

Nectar and Pollen Rewards of Invasive Plants and their Native Counterparts

Student: Kimberly A. Lorentz

Mentors: Dr. Rebecca E. Irwin and Dr. Jessamyn Manson

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Abstract

Many invasive plants are equipped with a suite of mechanisms that can allow them to out-compete native plants. Recently, there has been growing recognition that invasive plants may affect native plants by luring away shared pollinators. The goal of this research was to explore the properties that make invasive plants strong competitors for pollination services from insects and birds. I addressed the question: How commonly do invasive plants offer more attractive nectar and pollen rewards than their native counterparts? I examined quality and quantity of floral rewards from native-invader pairs by measuring nectar production rate, nectar concentration, pollen volume, and pollen protein content. For all of the response variables except nectar production rate, most or all of the invasive plants were more rewarding than their native counterparts. To assess whether invasives offering more rewarding nectar and pollen than natives is a general phenomenon will require the study of a broader selection of species pairs.

Key Words: pollinators, mating system, competition, preference, nectar, pollen

Introduction

Invasive species are a leading component of environmental change and a second leading cause of biodiversity loss worldwide (Houlahan and Findlay 2004). Invasives by definition, are species establishing in the wild beyond their natural distribution ranges following transportation of whole plants or propagules by humans or human related activities (Mooney and Hobbs 2000) and are typically associated with negative impacts on the environment, human activities, or human health (Lee 2002). Invasive plants are known to infest agricultural fields and reduce crop yields and cattle forage (Pimentel et al. 2000), restrict recreational access to infested areas, increase soil erosion, and form dense stands that dry and become fire hazards (Erickson and White 2007). Many invaders are equipped with a wide suite of mechanisms that help them out-compete native plants. These include resistance to native herbivores and disease as well dominating nutrients and space (Ledger and Forister 2005). There is also growing recognition that invasive plants can affect native plants by luring away shared pollinators (reviewed in Bjerknes et al. 2007); however, the mechanisms driving such changes in pollinator behavior are not well explored.

Several studies suggest that certain invasive plants may be superior competitors than their native congeners because they offer more attractive floral rewards for pollinators. Chittka and Schürkens (2001) found that the invasive forb *Impatiens glandulifera* (Balsaminaceae), offered significantly more nectar than the native and morphologically similar *Stachys palustris* (Lamiaceae), a trait which correlated with reduced pollinator visitation and subsequent seed set of co-occurring *S. palustris*. Similarly, Brown et al. (2002) observed that the highly invasive *Lythrum salicaria* (Lythraceae) has larger and more numerous flowers, and greater nectar and

pollen rewards, than native *Lythrum alatum*, making the invasive a putatively more attractive plant than its native congener. Furthermore, a study of invasive *Linaria vulgaris* (Plantaginaceae) demonstrated that it may be especially attractive to pollinators because its nectar has a higher sugar concentration than that of many native plants (Irwin, unpublished data). While these individual studies suggest that increased floral rewards may be a general pattern among invasive species compared to natives, a broad comparative investigation of pollen and nectar in native and invasive plants has yet to be done.

The purpose of this project was to explore nectar and pollen characteristics that make invasive plants strong competitors for the pollination services of insects and birds. I addressed the question: How commonly do invasive species offer more rewarding nectar and pollen than their native counterparts? I examined floral rewards (flower number and nectar and pollen quantity and quality) from a broad selection of native-invader species pairs. I quantified nectar and pollen rewards by measuring: nectar production rate, nectar concentration, total pollen volume, and collected pollen samples for future nitrogen content analysis (to measure protein content). I predicted that invasive species that are obligate outcrossers (Table 1) and rely solely on pollinators for reproduction will be significantly more rewarding than native plants. I also predicted that there would not be a strong difference between invasive plants that are self-compatible (Table 1) and their native congeners, since insects or other animals are not necessary to mediate pollination.

