

Biocontrol attack increases pollen limitation under some circumstances in the invasive plant *Centaurea solstitialis*

Sarah M. Swope

Received: 12 June 2013 / Accepted: 14 August 2013 / Published online: 14 September 2013
© Springer-Verlag Berlin Heidelberg (outside the USA) 2013

Abstract Herbivore damage often deters pollinator visitation and many invasive plants in North America are pollinator-dependent. This has important implications for the biological control of invasive plants because it means that agents that deter pollinators may have a larger than expected impact on the plant. Yet interactions between pollinators and biocontrol agents are rarely evaluated. *Centaurea solstitialis*, one of the most problematic invasive species in California, is dependent on pollinators for reproduction. I factorially manipulated infection by a biocontrol pathogen and pollen supplementation to test for (1) pollen limitation in *C. solstitialis*, (2) whether infection increased pollen limitation, and (3) whether this varied across a soil moisture gradient. Plants growing on north-facing slopes where soil moisture was higher experienced mild pollen limitation in the absence of the pathogen and more pronounced pollen limitation when they were infected. Plants on drier south-facing slopes did not suffer from pollen limitation but instead appeared to suffer from resource limitation. Pathogen infection directly reduced seed set in *C. solstitialis* by 67–72 %. On north-facing slopes, infection had an additional, indirect

effect by increasing the degree of pollen limitation plants experienced. The trait that mediates this indirect pathogen–pollinator interaction is the number of inflorescences plants produced: infected plants made fewer inflorescences which led to greater pollen limitation. Although in the present study this outcome is dependent on abiotic factors that vary over small spatial scales, exploiting other invasive plants' dependence on pollinators by selecting agents that deter visitation may enhance agent impact.

Keywords Biological control · Pathogen · pollen limitation

Introduction

Attack by enemies such as herbivores and pathogens can lower plant fitness by reducing growth and reproduction (Marquis 1984; Karban and Strauss 1993). Additionally, recent research has shown that enemy attack often alters the frequency and intensity of plants' interactions with their mutualists. For example, pollinators visit undamaged plants more frequently than they visit plants that have been heavily attacked by herbivores because damaged plants are less rewarding or less conspicuous to pollinators (Euler and Baldwin 1996; Strauss et al. 1996; Lehtilä and Strauss 1997; Mothershead and Marquis 2000; Barber et al. 2011; Swope and Parker 2012). Reduced pollinator visitation will lower plant fitness but only when seed production is limited by pollen receipt. If seed production is limited by resources, as might happen in more abiotically stressful sites, a reduction in pollinator visitation will not have fitness consequences for the plant.

Several studies have found that herbivory has a direct, negative effect on plant performance while simultaneously

Communicated by Elizabeth Elle.

Electronic supplementary material The online version of this article (doi:10.1007/s00442-013-2750-4) contains supplementary material, which is available to authorized users.

S. M. Swope (✉)
USDA ARS Great Basin Rangelands Research Unit,
920 Valley Rd, Reno, NV 89512, USA
e-mail: sswope@mills.edu; Sarah.swope@ars.usda.gov

Present Address:
S. M. Swope
Biology Department, Mills College, 5000 MacArthur Blvd,
Oakland, CA 94613, USA

reducing pollinator visitation (e.g., Lehtilä and Syrjänen 1995; Juenger and Bergelson 1997; Mothershead and Marquis 2000; Hladun and Adler 2009; Barber et al. 2011), three of which (Strauss and Murch 2004; Kessler et al. 2011; Swope and Parker 2012) found that herbivory further reduced plant fitness by increasing pollen limitation. Both herbivore-mediated plant–pollinator interactions and pollen-limited fecundity appear to be common in nature (reviewed in Knight et al. 2005, 2006), meaning that indirect interactions between enemies and pollinators may be important in ecological communities.

The question of how enemy attack might alter plant–pollinator interactions and subsequently individual fitness is important from both basic and applied perspectives. Invasion by exotic species is one of our most pressing conservation concerns, and biological control agents (hereafter biocontrol), the use of a host-specific enemy from the invader's native range to control it in the introduced range, is often cited as our best chance to control the most widespread and well-established invaders (van Driesche et al. 2008). The ideal invader has long been described as one that is not dependent on pollinators for reproduction (Baker 1965, 1974). Although this is true for some notorious invaders in North America, e.g., *Bromus tectorum* (Upadhyaya et al. 1986), *Alliaria petiolata* (Cavers et al. 1979), *Carpobrotus edulis* (Vilá et al. 1998), and *Hypericum perforatum* (Crompton et al. 1988), many exotic plants depend on pollinators for reproduction, including some very successful invaders, e.g., *Cytisus scoparius* (Parker 1997), *Lythrum salicaria* (Levin 1970; Mal et al. 1992), *Centaurea diffusa* and *C. maculosa* (Harrod and Taylor 1995), and the focus of this study, *Centaurea solstitialis* (Sun and Ritland 1998). Reichard (1994) found that over half of the successful invaders in North America depend on pollinators for successful reproduction and therefore invasion, yet the role of pollinators in the success of specific invaders has received relatively little attention (but see Ågren 1996; Parker 1997). Even less attention has been paid to the potential role of pollinators in invasive plant management using biological control agents.

