan USA Inc., Cranberry Twp., PA). *S. hendersonii* stigmas split into eight or more distinct sections, radiating outward from the style. Therefore, to prepare each stigma for SEM analysis, I mounted each of the sections

(under a dissecting microscope) onto double-sided tape on a single SEM tab, orienting each section so that its receptive side would be visible from a single plane of view. Each tab was assigned a random number that was used as a reference during SEM work to ensure a blind analysis of pollen. After mounting on the tab, specimens were air-dried for at least 24 hours before being coated with gold-palladium using a Quorum Technologies SC7640 Auto/Manual High Resolution Sputter Coater (Quorum Technologies, West Sussex, UK). Specimens were coated for between 120 and 180 seconds with the sputter coater set to 10 nm/min and the plasma current on 17 mA. I viewed each section under high magnification (500X-2000X) to count the number of *S. hendersonii* and *R. armeniacus* pollen grains adhering to it. All other pollen grains were counted and, whenever possible, identified to species using a pollen atlas for the common flowering species at the field sites (Appendix A).

### **Seed set**

To test the hypotheses that *S. hendersonii* is pollen-limited in the presence of *R. armeniacus* and that the extent of limitation is related to its distance from the invader, I used two pollination treatments: natural pollination and pollen supplementation. Because pollen limitation can vary significantly by site, season and even at a single site within a single season (Burd 1994), treatments were scattered throughout the field season from June to August, and limited only by the amount I could do each day. In all, I quantified the seed set of 383 naturally-pollinated flowers ( $25.53 \pm 1.63$  at each distance per site). This treatment allowed me to see if there was a distance effect due to any factors related to *R. armeniacus*,

including pollen limitation, competition for resources (e.g., light, nutrients, water), seed predation, and allelopathy.

To determine if any distance effects on *S. hendersonii* seed set resulted from pollen limitation specifically, I pollen-supplemented 383 flowers in a second treatment ( $25.53 \pm 1.63$  flowers per distance per site). By comparing the seed set of pollen-supplemented flowers at each distance to that of naturally-pollinated flowers on the same inflorescence, I could separate out the effects of other stresses from pollen limitation specifically. For example, if natural flowers showed a negative distance effect, but pollen-supplemented flowers showed no distance effect, it would suggest that the difference in seed set was indeed related to pollen limitation. Conversely, if natural flowers and pollen-supplemented flowers both showed a strong negative distance effect, it would imply that other factors were at play, such as resource competition, seed predation or allelopathy. Flowers were selected for treatment in pairs on a single inflorescence so that a pollen-supplemented flower could be compared to a flower in the same stage of maturity/receptivity as its naturally-pollinated counterpart, taking into account any individual plant effects.

To hand-pollinate the treatment flowers I collected pollen from ripe anthers on flowers in a different distance plot at the same site using fine-tipped forceps and stored it in an Eppendorf tube during transport. Although I removed pollen from experimental plants, I did not collect pollen from experimental flowers. Pollen was mixed in the tube and applied to the pollen-supplemented stigmas in a different distance treatment at that site using a clean pair of forceps. Total time between collection and delivery never exceeded one hour. With

this procedure, the pollen delivered to each stigma was fresh, but still represented several plants, each of which were different individuals from the target plants, to ensure out-crossing. Toward the end of the study, when few *S. hendersonii* flowers were still in bloom, pollen was sometimes harvested from flowers within the same distance plot; however, I was careful to avoid pollen-supplementing a flower with pollen from other flowers on the same plant.

To apply pollen, I used fine-tipped forceps to select a few anthers from the Eppendorf tube to gently brush against the stigmatic surface of the treatment flowers. This was repeated until the entire stigmatic surface was coated with pollen grains (Figure 3). Forceps were wiped clean between pollen applications to prevent contaminating the transport tube. Seed capsules in both treatments (pollen-supplemented and naturally-pollinated) were harvested as they reached maturity and placed in Eppendorf tubes or small plastic bags for storage at -20°C. Seed herbivory was scarce, but I discarded a couple of fruits that showed clear evidence of herbivory at the time of collection (presumably from songbirds). A viable seed count for each capsule was done under a dissecting microscope. Seed counts were done by depositing the seeds into a watch glass or clear bag. A viable seed count was done based on the overall appearance of the seed and in accordance with Marshall (1997). Specifically, shriveled, flattened or distinctly pale seeds were considered unviable (Figure 4).

### *Statistical analyses*

The reproductive success of both morph types (83% hermaphrodite, 17% female) was investigated in this study. Sex type is not known to affect reproductive output in this species



Figure 3. Typical pollen load on a *Sidalcea hendersonii* stigma before (left) and after (right) pollen supplementation. *Photo courtesy Merrill Peterson*

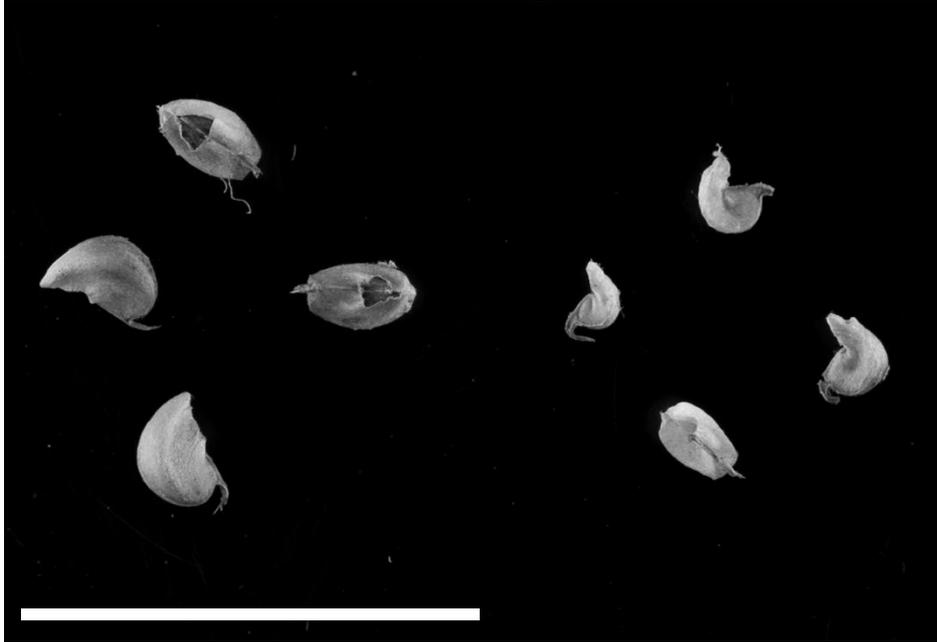


Figure 4. Viable seed counts were done of all flowers in the natural and pollen-supplemented treatments. Counts were done based on the appearance of the seed. Healthy, round, heavy seeds (left) were considered viable. Shriveled, pale or flattened seeds (right) were not included in the viable seed count. Scale bar represents 10mm. *Photo courtesy John Huddleston*

(Marshall 1997), thus for the remainder of the analyses, I pooled seed-set data for the two morph types. All visitation observations were done with hermaphroditic plants. Seven female stigmas (out of 54 total) were collected over the four days sampled, and these were also pooled with the stigmas from hermaphrodites in all analyses.

Visitation rates, pollen deposition, and seed set were all analyzed using ANOVA (SPSS v. 17.0). For visitation, date of observation was designated a random factor, while distance and site were fixed factors (Appendix B.1). Site is fixed because I did not select plots at random, but rather sought to incorporate a high degree of between-site variation. For deposition, date of collection was designated a random factor, while sex, site and distance were fixed (Appendix B.2). For seed set, plant and flower were designated random factors, while distance, site and treatment (naturally-pollinated or pollen-supplemented) were fixed (Appendix B.3). Because my naturally-pollinated and pollen-supplementation treatments were paired replicates representing two flowers at the same stage of maturity on the same inflorescence, this analysis is more powerful than randomly selected flowers on different plants, as is frequently seen in the literature.

## **Results**

### *Pollinator visitation: Number of visits*

A total of 15 hours over three days was spent observing pollinators on *S. hendersonii* inflorescences. During this time there were a total of 48 visitors ( $1.07 \pm 0.27$  visits/20 min.