Methods

Taxon sampling. I selected four species from the Colorado Noxious Weeds List (Colorado Department of Agriculture) that are common to the Gunnison basin (Taylor 1999). They represented four different families: Boraginaceae, Brassicaceae, Convolvulaceae, and Plantaginaceae. For three of the species, I selected native congeners from the same family that had similar morphologies (Table 2). For the one invasive species which had no native congener in the same family, I selected a native species pair based on shared habitat and pollinators as well as similar flower size, color, and shape. Although there are many invasive plants in the Compositae, these species were not chosen for study due to the difficulty in measuring their nectar and pollen rewards.

For each study species, I collected samples from at least three sites, ten plants per site. The research sites included 19 locations in the the following areas: Gothic townsite, Slate River Road, Washington Gulch, and Cement Creek.

Mean floral display. I counted the number of open flowers per plant . For *Linaria vulgaris*, I counted the number of flowers per ramet because ramets are connected by underground rhizomes. Mean floral display size was used it to estimate the nectar and pollen rewards available to pollinators on a per-plant basis (see below).

Pollen production. I removed all of the anthers from three buds per plant that were near full maturity. Buds were used instead of open flowers so that anthers were fully developed but not yet dehiscing. I dried the anthers in mircocentrifuge tubes for at least two weeks. I added 1500 uL of ethanol to each sample and sonicated it for five minutes to release any remaining pollen from the anthers. Once the pollen grains were in suspension using a vortex, I counted the number

of pollen grains in a five uL subsample with a hemacytometer under a dissecting microscope (Kerns and Inouye 1993). I counted four subsamples for the pollen collected from each flower and used the average to calculate the number of pollen grains per flower. Due to the time consuming nature of counting pollen, the data set will not be completed by the end of the AIR program but the results will appear in my senior thesis that will be finished by the end of this academic year.

Pollen volume. To measure average pollen grain volume for each species, I followed the methods of Roulston et al. (2000). I stained pollen samples from several different individual plants on slides with a basic fuchsin dye. I measured the polar and equatorial aspects of pollen grains with an ocular micrometer under a compound microscope at 400x magnification for approximately 10 haphazardly encountered pollen grains per slide for a total of 10 slides. The volume of each pollen grain was estimated using volumetric formulas for spheres ($\frac{1}{6}\pi p^3$), ellipsoids ($\frac{1}{6}\pi p_1 p_2 p_3$), or a combination of formulas when the pollen grains were not symmetrical. Once the pollen counts are complete, I will be able to calculate the total volume of pollen by multiplying the number of pollen grains per flower by the mean pollen grain volume. This will be calculated on a per-flower and a per-plant basis.

Pollen nitrogen/protein. I pooled pollen across plants within a site in order to collect pollen for nitrogen content. Nitrogen content will be measured using combustion at the Dartmouth College Analytical Laboratory. Pollen protein will be calculated from the nitrogen content estimates. I will not do this analysis for *Barbarea vulgaris* and *Draba* sp. due to the difficulty of collecting enough pollen from such very small flowers. This analysis will not be completed by the end of the AIR program but the results will appear in my senior thesis.

Nectar production rate. I used a microcapillary tube to drain the standing crop of nectar from all of the flowers on a plant. Often, the standing crop of nectar in flowers was zero. I covered the entire plant with a mesh bag to exclude pollinators and returned approximately 24 hours later to measure the volume of nectar in three flowers using a microcapillary tube and calipers. I always measured nectar between 9 and 11:00 AM to control for some variation in nectar production rate that occurs throughout the day. I did not measure nectar production rate or sugar concentration (described below) in *B. vulgaris* or *Draba* sp. because the flowers were too small given the field analytical techniques I used.