The impact of biocontrol agents on the target invader may be magnified if attack also deters pollinators. In one of the few studies to explicitly consider pollinators in invader–biocontrol systems, Swope and Parker (2012) found that attack by one biocontrol agent, a bud herbivore, reduced the number of inflorescences produced by the invasive plant *C. solstitialis* which in turn reduced pollinator visitation. Where *C. solstitialis* reproduction was pollen limited, attack by this agent had an additional impact on the plant beyond the direct effect of bud herbivory. Attack by a second agent, a seed predator, also deterred pollinator visitation and indirectly reduced seed production. In the case of the second agent, attack did not reduce the number of inflorescences the plants produced but damaged them in a way

that presumably made them less attractive to pollinators. Given the frequency of both herbivore-mediated plant–pollinator interactions and pollen-limited fecundity in natural populations, exploiting the dependence of invaders on their pollinators by using biocontrol agents that deter visitation may be an underappreciated avenue for control. Much of our understanding of how enemy attack alters plant–pollinator interactions comes from studies in which the enemy is an herbivorous insect; considerably less is known about how pathogen infection might alter plant–pollinator interactions (but see Roy 1996; Biere and Honders 2006).

The purpose of this study is to explore whether infection by the biocontrol pathogen *Puccinia jaceae* f.s. *solstitialis* affects the degree to which reproduction of its invasive host, the annual plant *C. solstitialis*, is limited by pollen receipt and whether this varies across a resource gradient. Specifically, I asked the following questions: (1) is *C. solstitialis* reproduction limited by pollen receipt, (2) does this change across a gradient of soil moisture (south- and north-facing slopes), and (3) does the addition of a biocontrol pathogen exacerbate the degree of pollen limitation? To answer these questions, I conducted a field experiment in which pollen addition and pathogen infection were manipulated in a factorial design, and I used two measures of plant reproductive success: percent seed set and total seed output per plant. Percent seed set sheds light on the degree to which enemy attack affects pollen limitation. Whole plant seed production is most relevant to addressing the question of whether agent-induced reductions in pollinator visitation are a promising avenue for invasive plant management.

Materials and methods

Study system

Centaurea solstitialis L. (Asteraceae, yellow starthistle) is considered one of the most problematic invasive plants in California (Pitcairn et al. 2006). It is native to Eurasia and, since its introduction in ~1869 (DiTomaso and Gerlach 2000), it has come to occupy 5.8 million ha in California (Pitcairn et al. 2006). It has an annual life cycle and is dependent on generalist insect pollinators for reproduction (Sun and Ritland 1998; Barthell et al. 2001). Four years of pollinator observations at this site show that >96 % of 2,017 floral visits were by *Apis mellifera* (Swope and Parker 2010a, 2012, Swope, unpublished data); the remaining visitors are two species of *Bombus*. *C. solstitialis* flowers in the middle of the summer drought when water is the limiting resource (DiTomaso and Healy 2007). As an annual, *C. solstitialis* has only one opportunity to reproduce, so there is no future cost to increased reproduction as a result of supplemental pollination (Montalvo and Ackerman 1990;

Zimmerman and Pyke 1988; Primack and Hall 1990) and plants produce inflorescences continuously until they reach the end of their lifecycle in later summer.

Since 1985, the USDA has released six species of biological control agent intended to control *C. solstitialis*. Three weevils (Coleoptera: Curculionidae) and three flies (Diptera: Tephritidae) have not satisfactorily controlled *C. solstitialis* despite evidence that their attack has reduced plant density, at least to some degree (Swope and Parker 2010b). As a result of the unsatisfactory level of control, the USDA continues to seek new biocontrol agents for *C. solstitialis* and, in 2003, introduced the foliar pathogen *P. jaceae* f.s. *solstitialis* (hereafter *Puccinia*). *Puccinia* infects the leaves of the plant during the winter months. The plant sheds its leaves, and along with it the infection, when it begins to flower in the summer. Infection causes pustules to develop on the leaves and this reduces the plant's photosynthetic capacity. *Puccinia* infection has been shown to reduce *C. solstitialis* taproot growth in the greenhouse (Shishkoff and Bruckart 1996), and the plant depends on its taproot to access deep soil moisture, especially during the summer drought when it flowers (DiTomaso and Healy 2007). Infected plants are smaller and produce fewer inflorescences compared to uninfected plants (Swope and Parker 2010a; Swope and Stein 2012), presumably because of their reduced photosynthetic capacity and/or because plants with shorter taproots are less able to access essential resources in the soil. *Puccinia* infection therefore has the potential to change plant reproductive output in two ways. Infected plants might be able to mature fewer pollinated ovules because they suffer greater resource limitation, or reductions in inflorescence number may result in lower pollinator attraction and thus greater pollen limitation.

