

The effects of the invasive plant *Linaria vulgaris* on native plant fitness and pollinator behavior

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Abstract

The invasion of the European perennial *Linaria vulgaris* to the alpine environment of Colorado may lead to competition for natural resources, space, and pollinators with native species. I measured the effects on fitness of *L. vulgaris* on three plant species native to Colorado, using both hand-pollination and controls to assess pollen limitation of seed set; plant size was measured for analysis as well, using root stalk diameter of *Ipomopsis aggregata* and number of flowering stalks of *Linum lewisii* and *Potentilla pulcherrima*, in the presence and absence of *L. vulgaris*. I also recorded pollinator visitations to my focal plants. The fruits for each plant were collected as they matured, and the number of aborted fruits were collected and recorded as well. The number of seeds was counted for each plant per collection day, as fruits often dehisced in the envelopes used to collect them. Analyses of plant size and mean floral display showed no significant difference in the presence of *L. vulgaris* as compared to its absence. These preliminary results suggest that *L. vulgaris* does not affect plant size or floral display in these three species.

Introduction:

As humans travel and alter natural landscapes, organisms are inevitably carried along and introduced into new environments. Some of these organisms have traits that favor their survival in unfamiliar territory, such as prolific breeding, a wide range of environmental tolerance, and a generalist diet (McNeely 2004). We are only now beginning to realize the effects of these alien species, both on the structure of the ecosystem they invade and on the changes in competition, behavior, and fitness of native species they interact with (Kearns, Inouye & Waser 1998; McNeely 2004). While it is natural for species to expand and change territories, global trade and human interference have greatly accelerated the rate of that spread (McNeely 2004). Introduced animals, plants, and microorganisms are linked to 39% of known animal extinctions since 1600 (Groombridge, ed. 1992). Biological invasions that affect mutualistic relationships (e.g. plant-pollinator interactions) are particularly important to us as well as to the ecosystem because of our economic reliance on their services (Kearns, Inouye & Waser 1998). Plants are dominant organisms in most terrestrial ecosystems in terms of both productivity and biomass, and effects

on their fitness and diversity may lead to consequences in subsequent trophic levels as well as abiotic factors such as soil composition. The impact of invasive plants on native species can lead to dramatic changes in the original ecosystem; changes that are often undesirable for economic, environmental, or conservation purposes.

Most past research has focused on biological invasions with respect to herbivory and competition. Studies have documented the effects of invasive plants on pollinator behavior (Chittka & Schürkens 2001), the influence of invasive plants on mutualistic relationships (Brown, Mitchell & Graham 2002), and the spread of invasives in general (Starfinger, Edwards, Kowarik, & Williamson 1998). Studies have also suggested that invasive plants can increase competition for pollinators and reduce native plant fitness (Chittka & Schürkens 2001). Memmot and Waser (2002) created a connectance web of 1429 animal species and 456 plants, 56 of which were introduced, in Illinois (a species list generated by Robertson, 1929). They made a database showing which animals visited which plants, and the result was an overall plant-pollinator community structure. Statistical analyses of Robertson's observational data revealed a roughly equal visitation rate of pollinators between invasives and natives; however, most visitors to the alien plants were the more generalized pollinators. It is unknown whether rates of visitation to native plants would have been higher in the absence of the invader.

Yet despite the numerous studies done, relatively little is known about the effects of invasive plants on plant-pollinator mutualisms (Irwin grant proposal 2005). This study investigated the effects of the invasive perennial *Linaria vulgaris* (Scrophulariaceae), also known as butter and eggs, on the pollination and reproduction of three plant species native to Colorado—*Ipomopsis aggregate* (Polemoniaceae), *Linum lewisii* (Linaceae), and *Potentilla pulcherrima* (Rosaceae)—by comparing plots of the natives with and without *L. vulgaris*. I was

interested both in the degree to which *L. vulgaris* alters pollen limitation of seed set to the natives, and the degree to which its presence alters native pollinator behavior. By including several species with which *L. vulgaris* shares pollinators and resources, I aimed to incorporate more of a community approach into my investigation. This study will provide valuable information on possible effects of introduced non-native plants into a native pollinator-plant community and will add to the growing body of knowledge concerning mutualisms within the context of biological invasions. I hypothesized that seed set of native species will be decreased in plots including *L. vulgaris* due to increased competition for resources between the natives and the invasive and also due to pollinator preference to the invasive species because of significantly higher nectar rewards in *L. vulgaris* (Irwin grant proposal 2005).

Methods:

My organisms of interest for this study are the invasive plant *Linaria vulgaris* and three native species: *Ipomopsis aggregata* (Polemoniaceae), *Linum lewisii* (Linaceae), and *Potentilla pulcherrima* (Rosaceae). *Linaria vulgaris* is a noxious perennial introduced into North America in 1874 as an ornamental plant (Beck 2004). The invasive is now present in all lower 48 states as well as Alaska and parts of Canada (USDA 2004), and colonizes rapidly in disturbed soil (Carpenter and Murray 1998; Irwin grant proposal 2005). *Ipomopsis aggregata* is self-incompatible and is pollinated primarily by hummingbirds and, to a lesser extent, bumblebees (Campbell & Halama 1993; Mayfield, Waser & Price 2001). *Linum lewisii*, an obligate outcrossing perennial, is pollinated by a variety of flies and bees (Kearns & Inouye 1994). *Potentilla pulcherrima* is visited by both flies and bees, although it is unknown whether these insects are effective pollinators.