Nectar sugar concentration. I used the nectar that was collected for nectar production rate measurements and a light refractometer to measure the refractive index. The refractive index of nectar is used as a measure of sucrose equivalents (Kerns and Inouye 1993). For nectar production rates that were too small, I could not get concentration estimates

Amount of sugar in nectar. The raw amount of sugar, in contrast to the percent sugar, is an important value to consider because it is liberated from the natural variation that comes from differences in temperature and humidity. Percent sugar changes as volume of nectar changes and nectar volume is sensitive to rain and evaporation. The amount of sugar (mg sugar/flower and mg nectar/plant) are a combination of nectar production rate and nectar concentration measurements. When these measurements are simply multiplied together, there is an increasingly large error at high percentages (Bolten et al. 1979). The percent sugar that was measured with a refractometer was converted to mg sugar/100 mL nectar using a conversion table that accounts for this error (Kerns and Inouye 1993). The mg sugar/100 mL nectar was multiplied by the nectar production rate volume measurements (μ l nectar/flower and total μ l/plant) to calculate the raw amount of sugar (mg sugar/flower and mg sugar/plant).

Data analysis. The independent variable in this study is the status of the plant (native or invasive) and the dependent variables are the measures of nectar and pollen quality and quantity, specifically nectar production rate, nectar concentration, amount of sugar in nectar, pollen volume, and pollen counts, and pollen protein content. I tested the null hypothesis that there is no difference between the invasive and native species. The data was normalized using by square root or log + 1 transformations. I used t-tests for each native-invader pair for all of the nectar variables and for pollen counts. I used a paired t-test to test whether pollen volume differed across species pairs. Although I ran a large number of analyses, it is not necessary to do a Bonferroni correction to correct p-values for bias (Moran 2003) because native-invasive pairs were tested independently. The resulting p-values were judged against a 5% significance level. Statistical analyses were performed in JMP.

Results

Pollen quantification. I found no difference in mean pollen grain volume for invader-native pairs across the species pairs (paired $t_4 = 1.16$, $P = 0.845$). However, I did find differences in the amount of pollen produced per flower for some species pairs. The invasive *Barbarea vulgaris* and *Linaria vulgaris* had significantly higher pollen counts than their native congeners (respectively, $t_{37} = -2.434$, $P = 0.0099$ and $t_{37} = -3.548$, $P = 0.0005$) while the invader *Cynoglossum officinale* has significantly less pollen than its native congener ($t_{10} = 12.085$, $P < 0.0001$; Figure 1).

Nectar production rate. *Linaria vulgaris* had 35% higher nectar production rate per flower than its native congener *Penstemon strictus* ($t_{58} = -2.606$, $P = 0.0116$) and was nearly tenfold

higher than *Mimulus guttatus* ($t_{56} = -9.747$, $P < 0.0001$). When examined at the plant level, *L. vulgaris* had a nectar production rate that was 75 times greater than *M. guttatus* ($t_{39} = -12.036$, $P < 0.0001$), but there was not a significant difference between *L. vulgaris* and *P. strictus* ($t_{54} = -1.235$, $P = 0.111$). *Cynoglossum officinale* and *Convolvulus arvensis* had a lower nectar production rate per flower than their congeners for both individual flowers (respectively, $t_{67} = 3.11867$, $P = 0.0013$ and $t_{28} = 6.16628$, $P = < 0.0001$) and across entire plants (respectively, $t_{56} = -2.476$, $P = 0.0082$ and $t_{21} = 3.803$, $P = 0.0005$).

Nectar sugar concentration. For the four complete pairs, the invasive species had a significantly higher mean sugar concentration than their native congeners (Figure 2).