(mean  $\pm$  S.E.) at 1m,  $0.8 \pm 0.13$  at 15m, and  $1.33 \pm 0.33$  at 50m). I also spent five hours over three days observing visitors to single *R. armeniacus* flowers. A total of 48 visitors ( $3.13 \pm 0.38$  visits/20 min.) were counted during these 20-minute observation periods at each of the five sites.

The first two observation days, July 10 and July 16, roughly coincided with the peak bloom time for *R. armeniacus* at the sites. By July 22-23, approximately half of *R. armeniacus* flowers were setting fruit. The total number of *S. hendersonii* inflorescences in bloom at each distance plot was tallied each day of the study and ranged from a single inflorescence to 58, with the highest density of flowers early in the study season (See Appendix C for flowering phenology of *S. hendersonii*). The above visitation rates were based on total visits to inflorescences with 3-5 flowers for *S. hendersonii* but only a single *R. armeniacus* flower. Because no pollinator visited all flowers on a single *S. hendersonii* inflorescence, one can extrapolate that *R. armeniacus* received substantially more than three times the number of visits per flower than *S. hendersonii*.

An ANOVA revealed no significant difference in the number of visitors to *S. hendersonii* as a result of proximity to *R. armeniacus* (Table 1). Multiple attempts to transform the data to remove a lack of homogeneous variance failed. A power analysis of the visitation ANOVA, using G\*Power (v. 3.1.0), revealed a 0.63 probability of detecting a large effect. This suggests that, given the substantial variance in pollinator visitation, my replication may have been insufficient to find an effect.

Table 1. Pollinator visitation frequency to *Sidalcea hendersonii* as a function of distance from the nearest *R. armeniacus* patch, site, date, and their interactions, based on observations on three observation dates in July 2009, determined by ANOVA.

Source	Type III SS	df	Mean square	<i>F</i>	p
Site	1.911	4	0.478	0.271	0.888
Distance	0.000	2	0.000	0.000	1.000
Site*Distance	9.556	8	1.194	1.254	0.332
Date	0.133	2	0.067	0.029	0.971
Site*Date	14.089	8	1.761	1.848	0.141
Distance*Date	5.867	4	1.467	1.539	0.238
Site*Distance*Date	15.244	16	0.953	-	-

### *Pollinator visitation: Types of visitors*

There was a great deal of overlap in the types of pollinators that visited the two plant species (Table 2), however the predominant visitor to each was different. European honeybee (*Apis mellifera*) was the predominant visitor to *R. armeniacus*, accounting for more than 68% of all visits. Andrenid bees and bumblebees each accounted for another 8% of all *R. armeniacus* visitors, while other species each contributed less than 5% of the total visits (Table 2). In contrast, visits to *S. hendersonii* were widely distributed among different pollinator species and types. The most prevalent visitors to *S. hendersonii* were sweat bees (Halictinae), contributing nearly 30% of all visits. A nectar-drinking ant (*Formica* sp., Hymenoptera: Formicidae) was also a frequent visitor (21%) as was the European skipper (*Thymelicus lineola*, Lepidoptera: Hesperiiidae) (15%). The introduced wharf borer (*Nacertes melanura*, Coleoptera: Oedemeridae) and leaf-cutter bees each accounted for 8% of visits, and andrenid bees and bumblebees each accounted for about 6% of visits (Table 2). This diverse assemblage suggests that *S. hendersonii* is well maintained in the existing native pollinator network, though it is notable that about one-third of all visits were by non-native insects.

Approximately 77% of the observed visits to *S. hendersonii* were by pollinator species that were also observed visiting *R. armeniacus*, indicating the potential for a high level of pollinator sharing. The only pollinator that visited *R. armeniacus* and not *S. hendersonii* was the European honeybee (*Apis mellifera*). Although abundant on *R. armeniacus* and several other flowering species surrounding the study plots, honeybees were

Table 2. Pollinators to *Rubus armeniacus* (RUAR) and *Sidalcea hendersonii* (SIHE) at the Ferndale, Wash., study sites based on observations on July 10, 16 and 22, 2009. Visits were recorded only for species active on the inner portions of the flowers. Values are the percentage each pollinator contributed to the total number of visits. Percentage visitation was not available (N/A) for pollinators that were observed before or after observation periods.

Pollinator	Order	RUAR	SIHE combined	SIHE 1m	SIHE 15m	SIHE 50m
<i>Bombus</i> spp. (bumblebees)	Hymenoptera	8.3%	6.3%	2.1%	2.1%	2.1%
<i>Naccerdes melanura</i> (orange wharf borer)	Coleoptera	2.1%	8.3%	--	2.1%	6.3%
<i>Thymelicus lineola</i> (European skipper)	Lepidoptera	2.1%	14.6%	6.3%	4.2%	4.2%
<i>Sphaerophoria</i> sp. Syrphinae (syrphid flies)	Diptera	2.1%	2.1%	2.1%	--	--
<i>Osmia</i> sp.? Megachilidae (leaf cutter bee)	Hymenoptera	4.2%	8.3%	2.1%	4.2%	2.1%
<i>Megandrena</i> sp.? Andrenidae (andrenid bee)	Hymenoptera	8.3%	6.3%	6.3%	--	--
Halictinae (sweat bees)	Hymenoptera	4.17%	29.2%	10.4%	6.3%	12.5%
<i>Symphoromyia</i> sp.? Rhagionidae (snipe fly)	Diptera	N/A	4.2%	2.1%	2.1%	--
<i>Apis mellifera</i> (European honeybee)	Hymenoptera	68.8%				
<i>Formica</i> sp. Formicidae (ant)	Hymenoptera		20.8%	4.2%	10.4%	6.3%
<i>Selasphorus rufus</i> (Rufous hummingbird)	Apodiformes		N/A	--	--	--
Total number of visitors		48	48			

never observed visiting *S. hendersonii* flowers. The only pollinators that were unique to *S. hendersonii* were an ant (*Formica* sp.) and the Rufous hummingbird (*Selasphorus rufus*).

### *Pollen deposition*

In all, 4,573 pollen grains were counted and 3,173 of these belonged to *S. hendersonii*. Conspecific pollen was present on 89% of *S. hendersonii* stigmas ( $58.76 \pm 8.63$ , mean per stigma  $\pm$  S.E.) (Figure 5). Because these flowers exhibit protandry, we can assume that the counts constitute very little pollen from the same flower. *R. armeniacus* pollen was the most prevalent heterospecific pollen type deposited on *S. hendersonii* stigmas, representing 19% of all pollen (Figure 5). At least one *R. armeniacus* pollen grain was recorded on 67% of all *S. hendersonii* stigmas ( $15.85 \pm 3.9$ , for all stigmas). *R. armeniacus* pollen was abundant (more than one-fifth of the total pollen) on 35% of all stigmas.

The majority (78%) of *S. hendersonii* stigmas also contained some level of heterospecific, non-*R. armeniacus* pollen. Pollen deposition from at least a dozen other flowering species in the study region collectively represented an average of 12% of the total pollen on all stigmas. On average, a stigma had 1.55 different species ( $\pm 0.19$ ). Most heterospecific deposition was low on a per-stigma basis. About 60% of all stigmas had fewer than three heterospecific pollen grains, although four stigmas held primarily heterospecific pollen, specifically white clover (*Trifolium repens*) (Table 3).

Interestingly, a one-way ANOVA showed no significant effect of distance from *R. armeniacus* on the amount of *R. armeniacus* pollen deposition on *S. hendersonii* stigmas.