To carry out my experiment, plots were set up in roughly 2×2 meters around the Rocky Mountain Biological Laboratory (RMBL) area, each of which contained at least one of the target native plant species. Stringettes made from 10-12cm pieces of string with the ends taped together with blue labeling tape were used to mark and label individual plants. The plants were then randomly assigned a treatment of either hand-pollination, for which I hand-pollinated all open flowers per plant three days a week, or control, for which I handled the plants in a similar manner to the other treatment without adding excess pollen. The treatments were paired by number, i.e. 1H, 1C; 2H, 2C; where H=hand-pollinated and C=control. Five plants were marked per treatment per species per plot. Treatments were crossed with the presence and absence of *L. vulgaris*. I found five sites which contained both *L. vulgaris* and at least one of my target species and five sites with only the native plants. The total sample size for each species was 80 plants: 40 plants in the presence of *L. vulgaris* and 40 plants in its absence.

Pollen for each species was obtained from a random collection of plants at least 2m from the plots by plucking the anthers with fine-point tweezers and collecting them in labeled Eppendorf tubes. Pollen was then applied with a camel-hair paintbrush to the stigma of each open flower. To prevent mixing of interspecific pollen, the paintbrush was sterilized with ethanol and dried before applying pollen to another plant species.

Before treatments began, measurements were taken of each plant to control for initial plant size. For *I. aggregata*, I measured the root stalk diameter; for *P. pulcherrima* and *L. lewisii* I counted the number of stalks per plant. In plots with *L. vulgaris*, I also estimated the density of the invasive using three 25cm by 25cm squares placed haphazardly but not overlapping within the plot and then averaging the densities of *L. vulgaris* for each plot.

In addition, I took note of pollinator visitation to my focal plants two days each week using a tape recorder to verbally record the visitor to at least family name, how long it stayed at each flower, and the number of flowers it visited per plant. At the beginning of each session I recorded the site, date, time, and weather conditions, and at the end of a 20-minute period I ended by repeating site name, saying the end time, and noting any changes in weather during the session. I visited my plots in a random order, and plots visited in the morning one day were switched to an afternoon visit the next observation day, and vice versa. For practice in identifying the pollinators that visit each of my target species, I observed already flowering plants not included in my sites.

Once my focal plants produced fruits, I collected and counted the number of successful and aborted fruits and counted the number of seeds per fruit. The response variables for this experiment were pollinator visitation rates and behavior, pollen receipt, and seed production. Treatment pairs (hand-pollinated, control) were the replicates for the experiment. A multiple analysis of variance (MANOVA) was used to compare visitation and behavior to native plants with and without *L. vulgaris*, and an analysis of variance (ANOVA) was used to compare seed production with and without *L. vulgaris*, and in control versus hand-pollinated plants.

Results

Plant size:

No significant difference was found between plant sizes in plots of *L. vulgaris* versus plots without the invasive (*I. aggregata*: $F_{1,73}=0.0023$, $P=0.9615$; *L. lewisii*: $F_{1,79}=0.6026$, $P=0.4399$; *P. pulcherrima*: $F_{1,79}=1.513$, $P=0.2224$).

Table 1. Plant Size

	<i>I. aggregata</i>		<i>L. lewisii</i>		<i>P. pulcherrima</i>	
	Absent	Present	Absent	Present	Absent	Present
mean	5.9285	5.9508	10.0000	9.0250	2.0000	1.7250
SE	0.3373	0.3110	0.8881	0.8881	0.1581	0.1581

Floral display:

No significant difference was found between mean floral displays of natives in plots of *L. vulgaris* versus plots without the invasive (*I. aggregata*: $F_{1,68}=0.0009$, $P=0.9758$; *L. lewisii*: $F_{1,53}=0.0149$, $P=0.4399$; *P. pulcherrima*: $F_{1,78}=0.4307$, $P=0.5136$).

Table 2. Mean Floral Display

	<i>I. aggregata</i>		<i>L. lewisii</i>		<i>P. pulcherrima</i>	
	Absent	Present	Absent	Present	Absent	Present
mean	4.9713	4.9513	2.9243	2.8526	1.8483	1.9433
SE	0.4930	0.4324	0.3914	0.4376	0.1030	0.1017

Seed set:

Data is still in the process of being collected.

Pollinator visitation:

Data still needs to be transcribed and analyzed.

Discussion

No effects were found from the presence of *L. vulgaris* in mean floral display or plant size for my three target species. However, while no differences were found for plant size, the measurements were taken in the beginning of the experiment, before flowers had begun to bloom. If these measurements were taken at least one other time later in the season, it would be possible to test for effects of growth rate in the plants rather than just initial plant size. If this study were

repeated, it would also be helpful to obtain information on nectar production in the native plants and in *Linaria vulgaris* as an additional measurement of resource competition.

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