Nectar sugar amount. For the mean amount of sugar produced per flower, *Linaria vulgaris* was 32 times greater than *Mimulus guttatus* and five times greater than *Penstemon strictus* (respectively, $t_{28} = -4.459$, $P < 0.0001$ and $t_{32} = -3.962$, $P = 0.0002$). On a per-plant basis, *L. vulgaris* was 280 times greater than *M. guttatus* and three times than *P. strictus* (respectively, $t_{18} = -4.05718$, $P = 0.0004$ and $t_{30} = -2.972$, $P = 0.0029$) and *Cynoglossum officinale* was 5 times greater than its congener ($t_{30} = -5.200$, $P < 0.0001$). *Convolvulus arvensis* was not significantly different from its congener on a per-flower basis ($t_9 = 1.841$, $P = 0.0947$) and it was 6 times less on a per-plant basis ($t_9 = 2.541$, $P = 0.0158$).

Discussion

I predicted that there would be a gradient of floral rewards that is dependent on mating system. Although the data generally matches my expected pattern, I am limited in my ability to assess the generality of the pattern because of the small sample size. Invasive *Linaria vulgaris*, which is self-incompatible, was more rewarding than its congeners for all response

variables. *Cynoglossum officinale*, a self-compatible invasive, had greater nectar concentration and raw amount of sugar but a lower nectar production rate and amount of pollen than its corresponding native plant. Thus the two completed pairs do show that dependency on pollinators for reproduction corresponds to the degree of increased floral rewards. My analyses of *Convolvulus arvensis* (self-incompatible) and *Barbarea vulgaris* (self-compatible) are not yet finished. A goal of this study was to use a broad sample of species to assess the generality of the pattern so it would have been advantageous to also consider more invader-native pairs such as self-incompatible *Matricaria perforata*-*Erigeron speciosus* and *Cirsium arvense*-native *Cirsium*, and self-compatible *Tragopogon dubius*-*Agroseris glauca* and *Erodium cicutarium*-*Geranium richardsonii*. However due to time constraints I had fewer study species than what was ideal and I am not able to suggest that this pattern would hold true for all invasive plants.

Overall, I found that invasives are usually more rewarding than natives in many, but not all, estimates of nectar and pollen quality and quantity. With regards to pollen rewards, my hypothesis that invasive species would have a greater amount of pollen per flower than natives was supported by the fact that two out of three invasives had higher pollen counts than their congeners. Although other studies have considered pollen protein content as a driving factor in pollinator plant preference (Roulston et al. 2000, Roulston and Cane 2000), pollen quantity has not been previously explored. These results warrant further investigation about the role that total pollen output has on pollinator preference and how increased pollen output could be an advantageous trait in invasive plants.

Measurements of nectar production rate were not in agreement with my hypothesis that invasive species should have a higher nectar production rate than their native congeners. In fact, two out of three completed pairs were the opposite of what I predicted, in that the native plants

had a higher nectar production rate per flower. However, it is important that this not be viewed in isolation, since nectar volume alone is not a good indication of nectar rewards. The quantity of nectar in a plant that is sheltered from pollinators still fluctuates through time as volume is supplied by condensation from humid air or precipitation or as it is lost by evaporation. Rates of secretion also show high variation between flowers and between plants and are highly dependent on microclimate (Corbet 2003).

Nectar sugar concentration was another response variable that cannot be considered by itself, even though all of the invasives followed my hypothesis and were more highly concentrated than their native congeners. Especially in open flowers that contain small amounts of nectar that is characteristic of insect pollination, concentration often fluctuates rapidly. Corbet (2003) advocates that studies of sugar content be based on measurements of concentration as well as volume in individual flowers.

My calculation of the raw amount of sugar is comprised of both nectar production rate and sugar concentration and therefore is the most important nectar response variable to consider. In accordance with my hypothesis, I found that two out of three invasives had more sugar on a per-plant basis than the native they were compared to.

The results of this study coincide with several species-level studies that also found invasive plants to have greater nectar rewards for pollinators than their native congeners (Chittka and Schürkens 2001, Brown et al 2002). It also confirmed previous observations that *L. vulgaris* has particularly highly concentrated nectar. Irwin speculated that the high sugar concentration might be why pollinators have a strong preference for *L. vulgaris* (unpublished data). The data from this study suggests that *L. vulgaris* is also highly rewarding in terms of nectar and pollen quantity. It would be interesting in future studies to consider which component or combination

of components of floral rewards drives the observed pollinator preference for *L. vulgaris*.