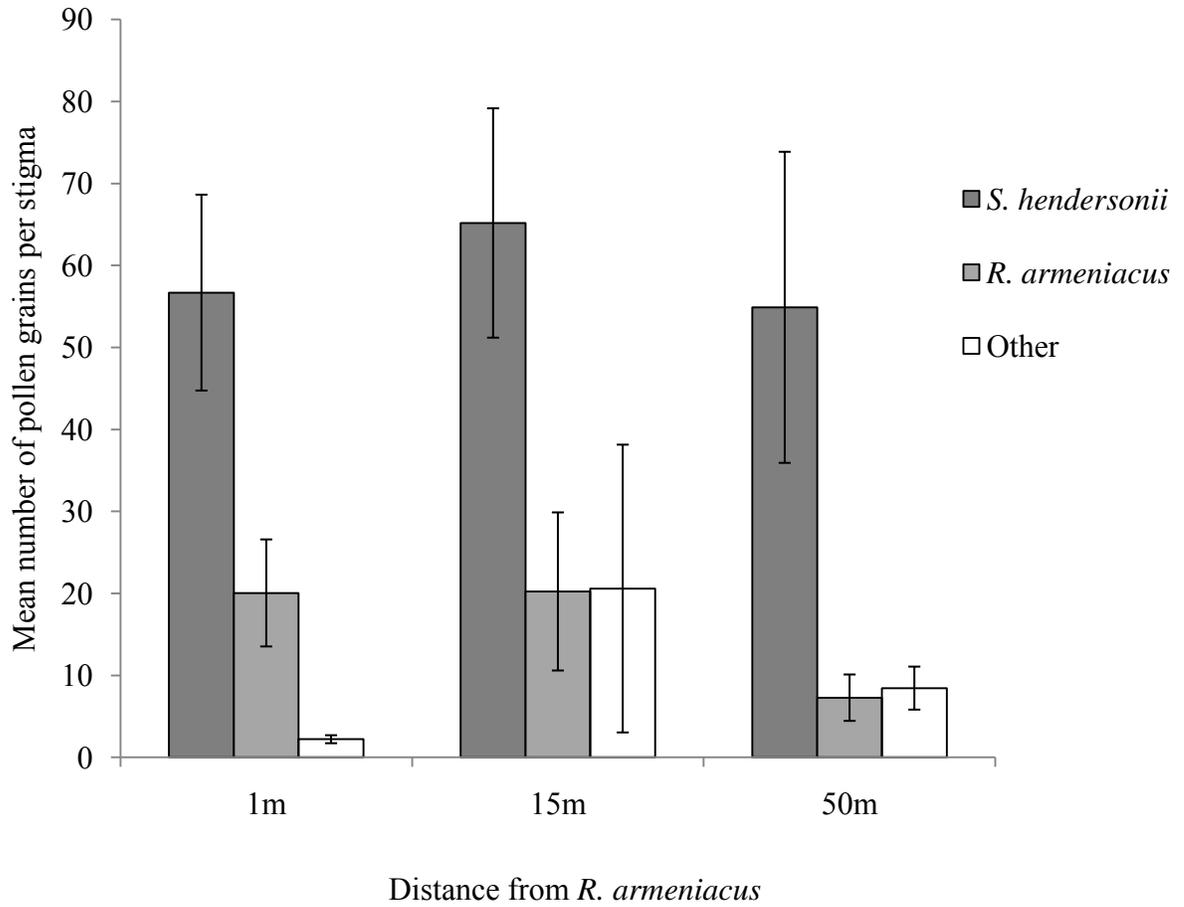


Figure 5. The mean number of pollen grains deposited on *S. hendersonii* stigmas as a function of distance from the nearest dominant *R. armeniacus* patch. Error bars are standard error.

Table 3. Composition of all non-conspecific non-*Rubus armeniacus* pollen deposited on all *Sidalcea hendersonii* stigmas. Collectively, the species below represent about 12% of the total pollen count on all *S. hendersonii* stigmas.

Pollen species	Percent of stigmas with this species	Mean number of grains/stigma $\pm$ SE
<i>Raphanus sativus</i>	30%	1.24 $\pm$ 0.81
<i>Phalaris arundinacea</i>	24%	0.67 $\pm$ 0.49
<i>Trifolium repens</i>	17%	5.48 $\pm$ 10.98
<i>Vicia sativa</i>	11%	0.30 $\pm$ 0.49
<i>Cirsium arvense</i>	9%	0.85 $\pm$ 2.42
<i>Spiraea splendens</i>	6%	0.11 $\pm$ 0.14
<i>Taraxacum officinale</i>	6%	0.06 $\pm$ 0.00
<i>Polygonum persicaria</i>	6%	0.07 $\pm$ 0.08
<i>Plantago major</i>	4%	0.04 $\pm$ 0.00
<i>Hieracium cynoglossoides</i>	4%	0.04 $\pm$ 0.00
<i>Ranunculus repens</i>	2%	0.02 n/a
Other (various unidentified)	28%	1.26 $\pm$ 0.76

N = 54 stigmas

Site, date and interactive effects were also not significant (Table 4). A separate one-way ANOVA showed no significant effect of distance from *R. armeniacus* on the amount of conspecific pollen deposition on *S. hendersonii* stigmas. Site, date and interactive effects were also not significant in this analysis (Table 5). Multiple attempts to transform the data to remove a lack of homogeneous variance failed. The power analysis for both deposition ANOVAs revealed a 0.78 probability for detecting a large effect, showing that replication was probably sufficient.

### *Seed set*

A one-way ANOVA revealed a significant effect ( $p < 0.001$ ) of pollination treatment (natural vs. pollen-supplemented) on seed set by *S. hendersonii* (Table 6, Figure 6). A total of 383 flowers were hand-pollinated compared to 383 paired, naturally-pollinated flowers on the same inflorescences. Flowers in the pollen-supplemented treatment had an average of 4.8 ( $\pm 0.18$ , SE) seeds per fruit across all sites and distances, whereas those in the natural treatment had an average seed set of 3.5 ( $\pm 0.21$ ). The difference between pollen-supplemented and naturally-pollinated flowers represents an average seed set increase of 37% across all sites and distances in response to pollen supplementation (Figure 7).

Multiple attempts to transform the data to remove a lack of homogeneous variance failed. Plant was significant ( $p < 0.001$ ), an expected outcome that is likely evidence of genetic and environmental differences between plants. However, the difference in seed set between the natural and pollen-supplemented treatments did not vary with distance from

Table 4. Deposition of *Rubus armeniacus* pollen on *Sidalcea hendersonii* stigmas as a function of distance, site, date and their interactions for a total of 60 flowers collected on four dates in July 2009, determined by ANOVA.

Source	Type III SS	df	Mean square	F	p
Site	1766.941	2	883.471	1.906	0.231
Distance	1525.667	4	381.417	0.615	0.661
Site*Distance	3078.104	3	1026.035	5.329	0.596
Date	9825.642	8	1228.205	1.397	0.266
Site*Date	2802.805	6	467.134	0.531	0.777
Distance*Date	7498.548	12	624.879	0.711	0.723
Site*Distance*Date	14943.834	17	879.049	-	-

Table 5. Deposition of conspecific pollen on *Sidalcea hendersonii* stigmas as a function of distance, site, date and their interactions for flowers collected on four dates in July 2009, determined by ANOVA.

Source	Type III SS	df	Mean square	<i>F</i>	p
Site	312.713	2	156.356	0.061	0.942
Distance	34443.123	4	8610.781	2.537	0.097
Site*Distance	10642.294	8	1330.287	0.384	0.915
Date	5436.366	3	1812.122	0.733	0.619
Site*Date	15528.148	6	2588.025	0.746	0.621
Distance*Date	40743.605	12	3395.300	0.979	0.503
Site*Distance*Date	58957.241	17	3468.073	-	-

Table 6. Effects of pollination treatment (natural vs. pollen-supplemented), distance from nearest *R. armeniacus* patch, site, and their interactions on seed set of *Sidalcea hendersonii* flowers between June and August 2009, determined by ANOVA.