Study site

This experiment was conducted in two *C. solstitialis* populations in Mt. Diablo State Park (37°51'N, 121°55'W, 335 m asl; Contra Costa County, California, USA) where *C. solstitialis* is considered the highest priority invader by Park managers (Marla Hastings, personal communication). Because soil moisture is the limiting resource during the flowering period and because I was interested in assessing how pathogen infection influences pollen limitation across a resource gradient, I conducted this experiment on adjacent north- and south-facing slopes in two populations. I located invasions on each of two hills, 4.47 km apart, both with narrow ridges and steep north- and south-facing slopes. *C. solstitialis* has been established at both locations for decades (Ertter and Bowerman 2002; Marla Hastings, personal communication). North- and south-facing slopes often differ from one another in numerous traits, such as soil depth and texture, bedrock exposure, and solar radiation, all of which

are likely to influence soil moisture. Plants on south-facing slopes ought to experience drier conditions than plants on north-facing slopes in the northern hemisphere. I randomly selected 50 naturally recruiting plants on each slope in both populations. Plants were located 25 m downslope from and parallel to the ridge. Half of plants on each slope were randomly assigned to the +*Puccinia* group and the other half were assigned to the uninfected control group. *C. solstitialis* size and density vary with slope aspect. Plants on the north-facing slopes were smaller and density higher than on the south-facing slopes (see “Discussion”).

Two of the insect biocontrol agents (discussed above) are present in both study populations, *Eustenopus villosus* (Coleoptera: Curculionidae) and *Chaetorellia succinea* (Diptera: Tephritidae). Attack by these agents would complicate the results of this experiment, so, to protect inflorescences from these predators, I bagged entire plants with a lightweight bridal veil covering to prevent the insects' access to the plants. As soon as petals emerged, but several days before full petal expansion, I removed the bridal veil covering to expose the inflorescences to pollinators. Both of these seed predators prefer to lay eggs in young, small buds prior to petal emergence, and generally ignored the experimental plants after the bridal veil had been removed.

Experimental pathogen infection

Plants were infected with *Puccinia* by spraying them to run-off with a solution of 300 mg of urideniospores in a solution of 300 mL of distilled water and 6 drops of the wetting agent Tween20 (polyoxyethylene sorbitan monolaurate; Acros Organics, Morris Plains, NJ, USA). *Puccinia* spores were provided by the California Department of Food and Agriculture's Integrated Pest Control program which rears biocontrol agents. Plants in the uninfected control group were sprayed with water and Tween20. All plants were inoculated (or sprayed with water and Tween20) on 8 January 2011 and again on 16 February 2011. Plants were inspected at 3-week intervals from the time of first inoculation to the disappearance of pustules (early June) to confirm that plants in the +*Puccinia* group had been successfully infected and that uninfected plants had not. Every plant in the +*Puccinia* group developed pustules on its leaves and none of the plants in the uninfected control group showed any pustule development on the leaves.

Quantifying water stress

I measured soil moisture at three times during the growing season. Once at the time of the first inoculation (8 January 2011), again at the time of the second inoculation (16 February 2011) and finally at the peak of flowering in late July (26 July 2011). To quantify the degree to which plants on the

two slopes experienced water stress, one leaf from each plant was removed as close to flowering as possible but while leaves were still green (second week of June, approximately 5 weeks before peak flowering) and analyzed for $\delta^{13}\text{C}$. Analysis of $\delta^{13}\text{C}$ was conducted by the Colorado Plateau Stable Isotope Laboratory, Northern Arizona University.

Experimental pollen addition

Half of the infected and half of the uninfected plants on each slope were randomly assigned to either the open pollination treatment or to the supplemental pollination treatment. Inflorescences of plants in both groups were visited by pollinators; for the plants assigned to the supplemental pollination group, pollen was added by gently brushing one inflorescence from each of three donor plants across the receptive stigmas of the experimental inflorescences. I used a hand lens to confirm that pollen had been transferred to the stigmas following pollen addition.

In this study, the number of inflorescences per plant ranged from 1 to 32 with a mean of 6.38 ($\pm 6.18\text{SD}$) making it possible to assess pollen limitation at the level of the whole plant rather than inferring it from adding pollen to individual inflorescences as is often necessary when plants produce more inflorescences than is reasonably possible to experimentally treat (e.g., Parker 1997).

Pollen donors were randomly selected from plants on the same slopes as the pollen recipients within each study population, e.g., plants on the south-facing slope received pollen only from donors also growing on the south-facing slope at that location and not from plants on the north-facing slope to avoid confounding problems associated with any local adaptation that might exist. Donor plants were located at least 3 m from the experimental plant to minimize the chance of biparental inbreeding (Sun and Ritland 1998). Pollen donors were covered with a bridal veil enclosure the day before the pollen addition treatment was applied to prevent pollinators from removing pollen before I was able to collect donor inflorescences. Pollen additions were conducted between 0700 and 0900 hours at each location; this timing was consistent with my observations of peak pollinator activity in the two study populations.