Additionally, while sugar content of nectar is usually of primary interest because sugars provide the energy that fuels activity or provisions the larvae (Corbet 2003), it is certainly not the only thing pollinators find valuable. Studies have shown that some nectivores prefer nectar with high amino acid content (Blüthgen and Fiedler 2004, Schütz and Erhardt 2005). Likewise pollen is known to have attractive components besides protein including vitamins, growth regulators, and lipids (Herbert et al. 1980). Future studies might also explore these other attractive components as they relate to pollinator preference and invasive plants.

The suggestion that invasive plants are more rewarding for pollinators than native plants leads to several interesting questions about the nature of floral rewards. For example, do increased floral rewards develop as a successful invasive plant adapts to its new range, or are they an inherent trait of the invasive plant and therefore facilitate its invasion? This question could be illuminated by comparing floral rewards for invasive plants in their native range and in their invasive range. Understanding the plasticity of floral rewards in invasives and how they compare to native plants could provide novel predictive insight into which introduced plants are most likely to become invasive in their new range.

Another interesting concept to consider is how increased nectar and pollen rewards in invasives affects the fitness of the surrounding native plant community. Most studies have found that invasive plants compete with native plants for pollinator visits but the reproductive output in the native species was not necessarily reduced (reviewed by Bjerknes et al. 2007). Conversely, one study found that a native species suffered significantly reduced seed set in the presence of an aggressive invading congener that shares pollinators (Brown et al. 2002). Future studies might

look into how nectar and pollen rewards relate to studies that examine pollination and subsequent seed set, like those of Brown et al. (2002).

Lastly, my results introduce some noteworthy management implications for invasive plants. Because the invasive species in this study appear to be more rewarding than natives, then eradication could mean eliminating a high quality food source for pollinators, like bumble bees. For example, although *L. vulgaris* is not the only plant from which bumble bees collect nectar and pollen, it is often their preferred source for floral rewards. Therefore, if all *L. vulgaris* was eliminated from an area where it is dominant now, it is reasonable to hypothesize that the bumble bee population might decrease in the following years due to reduced food supply for larvae.

Conclusions

A comparison of native-invader pairs found that invasive plants often have higher quality nectar rewards and higher quantities of pollen than native congeners. The narrow sample size limits my ability to assess how universal this phenomenon is. Future study should focus on broadening the sample size and also considering other components of nectar and pollen rewards.

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Tables

Table 1. Mating system of invasive species .

<i>Linaria vulgaris</i> (Stout et al. 2000)	<i>Cynoglossum officinale</i> (Klinkhamer and Dejong 1987)
<i>Convolvulus arvensis</i> (Westwood et al. 1997)	<i>Barbarea vulgaris</i> (van Leur et al. 2006)

Table 2. Invasive and Native Congeners

Invasive	Native
<i>Linaria vulgaris</i> (Plantaginaceae)	<i>Penstemon strictus</i> (Plantaginaceae), <i>Mimulus guttatus</i> (Scrophulariaceae)
<i>Convolvulus arvensis</i> (Convolvulaceae)	<i>Geranium richardsonii</i> (Geraniaceae)
<i>Cynoglossum officinale</i> (Boraginaceae)	<i>Mertensia ciliata</i> (Boraginaceae)
<i>Barbarea vulgaris</i> (Brassicaceae)	<i>Barbarea orthoceras</i> or <i>Draba</i> sp. (Brassicaceae)

Figures

Figure 1. Mean pollen counts per flower for native-invader pairs. Blue bars represent the invasive species and red bars represent their native congeners. See Results section for summary statistics.