Source	Type III SS	df	Mean square	<i>F</i>	p
Site	26.539	4	6.635	1.289	0.274
Distance	18.319	2	9.160	1.778	0.170
Site*Distance	90.766	8	11.346	2.196	0.027
Treatment	182.542	1	182.542	35.445	<0.01
Site*Treatment	14.547	4	3.637	0.706	0.588
Distance*Treatment	13.883	2	6.941	1.348	0.261
Site*Distance*Treatment	38.416	8	4.802	0.930	0.492
Plant	1562.829	278	5.622	1.524	<0.01

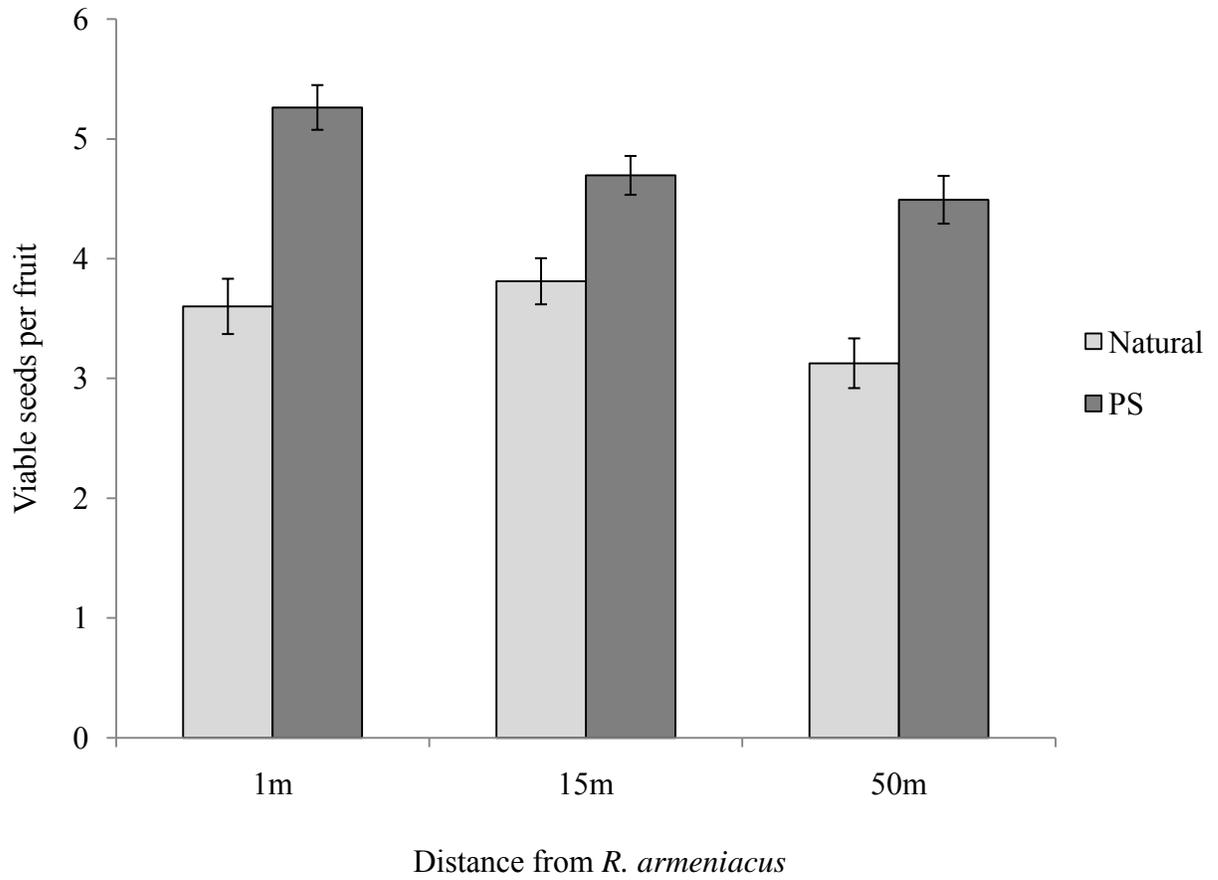


Figure 6. *Sidalcea hendersonii* seed set in bagged, pollen-supplemented and natural treatments as a function of distance from the nearest *R. armeniacus* patch. Error bars are standard error.

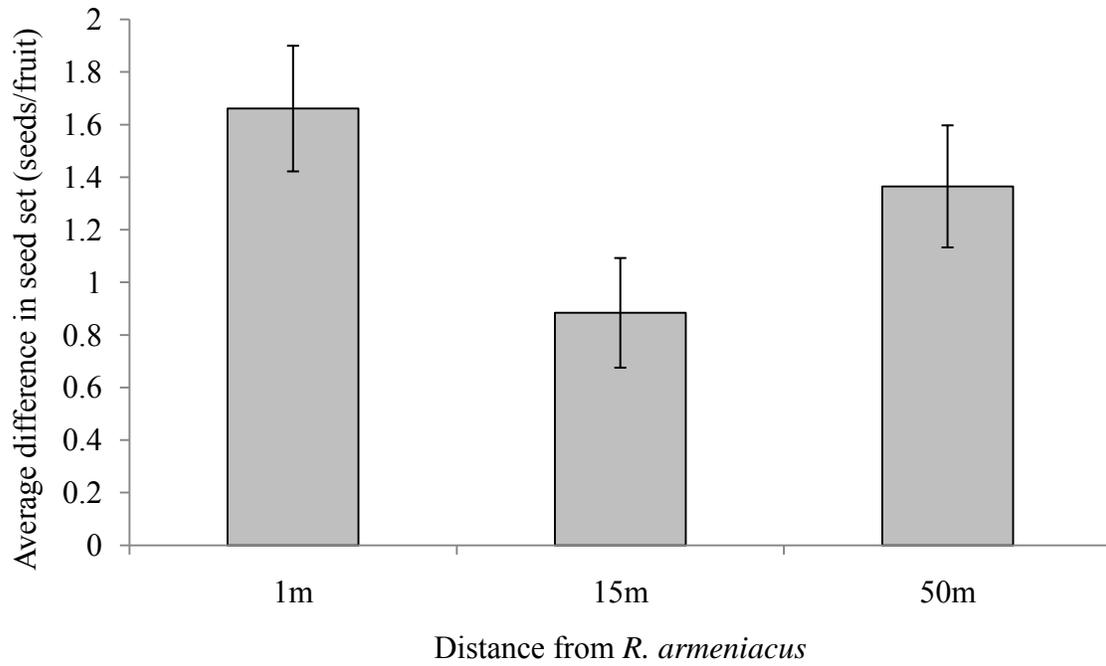


Figure 7. Average increase in seed set between paired flowers in the natural treatment and flowers in the pollen-supplemented treatment. Error bars represent standard error.

*R. armeniacus* (Table 6). A power analysis for the seed set ANOVA revealed a 0.99 probability for detecting a medium-sized effect, showing that replication was sufficient to detect any potential distance effects. There was a significant site-by-distance interaction ( $p=0.027$ ), indicating that the effect of distance varied among sites. For example, natural seed set was highest at the 1m plots at sites 3 and 5, highest at the 15m plots at sites 2 and 4 and highest at the 50m plot at site 1. Only one distance plot did not exhibit pollen limitation; the mean seed set in the natural treatment was slightly higher than the pollen-supplemented treatment at the 50m plot at site 1.

### **Discussion**

This proximity-based study found no direct evidence of invader-mediated reproductive decline in a rare, endemic wildflower. However, my observations and analyses do suggest great potential for *S. hendersonii* pollen limitation in *R. armeniacus*-invaded habitats, and the results do not rule out such an effect.

#### *Potential for negative effects*

If *R. armeniacus* grows in proximity to *S. hendersonii*, there is the potential for pollinator competition and pollinator sharing. *R. armeniacus* provides the same basic resources to pollinators, its flowering phenology overlaps with *S. hendersonii* and it frequently becomes the dominant floral resource where it invades. Based on my pollinator observations, *S. hendersonii* received less than one-third the total pollinator visits of the co-flowering invader, *R. armeniacus*. The disparity in visitation frequency may be the best illustration of the attractiveness of this widespread invader. Unfortunately, no previous published studies have quantified levels of visitation for *S. hendersonii*, so it is impossible to

know what level of visitation is “normal.” Regardless, about three-quarters of all individual visitors to *S. hendersonii* during this study were from species that also visit *R. armeniacus*. This sets up the potential for extensive pollinator competition. My study also revealed inconstant pollinator behavior in a *R. armeniacus*-invaded system. Evidence for host switching between *R. armeniacus* and *S. hendersonii* specifically is supported by the stigma analysis, which revealed that 67% of *S. hendersonii* stigmas received *R. armeniacus* pollen. *S. hendersonii* is an obligate out-crosser, which means that decreases in pollen quantity or quality can be quantified by decreased seed set. A separate, concurrent experiment gauging the seed set of pollinator-excluded flowers confirmed reliance on out-crossing (Appendix D).