As inflorescences matured in the field, they were collected and stored in separate coin envelopes for dissection in the laboratory. Inflorescences were collected only once the pedicel had desiccated and the petal cap had loosened but not fallen off, so their removal is very unlikely to have caused a compensatory effect. Seeds are viable at this time (Swope, unpublished data). Viable seeds are easy to distinguish from unfilled ovules under a dissecting scope based on size, shape and color. On the rare occasions in which viability was uncertain, I germinated seeds in a Petri dish. This allowed me to accurately quantify the total number of

ovules, filled and unfilled, as well as the total number of viable seeds each plant produced.

Measures of pollen limitation

The degree of pollen limitation was assessed in two ways. First, I calculated the percent seed set for each plant. This assessment assumes that inflorescences on plants in each treatment group make the same number of ovules per inflorescence and this was confirmed (mean ovules per inflorescence ranged from 32.7 to 34.1 in the different treatment groups; $F_{1,1134} = 0.087$, $p = 0.81$). The second measure of pollen limitation was the total viable seeds per plant. This is a less frequently used measure of pollen limitation (Knight et al. 2005), but most relevant to the question of whether a biocontrol agent can indirectly reduce invader performance by altering its interactions with its pollinators because we are more concerned with an invasive plant's per capita seed production than we are with its proportional seed set. An increase in the percent seed set and/or in the number of seeds per plant in response to pollen addition would indicate that reproduction is limited by pollen receipt. No increase in seed set despite supplemental pollination indicates that reproduction is limited by something other than pollen receipt, most likely resources.

Statistical analyses

I used one-way ANOVA to determine if there were significant differences in percent soil moisture on the two slopes at both populations, and I used general linear models (GLM) to determine if slope aspect or pathogen infection affected $\delta^{13}\text{C}$ in the plants as a measure the degree of drought stress plants in the different treatment groups were experiencing.

I used GLM to determine if population, slope aspect, pathogen infection or pollen addition (fixed, independent variables) affected seed set. The total number of inflorescences per plant was a covariate in the analysis. It has been shown here (see "Results") and elsewhere (Swope and Parker 2010a; Swope and Stein 2012) that pathogen infection reduces the number of inflorescences that plants produce, and that plants with more inflorescences may attract more pollinator visits independent of the other factors. Additionally, aboveground biomass and the number of inflorescences are highly correlated ($r = 0.950$, $p = 0.0001$), and larger plants likely have greater access to resources. The use of this covariate reveals the influence of pathogen infection on pollen limitation beyond its direct effect of reducing the number of inflorescences the plant produces. The response variables were the proportion of seed set by the whole plant (arcsine transformed) and the number of viable seeds per plant (Ln transformed).

Results

There was no significant effect of “population” as a factor in the analysis, so I combined the data from the two populations to minimize the complexity of the results and increase statistical power.

Soil moisture and water stress

There was no difference in soil moisture on the two slopes at the first two sampling periods (8 January and 16 February 2011), which occurred during the rainy season. During the summer drought (flowering season; 26 July 2011), soil moisture was higher on the north-facing slopes (9.74 ± 2.51 %SE) than it was on the south-facing slopes (5.78 ± 0.36 %) ($F_{1,11} = 9.689$, $p = 0.02$) (see Fig. S1 in the electronic supplementary material). Slope aspect had a significant effect on the degree of water stress plants experienced ($F_{1,98} = 0.028$, $MS = 71.101$, $p = 0.0001$). Plants growing on the south-facing slopes had the highest $\delta^{13}\text{C}$, indicating that they have higher water use efficiency and were operating under a greater water deficit than plants on north-facing slopes (Fig. 1). Pathogen infection had no effect on the amount of water stress plants experienced ($F_{1,98} = 0.113$, $MS = 0.81$, $p = 0.74$) and the interaction between slope aspect and pathogen infection was not significant ($F_{1,98} = 0.022$, $MS = 0.016$, $p = 0.88$).

Number of inflorescences

The number of inflorescences per plant was used a covariate in the analysis to better assess the effect of pathogen infection and pollen supplementation on plants growing on slopes with different aspects. In the absence of infection,