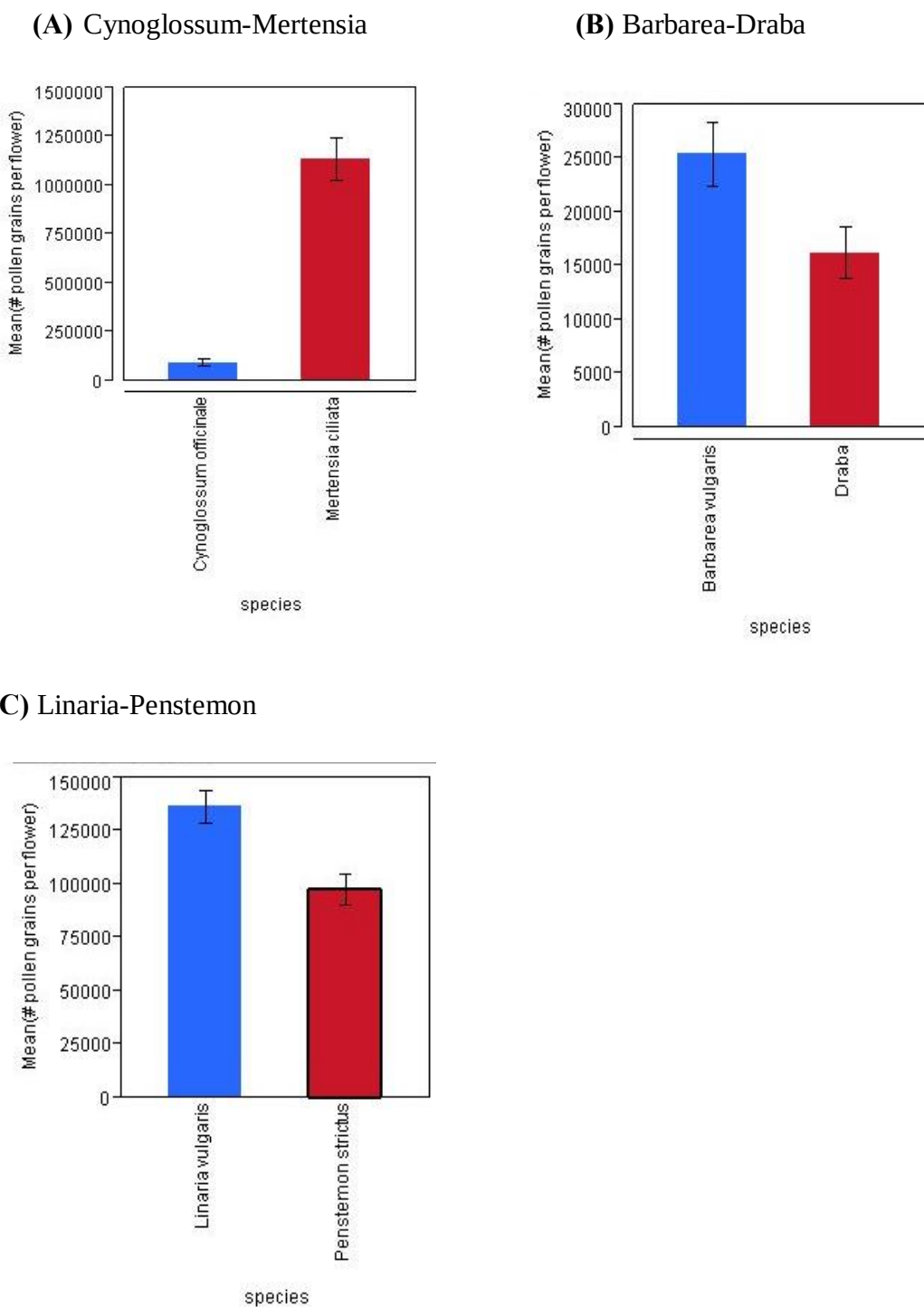


Figure 2. Mean sugar concentration for native-invader pairs.