#### *Lack of a distance effect*

Contrary to predictions, proximity to *R. armeniacus* over distances ranging from 1m to 50m had no significant effect on the number of visits, composition of pollen deposited, or seed set. The lack of a distance effect has three potential explanations. First, it is possible that *R. armeniacus* has no effect on the reproductive success of *S. hendersonii*, despite their shared pollinators and the frequent deposition of *R. armeniacus* pollen on *S. hendersonii* stigmas. The lack of an effect of distance from *R. armeniacus* on seed set in either the natural or pollen-supplemented treatments (Figure 6) suggests that there is neither a pollen-related distance effect, nor any other resource-related effect as a function of distance. Further evidence against pollen limitation due to *R. armeniacus* specifically is that the primary pollinator for *R. armeniacus* is an alien insect, the European honeybee (*Apis mellifera*), whereas the primary pollinators for *S. hendersonii* were native sweat bees (Halictinae) and *Formica* ants (Formicidae). Interestingly, *A. mellifera* was never observed pollinating *S.*

*hendersonii* at the site, although it has been reported in wild *S. hendersonii* populations in British Columbia (Marshall 1997).

Alternatively, the lack of a distance effect in my study may be attributable to negative effects of *R. armeniacus* on the reproductive success of *S. hendersonii* that extend over a large area, and thus effects were similar across all distances that were included in this particular study. In addition to the magnitude of the mean pollen limitation revealed by the pollen-supplementation experiment, evidence for a widespread effect is that more than half (62%) of all “paired” pollen-supplemented flowers set higher seed than their counterparts in the natural-pollination treatment and only one distance plot did not show a mean increase in seed set when pollen-supplemented. A third possible explanation, that the experiment was not sufficiently replicated to detect an effect of distance from *R. armeniacus*, is not supported by the power-analysis results (0.99 probability for detecting a medium-sized effect).

#### *Pollen limitation in context*

Pollen limitation is a common occurrence in plant communities, as evidenced by a review by Knight et al. (2005) that estimated that 63% of angiosperms were significantly pollen-limited. They looked at studies of 482 plants and concluded that pollen limitation is widespread and common in many plant families. Pollen limitation is of particular concern in invaded ecosystems, where novel plant-pollinator interactions may be formed to the detriment of native flora (Kearns et al. 1998). Interspecific interactions such as pollinator competition, pollinator sharing and changes in overall pollinator abundance and diversity can affect the reproductive success of native flora (Rathcke 1988, Morales and Traveset 2009).

For wildflowers such as *S. hendersonii* that are already imperiled, added stress on reproductive capacity is of utmost concern (Gisler and Love 2005).

The 37% increase in seed set that I observed in pollen-supplemented *S. hendersonii* plants compared to those in the natural pollination treatment is similar in magnitude to the degree of pollen limitation documented in other studies of the effects of invasives on native seed set. For example, Brown et al. (2002) found ~22% to 34% reduced seed set in native *Lythrum alatum* when grown in *L. salicaria*-invaded plots. Kandori et al. (2009) found 2% to 35% reduced seed set in a native dandelion in the presence of invasive *Taraxacum officinale*. And Flanagan et al. (2009) found that *Mimulus ringens* seed set was reduced by 34% when the wildflower grows in the presence of invasive *L. salicaria* because pollinators lost *M. ringens* pollen during visits to the invader. Nonetheless, because pollen limitation can occur even in uninvaded systems (Ashman et al. 2004, Knight et al. 2005), it would be premature to conclude that the pollen limitation of *S. hendersonii* in this study is due to interactions with *R. armeniacus* or other invasive plants. For example, Bell et al. (2005) found that a native *Mimulus* also suffered a 37% reduced seed set in the presence of a commonly sympatric native *Lobelia*.

Although few fruits in the natural-pollination treatment set maximum seed (8-9 seeds/capsule), the average natural-pollination seed set in my study population, 3.52 ( $\pm 0.12$  SE) falls within what has been observed in wild populations. Marshall (1997) reported viable seed counts and weevil predation rates in naturally-occurring British Columbia populations of *S. hendersonii* at Comox Spit, Ladner, Port Alberni and Sayward. The average seed set in these locations, prior to weevil predation, was 3.60 ( $\pm 0.12$ , SD). *R. armeniacus* was only

present at one of the sites, although all sites had some level of invasion by non-native plants. Researchers are also collecting data on imperiled Oregon populations. Without widespread quantification of pollen limitation in *S. hendersonii*, it is difficult to determine if the level of pollen limitation documented in the present study is on par with or substantially different from the degree to which pollen limitation impacts this species in other habitats. The paucity of such data generally challenges research on the impacts of invasive plants on pollination limitation, given that studies of pollen limitation in natural systems are rare and the degree of pollen limitation in such systems is likely to be influenced by the stochastic nature of pollination ecology (Knight et al. 2005, Elliot and Irwin 2009). Nonetheless, the fact that seed set in my *R. armeniacus*-invaded sites was similar to that in sites in B.C. without *R. armeniacus* (Marshall 1997) suggests that the presence of *R. armeniacus* has not severely impacted the reproductive success of *S. hendersonii*.

### **Pollen deposition**

The majority of pollen found on *S. hendersonii* stigmas was conspecific, however heterospecific pollen was present on 76% of *S. hendersonii* stigmas. *R. armeniacus* was the most common of these, being found on 67% of all stigmas analyzed. *S. hendersonii* stigmas also had pollen from several other showy invaders present in the study area, including *Raphanus sativus*, *Cirsium arvense*, *Trifolium repens* and *Vicia sativa*, evidence that individual pollinators were inconstant foragers in this system. Also, several unidentified pollen grains, presumably from wind-pollinated species, were found on many stigmas. Although in most cases such deposition was minor (fewer than 3 pollen grains per stigma), previous studies have shown that even single grains of allelopathic grass pollen can compromise the germination of conspecific pollen (Murphy and Aarssen 1995).

### Seed set limitations

The average seed set of the pollen-supplemented group,  $4.8 (\pm 0.18, \text{SE})$  seeds per flower, is lower than might be expected by hand-pollination, given that *S. hendersonii* flowers are capable of producing up to 9 seeds (Roush 1931, Marshall 1997). Many wild populations do not set maximum seed (Beekman and Ratnieks 2000). This may be because of resource deficiencies or possibly it is an adaptation that helps extend the life of the parent plant (Proctor et al. 1996). Thus, the sub-maximal average seed set observed may be related to a variety of abiotic, site- and plant-specific factors. First, the study plants (all being juveniles) may not have allocated as much of their resources to floral display or nectar, pollen or ovule production in their first year, but rather to investment in vegetative growth and root formation. None of the plants in the study grew more than 0.7m tall, whereas the typical size of mature *S. hendersonii* plants is 1.5m (Clark 1976, Gisler and Love 2005). Evidence that *S. hendersonii* seed set may be low early in its first year comes from an experimental population set up in a greenhouse by Marshall (1997), where hand-pollinated hermaphrodite flowers on first-year plants set an average of  $2.27 (\pm 2.38 \text{ st. dev.})$  seeds per fruit and hand-pollinated females flowers on first-year plants set an average of  $4.35 (\pm 3.25 \text{ st. dev.})$  seeds per fruit. These low averages suggest that *S. hendersonii* is reproductively challenged, but not due to pollen limitation.

Other factors at play in my population include: general resource limitations, pollen allelopathy from invasive sympatric species, stress from being transplanted or unobserved pollen and/or seed herbivory. Another consideration when examining overall reproductive ability is that at the time of this study's initiation all sites were covered with invasive grasses and site preparation included removal of a good portion of the A-horizon to remove root

mass. Thus, the soil may have been deficient in essential nutrients and lacking structure. Additionally, most of the plots were likely on the dry end of *S. hendersonii*'s preferred habitat spectrum (Gisler and Love 2005), particularly as the summer progressed. Lastly, although I weeded the interior of the plots throughout the season, there was substantial heterospecific seedling recruitment in and around the plots, which may have exerted competitive pressure on the roots of study plants, specifically during the flowering and fruiting periods.