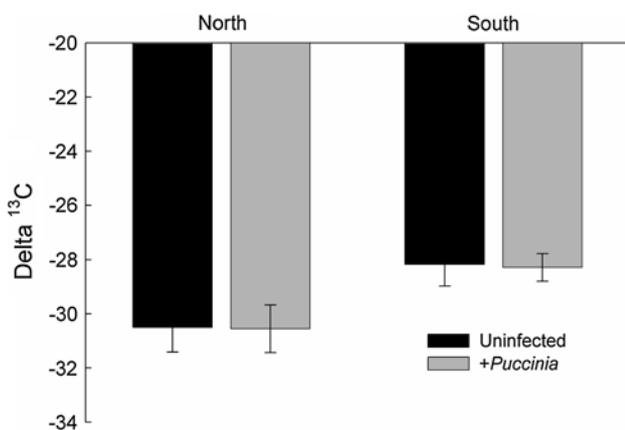


Fig. 1 Effect of slope aspect (north vs. south) and infection by the biocontrol pathogen *Puccinia jaceae solstitialis* on foliar $\delta^{13}\text{C}$ (‰) in *Centaurea solstitialis*. Bars mean \pm 1SE ($n = 98$)

plants growing on the south-facing slopes produced more inflorescences (15.9 ± 1.3) than those growing in the north-facing slopes (5.1 ± 2.2). Pathogen infection also reduced the number of inflorescences plants produced. Infected plants on the south-facing slopes produced an average of $6.1 (\pm 0.6)$ inflorescences per plants and plants on the north-facing slopes produced $1.5 (\pm 0.08)$ inflorescences.

Percent seed set

Pathogen infection reduced the percentage of seeds set by plants (Table 1; Fig. 2). Under natural conditions, i.e., open pollination, plants on the north-facing slopes set an average of 76.3 % (± 3.9 %) of their seeds when they were uninfected but only 64.7 % (± 3.8 %) when they were infected with *Puccinia*. Uninfected plants on the south-facing slopes set 67.6 % (± 4.2 %) of their seed and infected plants set 58.7 % (± 4.3 %). This represents a 15 % reduction in percent seed set for infected plants on north-facing slopes and a 13 % reduction for infected plants on south-facing slopes compared to the uninfected plants on the same slopes.

Slope aspect also affected percent seed set, but to a lesser degree than did pathogen infection (Table 1; Fig. 2). On average, plants on the north-facing slopes set a higher percentage of seed (mean \pm 1SE: 76.9 ± 1.6 %) than plants on the south-facing slopes (62.1 ± 1.5 %).

The addition of pollen significantly increased the percentage of seeds set for plants on the north-facing slopes but not the south-facing slopes (Tables 1, 3; Figs. 2, 3). Because I was interested in whether pathogen infection increased pollen limitation, I reanalyzed the data using only plants on the north-facing slopes, where reproduction was pollen-limited. I also analyzed the data with and without the number of inflorescences as a covariate to assess the effect of the pathogen on percent seed set via its direct effect (number of inflorescences) and to determine whether it had any effect on seed set aside from this direct effect of reduced inflorescence number. On the north-facing slopes, the addition of pollen increased seed set by an average of 7.25 % (open pollination = 76.3 % seed set; supplemental pollination = 83.6 %). Among plants infected with *Puccinia*, the addition of pollen increased seed set by 14.7 %, twice as much as it did among the uninfected plants (open pollination = 64.7 % seed set; supplemental pollination = 79.4 %). However, the pathogen \times pollination interaction was not significant regardless of whether the number of inflorescences was included as a covariate (Table 2). The number of inflorescences was marginally significant ($p = 0.058$) and had only a small effect relative to the main effects of pathogen infection and pollen addition (Table 2).

Table 1 Summary of effect of slope aspect, infection by the biocontrol pathogen *Puccinia jaceae* f.s. *solstitialis* and pollen supplementation on proportional seed set in the invasive weed *Centaurea solstitialis*

Source	df	SS III	MS	F	P
Total inflorescences	1	0.221	0.221	3.629	0.058
Slope aspect	1	0.529	0.529	8.683	0.004
Pathogen infection	1	0.857	0.857	14.063	<0.0001
Pollen supplementation	1	0.277	0.277	4.552	0.034
Slope aspect × pathogen	1	0.014	0.014	0.223	0.64
Slope aspect × pollination	1	0.375	0.375	6.160	0.014
Pathogen × pollination	1	0.019	0.019	0.309	0.58
Slope aspect × pathogen × pollination	1	0.045	0.045	0.735	0.39
Residual	197	12.005	0.061		

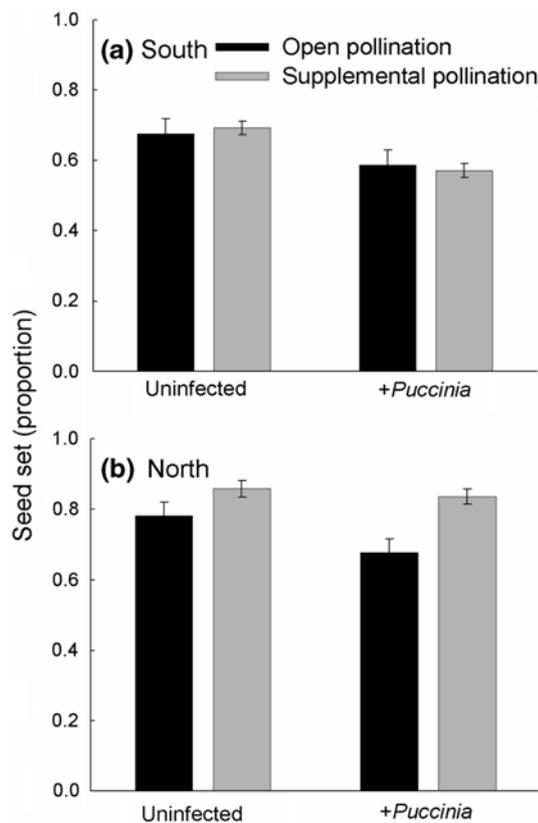


Fig. 2 Effect of presence of biocontrol pathogen *Puccinia* and pollen addition on seed set (proportion) in *C. solstitialis* for plants growing on **a** south- and **b** north-facing slopes. Bars mean \pm 1SE ($n = 205$)

Number of seeds set

Pathogen infection reduced total seed production on both slopes (Table 3; Fig. 3). On the north-facing slopes, uninfected plants produced, on average, 161 ± 7.5 seeds per plant while their infected counterparts on the same slopes produced only 45 ± 2.9 seeds per plant. This represents a 72 % decrease in seed production due to pathogen infection on north-facing slopes. On the south-facing

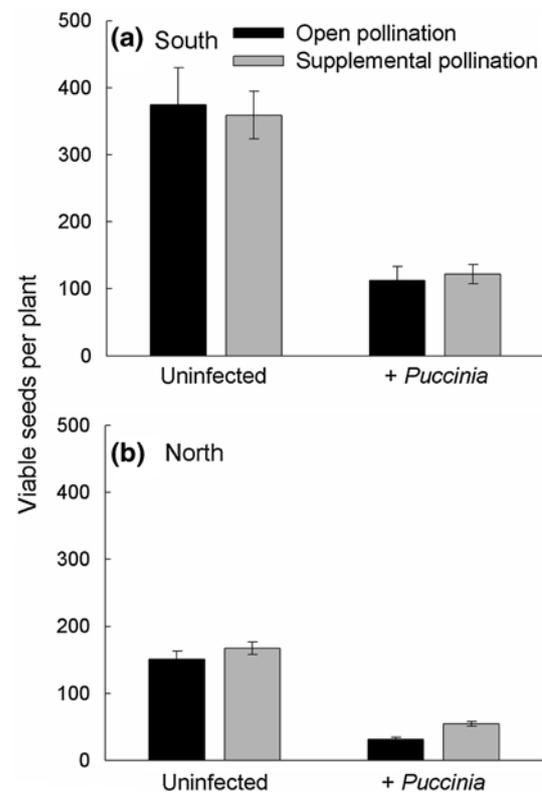


Fig. 3 Effect of presence of biocontrol pathogen *Puccinia* and pollen addition on the number of viable seeds produced by *C. solstitialis* for plants growing on **a** south- and **b** north-facing slopes. Bars mean \pm 1SE ($n = 121$)

slopes, the effect of pathogen infection on seed production was similar in magnitude (67 % reduction) as it was on the north-facing slopes. Uninfected plants produced on average 363 ± 29.8 SE seeds per plant and infected plants produced 118 ± 11.8 seeds per plant. The interaction between pathogen infection and slope aspect was significant. The 3-way interaction between slope aspect, pathogen infection, and pollen addition was also significant.

Table 2 Results from ANOVA testing for effects of infection by the biocontrol pathogen *Puccinia jaceae* f.s. *solstitialis* and pollen supplementation on proportional seed set in the invasive weed *Centaurea solstitialis* growing on north-facing slopes where the reproduction was pollen-limited, with (upper table) and without (lower table) the number of inflorescences as a covariate

Source	<i>df</i>	SS III	MS	<i>F</i>	<i>P</i>
Total inflorescences	1	0.466	0.466	5.695	0.019
Pathogen infection	1	0.911	0.911	11.129	0.001
Pollen supplementation	1	0.937	0.937	11.446	0.001
Pathogen × pollination	1	0.086	0.086	1.055	0.306
Residual	117	9.574	0.082		
Source	<i>df</i>	SS III	MS	<i>F</i>	<i>P</i>
Pathogen infection	1	0.593	0.593	6.975	0.009
Pollen supplementation	1	0.905	0.905	10.642	0.001
Pathogen × pollination	1	0.086	0.086	1.013	0.316
Residual	118	10.040	0.085		

Table 3 Summary of effect of slope aspect, infection by the biocontrol pathogen *Puccinia jaceae* f.s. *solstitialis* and pollen supplementation on whole-plant viable seed production in the invasive weed *Centaurea solstitialis*