Pollen allelopathy was not investigated in this study, however *P. arundinacea* and several other unidentified but seemingly wind-dispersed pollen grains (<15µm in diameter) were found on about 35% of stigmas. Effects of heterospecific pollen deposition may contribute to the low seed set in even the pollen-supplemented treatment. The biochemical effects of *P. arundinacea* deposition in particular is worth investigating because this invader is already known to invade *S. hendersonii*'s preferred coastal and meadow habitats (U.S. Dept. of the Interior 2006). Marshall (1997) did not identify grasses to genus at her British Columbia sites, so it is unknown if *P. arundinacea* was present. Lastly, seed or pollen predation may have been an unnoticed stress at the site. A couple of click beetles (Elateridae) were found nestled in the flowers I collected for pollen analysis, presumably feeding on pollen. Thus, pollen that was successfully deposited may have been eaten before pollen-tube germination. This would have been true in both pollen-supplemented and natural treatments.

Like its congeners, *S. hendersonii* is an obligate out-crosser (Roush 1931). The results of the bagged-flower experiment confirmed previous results (Marshall 1997) that seed set is drastically reduced in the absence of pollinators. Seed set in this treatment averaged 0.64

seeds per fruit, an inflated average since some pollinators were able to penetrate the pollinator-exclusion bags (Appendix D). This dependence on pollinators has important implications for an already rare species, particularly in light of the current state of pollinators (Lopezaraiza-Mikel et al. 2007). Pollinator populations are threatened in many regions due to climate change, habitat loss, and disease (Sárosspataki et al. 2005, Memmott et al. 2007, Hoehn et al. 2008). Destabilization of pollen webs is likely to have the greatest effects on rare, obligate out-crossers such as *S. hendersonii* because these plants cannot self-fertilize and pollen donors will be scarce in small, isolated populations (Waites and Agren 2004) or those impacted by habitat fragmentation (Brown and Kephart 1999). This situation can be further exacerbated if neighboring plants present a greater floral reward (Chittka and Schürkens 2001). Additionally, isolated *S. hendersonii* populations (Gisler and Love 2005) may be subjected to Allee effects (Groom 1998). For example, in remote populations, females may receive very little pollen and hermaphrodites may receive mostly pollen from other flowers on the same plant (Kearns et al. 1998). The former would lead to reduced seed set due to lowered pollen quantity, and the latter could result in inbreeding depression.

The level of pollen limitation of any population varies by season (Burd 1994, Brown et al. 2002, Knight et al. 2005), partly as a result of the great fluctuation in pollinator composition and abundance from season to season and from year to year (Proctor et al. 1996). Because different pollinator species may dominate at a given time, pollinator sharing and pollinator competition will differ depending on the players involved. The introduction of non-native agricultural and invasive pollinators (Kearns et al. 1998) adds another layer of new interactions to the mix. Perennials like *S. hendersonii* have an inherent advantage in this dynamic system because pollen limitation would need to be persistent for multiple years to

have long-term effects on population size and genetic diversity. Thus, despite the many potential causes of non-maximal seed set in my study population and in the B.C. populations studied by Marshall (1997), this low level of seed may be a fundamental aspect of *S. hendersonii* reproduction strategies.

#### *Restoration and conservation of a rare endemic*

Conservationists who intend to restore native wildflowers and shrubs should take into consideration the reproductive characteristics of the plant in question, the health of local pollination guilds and the extent of local plant invasions – not only in the immediate vicinity of the planting work, but in the floral neighborhood. *R. armeniacus* is a common invader of natural habitats in the Pacific Northwest and co-occurs with *S. hendersonii* (Marshall 1997). *R. armeniacus* creates dense monocultures and out-competes native species, including other *Rubus* species such as *R. parviflorus*, *R. spectabilis* and *R. ursinus* (Fierke and Kauffman 2006) that have similar floral morphology and attract similar pollinators, but flower earlier in the season (Hitchcock and Cronquist 1973). Thus, encroachment represents a change in understory cover as well as a change in floral timing. Not only are the remaining late-flowering species forced to compete with the showy invader for pollinator attention, but early-flowering species can be reduced or completely lost from a system, which may affect the overall distribution and diversity of pollinators (Lazaro et al. 2009). Recent evidence that supports the resiliency of pollinator networks was documented by Montserrat et al. (2009), who found abundant reshuffling of pollination networks in Europe in response to invasion. Despite the turnovers in dominant pollinator species, they deemed most of these pollination webs generally robust and noted that pollination of native plants was maintained when the level of plant invasion was moderate (Montserrat et al. 2009). That said, the infiltration of

showy non-native plants like *R. armeniacus* into native pollination webs may also be accompanied by an increase in generalist non-native pollinating insects. An influx of such generalist species may service most plants, however the increase in novel pollinator species may displace native pollinators, reducing the overall diversity of the pollination network (Waser et al. 1996, Kearns et al. 1998, Aizen et al. 2008a).

Establishment of *S. hendersonii* study plants was successful at all of my study sites, despite very different soil, moisture and light conditions; in general the plants proved to be quite hardy. They recovered well from drought stress, persevered in the face of minor slug and insect herbivory and all produced flowers and viable seed in their first year. None were lost during the experiment, though caging is important while populations get established, as several un-caged test plants were quickly stripped by resident deer. The best evidence of resiliency in the face of pollinator competition with sympatric invasives is that in this study system *S. hendersonii* was successfully serviced by many pollinators representing several taxa. Although the pollinators observed on *S. hendersonii* plants are generalists that visited other species in the study region, the overall diversity of the assemblage – including birds, nectar-loving ants, several bees, a beetle and a butterfly – suggests that the experimental *S. hendersonii* plants integrated well into the existing pollinator network. The variety and abundance of visitors provides evidence that *S. hendersonii* populations will be well serviced by pollinators even when it is restored to areas in which it has not existed for several decades or areas that lack the native suite of pollinators with which the species may have co-evolved.

The high level of pollinator diversity enjoyed by *S. hendersonii* at my sites may have been due to the presence of *R. armeniacus*, which provides floral resources in areas that have

been too disturbed by human development to support native flora, such as roadsides, urban and agricultural areas (Graves and Shapiro 2003). In the wake of increased stress on pollinator communities (Sároszpataki et al. 2005), some invasive species may also provide foraging corridors between habitat fragments (Wilcock and Neiland 2002). However, it is important to recognize that the influx of showy invasive plants does not necessarily equate to a boost in native pollinator populations. Observations at the study site revealed that 33% of all visits to *S. hendersonii* and 73% of all visits to *R. armeniacus* were by non-native pollinators, specifically *Apis mellifera*, *Nacerdes melanura* and *Thymelicus lineola*. It is notable that *T. lineola* is a recent invader, having moved into the region within the last decade (Merrill Peterson, personal observation). Thus, while invasive plants may support pollinators in florally-impoverished sites, the assemblages of those pollinators may include a high proportion of non-native species.

Lastly, the main source of *S. hendersonii* seed predation reported in British Columbia and Oregon populations are weevils (*Macrorhoptus sidalcea* and *Anthonomus melancholicus*) (Marshall 1997, Gisler and Love 2005). These weevils caused up to 73% of total seed mortality in B.C. populations (Marshall 1997, Marshall and Ganders 2001). The fact that weevils were not observed at my study sites suggests that restored *S. hendersonii* populations may actually enjoy greater reproductive fitness than remnant endemic populations, at least in the short term.

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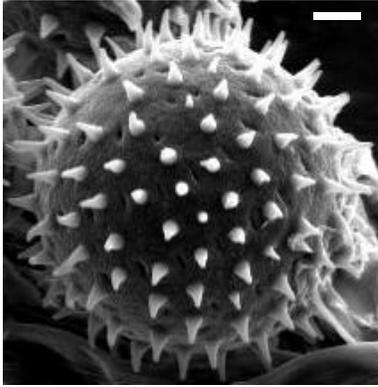
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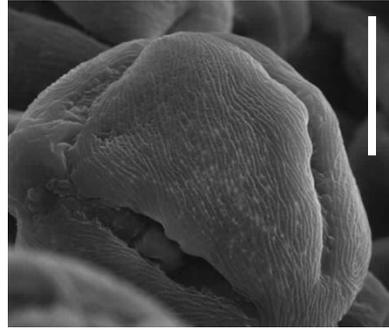
## Appendix A

Appendix A. A pollen atlas for the common flowering plants at the Ferndale, Wash., study sites in the summer of 2009. Pollen was collected directly from anthers and all questionable or poor samples were cross-referenced with pollen from Western Washington University's herbarium. All scale bars represent 10 $\mu$ m. The \* signifies a non-native species.

### I. Focal species

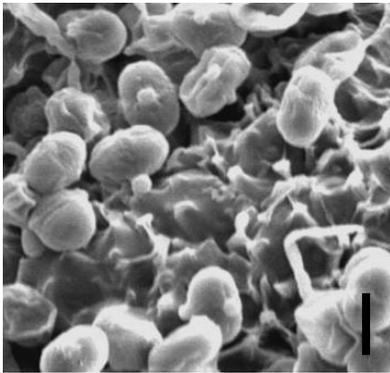


*Sidalcea hendersonii*  
Henderson's checkermallow

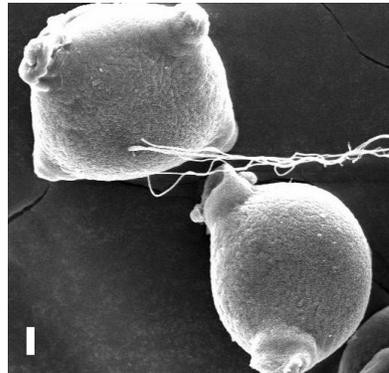


\**Rubus armeniacus*  
Himalayan blackberry

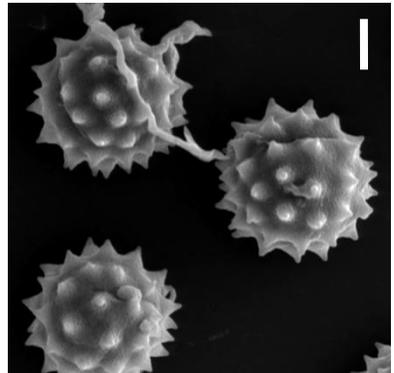
### II. Other common species



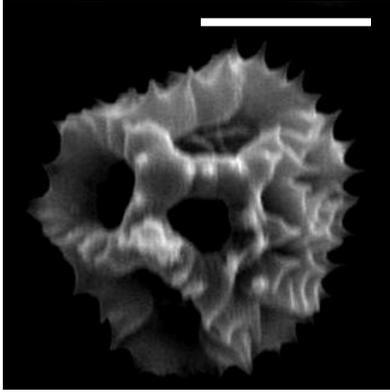
*Centaurium muehlenbergii*  
Monterey centuary



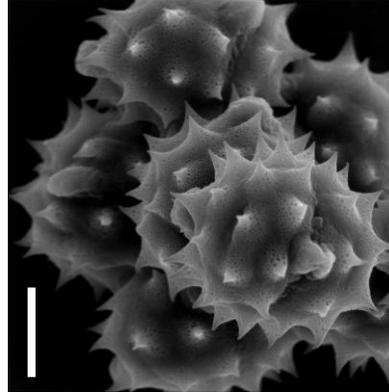
*Chamerion angustifolium*  
Fireweed



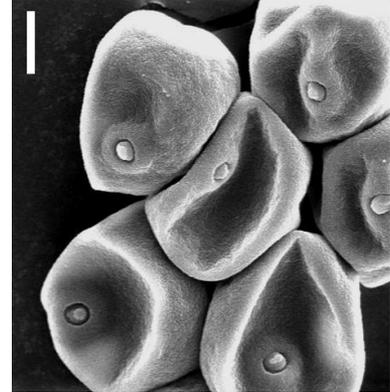
\**Cirsium arvense*  
Canada thistle



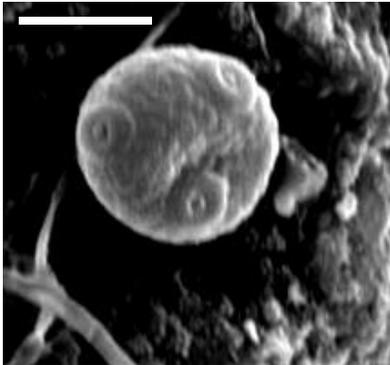
*Hieracium cynoglossoides*  
Hound's-tongue hawkweed



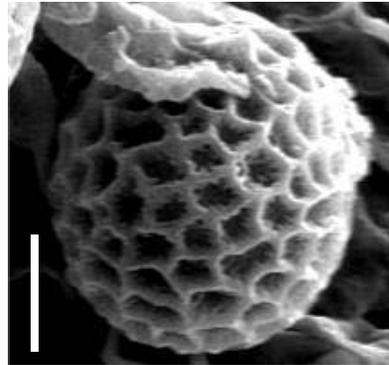
\**Leucanthemum vulgare*  
Oxeye daisy



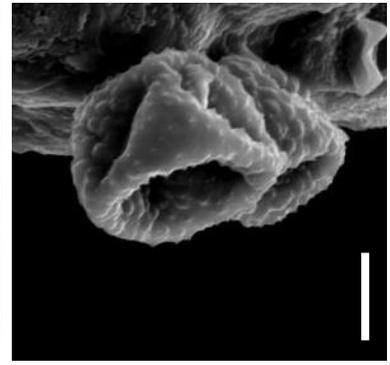
\**Phalaris arundinacea*  
Reed canary grass



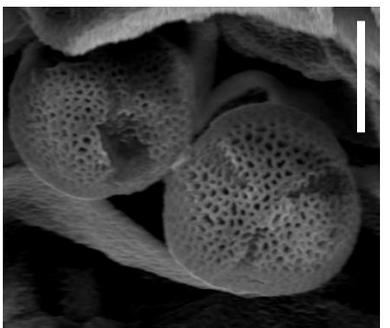
\**Plantago major*  
Broadleaf plantain



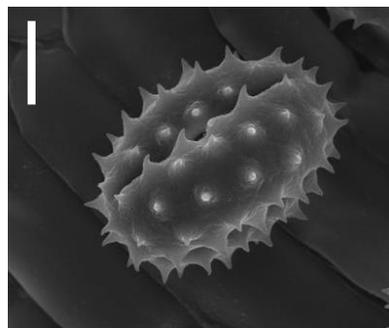
*Polygonum persicaria*  
Lady's thumb knotweed



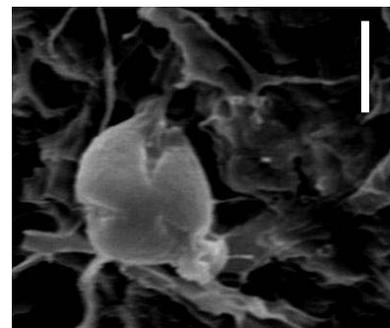
\**Ranunculus repens*  
Common buttercup



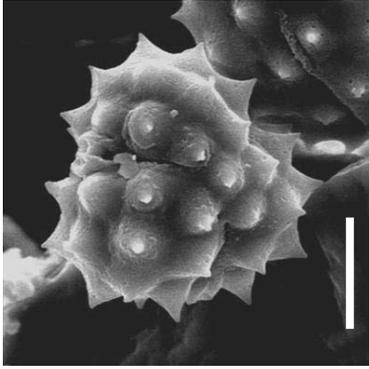
\**Raphanus sativus*  
Wild radish



\**Senecio jacobaea*  
Tansy ragwort



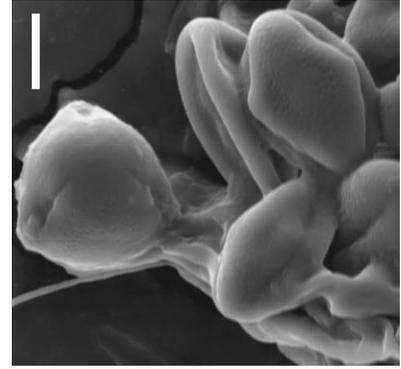
*Spiraea splendens*  
Rosy spiraea



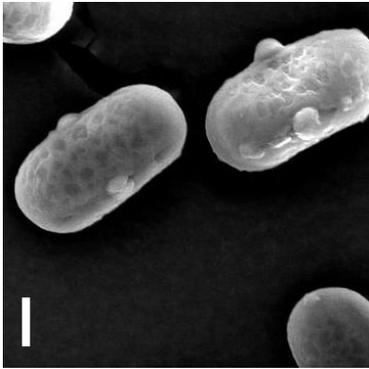
*\*Tanacetum vulgare*  
Common tansy



*\*Taraxacum officinale*  
Common dandelion



*\*Trifolium repens*  
White clover



*\*Vicia villosa*  
Woolly vetch

## Appendix B

Appendix B.1. ANOVA model for pollinator visitation to *Sidalcea hendersonii* as observed during three days of observation in July. There were five sites, each with three distance treatments (1m, 15m, 50m) for a total of 45 20-minute observations. Site (S) and distance (D) factors are fixed; date (A) is random.