Source	<i>df</i>	SS III	MS	<i>F</i>	<i>P</i>
Total inflorescences	1	44.415	44.415	709.497	<0.0001
Slope aspect	1	0.861	0.861	13.751	<0.0001
Pathogen infection	1	0.342	0.342	5.469	0.02
Pollen supplementation	1	1.201	1.201	19.183	<0.0001
Slope aspect × pathogen	1	0.462	0.462	7.375	0.007
Slope aspect × pollination	1	1.069	1.069	17.072	<0.0001
Pathogen × pollination	1	0.543	0.543	8.673	0.004
Slope aspect × pathogen × pollination	1	0.506	0.506	8.081	0.005
Residual	197	12.332	0.063		

As with percent seed set, I reanalyzed the data using only plants from the north-facing slopes (because plants on the south-facing slopes were not pollen limited) to assess whether pathogen infection increased pollen limitation (Table 4). On average, pollen addition increased seed set among uninfected plants by 10 % (open pollination = 150.8 ± 12.4 seeds per plant; supplemental pollination = 167.7 ± 9.3 seeds per plant). The addition of pollen caused a nearly 72 % increase in seed set when the plants were infected with the pathogen (open pollination = 31.7 ± 3.1 seeds per plant; supplemental pollination = 54.5 ± 3.8 seeds per plant). The interaction between pathogen infection and pollen addition was significant.

The total number of inflorescences per plant (the covariate) had a large effect on per capita seed production, and the effect of the pathogen was contingent upon whether the covariate was included in the analysis. The main effect of pathogen infection was not significant when the covariate was included, but was significant when it was not (Table 4), while the interaction between pollen addition and pathogen infection was significant regardless of whether the covariate was included in the analysis.

Discussion

I tested for the presence of pollen-limited reproduction in the invasive plant *C. solstitialis* and whether infection by a biocontrol pathogen alters the degree of pollen limitation the plant experienced. I conducted this experiment on plants growing on drier, south-facing slopes, and on north-facing slopes with higher soil moisture levels. Pathogen infection reduced the number of inflorescences plants produced on both north- and south-facing slopes. This is consistent with previous work that has shown that pathogen infection has a direct negative effect on *C. solstitialis* performance (Swope and Parker 2010a; Swope and Stein 2012). Plants on the south-facing slopes suffered from greater water stress than plants on the north-facing slopes, but pathogen infection did not increase water stress on either slope. Plants on the north-facing slopes experienced pollen-limited seed set but plants in the south-facing slopes did not. When measured on a per capita basis, pathogen infection increased pollen limitation, an effect that appears to be driven entirely by the direct effect of the pathogen on the number of inflorescences plants produced.

Table 4 Tests of significant effects of infection by the biocontrol pathogen *Puccinia jaceae* f.s. *solstitialis* and pollen supplementation on whole-plant viable seed production in the invasive weed *Centaurea solstitialis* growing on north-facing slopes where the plant was pollen-limited, with (upper table) and without (lower table) the number of inflorescences as a covariate

Source	<i>df</i>	SS III	MS	<i>F</i>	<i>P</i>
Total inflorescences	1	10.623	10.623	163.473	0.0001
Pathogen infection	1	0.141	0.141	2.163	0.144
Pollen supplementation	1	3.231	3.231	49.722	0.0001
Pathogen × pollination	1	1.485	1.485	22.849	0.0001
Residual	117	7.603	0.065		
Source	<i>df</i>	SS III	MS	<i>F</i>	<i>P</i>
Pathogen infection	1	54.691	54.691	354.067	0.0001
Pollen supplementation	1	3.523	3.523	22.805	0.0001
Pathogen × pollination	1	1.488	1.488	9.630	0.002
Residual	118	18.227	0.154		

Resource limitation

Plants on the south-facing slopes did not experience pollen limitation in either the presence or the absence of the pathogen, a finding that is consistent with resource-limited reproduction. Infected plants on these drier slopes set a lower percentage of seed and produced fewer total seeds than their uninfected counterparts. The fact that there was no difference in the total number of ovules per inflorescence (maximum reproductive potential) suggests that infected plants may suffer from greater resource limitation than uninfected plants on these drier slopes, although these data cannot conclusively answer this question. During the flowering season, soil moisture was lower on the south-facing slopes than it was on the north-facing slopes, and plants growing there suffered greater water stress as indicated by their significantly higher $\delta^{13}\text{C}$. Surprisingly, plants infected with the pathogen did not have higher $\delta^{13}\text{C}$ values than uninfected plants as would be expected if pathogen infection reduces the plant's access to soil moisture. These results could be interpreted to mean that pathogen infection has no influence on plants' access to soil water or that soil moisture is not the limiting resource, but this seems unlikely as it is contrary to previous work (Shishkoff and Bruckart 1996; DiTomaso and Healy 2007) that explicitly tested for resource limitation, which this study did not.