$$Y_{ijk} = \mu + S_i + D_j + SD_{ij} + A_k + SA_{ik} + DA_{jk} + SDA_{ijk}$$

Source	df	EMS	F ratio; df
$S_i$	4	$\sigma_{SDA}^2 + 3\sigma_{SA}^2 + 9\theta_S^2$	MSS/MSSA; 4,8
$D_j$	2	$\sigma_{SDA}^2 + 5\sigma_{DA}^2 + 15\theta_D^2$	MSD/MSDA; 2,4
$SD_{ij}$	8	$\sigma_{SDA}^2 + 3\theta_{SD}^2$	MSSD/MSSDA; 8,16
$A_k$	2	$\sigma_{SDA}^2 + 5\sigma_{DA}^2 + 15\sigma_A^2$	MSA/MSDA; 2,4
$SA_{ik}$	8	$\sigma_{SDA}^2 + 3\sigma_{SA}^2$	MSSA/MSSDA; 8,16
$DA_{jk}$	4	$\sigma_{SDA}^2 + 5\sigma_{DA}^2$	MSDA/MSSDA; 4,16
$SDA_{ijk}$	16	$\sigma_{SDA}^2$	

Appendix B.2. ANOVA model for pollen deposition on *Sidalcea hendersonii* stigmas collected on four days in July 2010 from five sites, each with three distance treatments. Site (S) and distance (D) are fixed factors, date (A) is random. A total of 54 stigmas were collected (six distance plots lacked a third replicate stigma).

$$Y_{ijk} = \mu + S_i + D_j + SD_{ij} + SDA_{(ij)k}$$

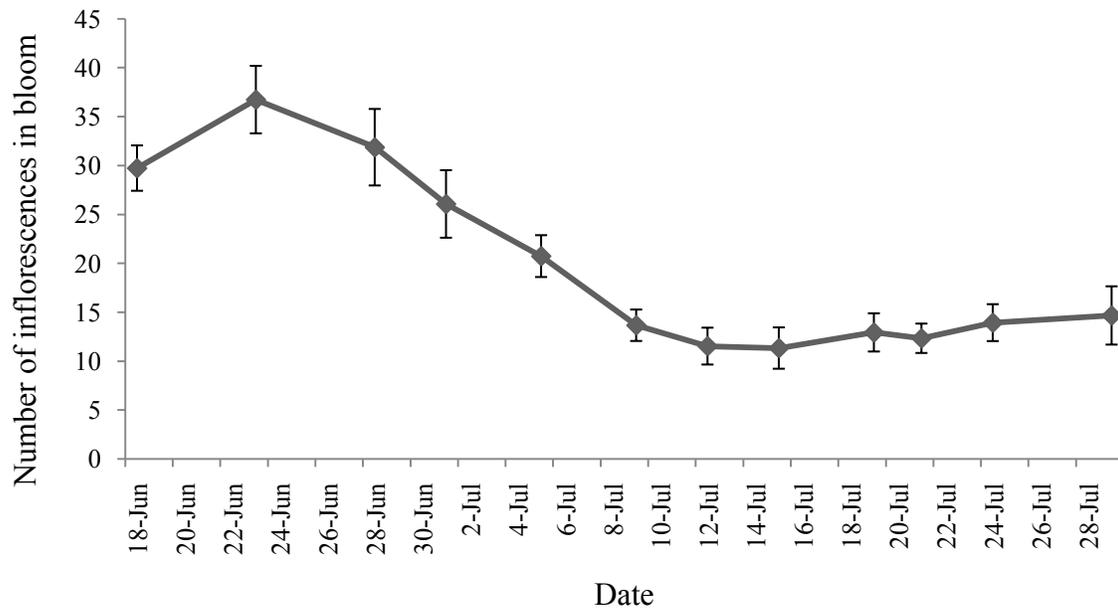
Source	df	EMS	F ratio; df
$S_i$	4	$\sigma_{SDA}^2 + 12\theta_S^2$	MSS/MSSDA; 4,45
$D_j$	2	$\sigma_{SDA}^2 + 20\theta_D^2$	MSD/MSSDA; 2,45
$SD_{ij}$	8	$\sigma_{SDA}^2 + 4\theta_{SD}^2$	MSSD/MSSDA; 8,45
$SDA_{ijk}$	45	$\sigma_{SDA}^2$	

Appendix B.3. ANOVA model for *Sidalcea hendersonii* seed set in natural vs. pollen-supplemented treatments at five sites, each with three distance plots (1m, 15m, 50m). Site (S), distance (D) and treatment (T) are fixed factors, plants (P) and flowers (F) are random factors. Each treatment is given to a total of 360 flowers, at least two flowers on each of 12 plants (actual numbers in each distance plot varied).

$$Y_{ijklm} = \mu + S_i + D_j + SD_{ij} + T_k + ST_{ik} + DT_{jk} + SDT_{ijk} + P_{(ijk)l} + F_{(ijkl)m}$$

Source	df	EMS	F ratio; df
$S_i$	4	$\sigma_F^2 + 2\sigma_P^2 +$	MSS/MSP; 4,330
$D_j$	2	$\sigma_F^2 + 2\sigma_P^2 +$	MSD/MSP; 2,330
$SD_{ij}$	8	$\sigma_F^2 + 2\sigma_P^2 + 48\theta_{SD}^2$	MSSD/MSP; 8,330
$T_k$	1	$\sigma_F^2 + 2\sigma_P^2 + 360\theta_T^2$	MST/MSP; 1,330
$ST_{ik}$	4	$\sigma_F^2 + 2\sigma_P^2 + 72\theta_{ST}^2$	MSST/MSP; 4,330
$DT_{jk}$	2	$\sigma_F^2 + 2\sigma_P^2 + 120\theta_{DT}^2$	MSDT/MSP; 2,330
$SDT_{ijk}$	8	$\sigma_F^2 + 2\sigma_P^2 + 24\theta_{SDT}^2$	MSSDT/MSP; 8,330
$P_{(ijk)l}$	330	$\sigma_F^2 + 2\sigma_P^2$	MSP/MSF; 330,360
$F_{(ijkl)m}$	360	$\sigma_F^2$	

### Appendix C



Appendix C. Flowering phenology of *Sidalcea hendersonii* across all five sites and all three distance plots therein. Error bars are standard error.

## Appendix D



Appendix D. To corroborate previous evidence (Marshall 1997) that *S. hendersonii* is an obligate out-croser, I quantified seed set in the absence of pollinators by bagging flowers with pink mesh (~1mm) draw-string bonnets when the flowers were in the bud stage. I bagged a total of 198 flowers ( $12.62 \pm 0.99$  (Mean  $\pm$  S.E.) at each distance at each site) throughout the season, 28 female and 161 hermaphroditic.

The results indicate that *S. hendersonii* is an obligate out-croser as seed set in this treatment averaged 0.64 seeds per fruit (c.f., Figure 6). Only 11% of the bagged female flowers produced seeds while 27% of the bagged hermaphrodites produced at least one seed. All seeds that were produced by flowers in this treatment may be attributed to either “selfing” (in the case of the hermaphrodites) and/or pollinators subverting the mesh material (both sex morphs). Rufous hummingbirds, specifically, were observed penetrating the bags on several occasions.