Alternatively, these results may be attributable to the timing of leaf collection for this analysis. During the rainy season, soil moisture was high and may not have been limiting to either infected or uninfected plants at that time. Leaves were collected for the $\delta^{13}\text{C}$ analysis shortly after the rains had stopped but before the summer drought had begun in earnest, and it is possible that differences in the degree of water stress experienced by infected and uninfected plants had not yet emerged. Because *C. solstitialis* drops its leaves before it flowers, it was not possible to collect leaves for this analysis when soil moisture was lowest and drought stress highest, and thus failed to capture the

real differences in water stress plants infected and uninfected plants experienced during flowering. One way to resolve this would be to explicitly test for resource limitation by adding resources (water) that might be limiting seed production during flowering.

Pollen limitation

Plants growing on the north-facing slopes were pollen-limited, but plants on the south-facing slopes were not. I tested whether pathogen infection increased pollen limitation for plants on north-facing slopes using two response variables, percent seed set and per capita seed set. Percent seed set describes the complex direct and indirect interactions between plants, their enemies and their mutualists. For both infected and uninfected plants, the addition of pollen increased percent seed set, while pathogen infection independently reduced the percentage of seeds set. Although uninfected plants appeared to have a larger response to pollen addition (uninfected plants set 7.3 % more seed when they received supplemental pollen and infected plants set 14.7 % more seed when pollen was added), the interaction between pathogen infection and pollen addition was not significant. In other words, the effect of adding the pathogen biocontrol agent was additive rather than super-additive. This was true even when I controlled for reduced inflorescence production among infected plants by including the number of inflorescences per plant as a covariate in the analysis. In sum, when measured on a proportional basis, plants were pollen-limited, and pathogen infection had the additional, independent effect of reducing percent seed set, though pathogen infection did not increase the degree of pollen limitation.

Per capita seed production is the metric of greatest relevance to invasive species management using biocontrol agents. As with percent seed set, both infected and uninfected plants on the north-facing slopes produced significantly more seed when pollen was added. But, unlike

percent seed set, infected plants had a significantly larger response to pollen addition than uninfected plants. Infected plants increased seed production by 77 % when pollen was added, while uninfected plants increased seed production by only 10 %. When measured on a per capita basis, pathogen infection had a direct effect on seed production as well as an indirect effect by increasing the degree of pollen limitation. Pathogen infection increased pollen limitation solely by reducing the number of inflorescences that plants produced, as indicated by the fact that the main effect of the pathogen was not significant when inflorescence number was included as a covariate in the analysis but had a large and highly significant effect when it was not.

Biocontrol practitioners and resource managers are most interested in the effect of pathogen infection on seed production under natural conditions, i.e., open pollination. In this study, when exposed to natural pollination, infected plants produced only 21 % as many seeds as uninfected plants. This reduction is driven in part by the direct effect of the pathogen as well as by its indirect effect via pollination. Previous work has found that the number of inflorescences correlates with pollinator visitation (Swope and Parker 2012), suggesting that the greater degree of pollen limitation found among infected plants is likely due to declines in pollinator visits as a result of producing fewer inflorescences.

Although plants on the north-facing slopes set a higher percentage of seed than plants on the south-facing slopes, they produced fewer total seeds. This non-intuitive result arises because of differences in the total number of inflorescences made by plants on the different slopes. *C. solstitialis* density is approximately three times higher on the north-facing slopes, and this reduces plant size and the number of inflorescences per plant (Swope et al., in review; Swope, unpublished data). Pathogen infection also reduced plant size and the number of inflorescences that plants made, so that infected plants on the north-facing slopes made the fewest inflorescences and therefore the fewest seeds, despite having the highest proportional seed set. Reproduction in plants on the south-facing slopes is not pollen-limited and instead appears to be resource-limited. Plants on south-facing slopes produced on average more seeds than those on north-facing slopes, and this appears to be due to the fact that they also produce more inflorescences in these lower density populations.

Implications for biocontrol

Infection by the pathogen biocontrol agent reduced per capita seed set in *C. solstitialis* both directly (on both slopes) and indirectly via pollen limitation on the north-facing slopes. Because indirect interactions are ubiquitous in nature (see reviews by Stout et al. 2006; Morris et al.

2007) and their effects can be large in magnitude, as seen here and elsewhere (Swope and Parker 2012), they ought to be explicitly considered when assessing a prospective biocontrol agent. Doing so will reduce the likelihood of selecting agents that interfere with one another (an argument that has been made elsewhere; e.g., Swope and Parker 2010a), but may also allow us to identify unexpected pathways that practitioners can exploit to increase agent impact (as in Swope and Parker 2012). This may be an especially rewarding approach for pollinator-dependent invaders given that pollinators have been shown to be sensitive to herbivore damage across a broad taxonomic range (e.g., Euler and Baldwin 1996; Strauss et al. 1996; Lehtilä and Strauss 1997; Mothershead and Marquis 2000).

Nevertheless, a cautionary note is warranted. The outcome of indirect interactions may be sensitive to abiotic factors that can vary over short distances and time scales. Here, the impact of the pathogen via the pollinators was dependent on slope aspect. Swope and Parker (2010a) found no effect of *Puccinia* infection on *C. solstitialis* seed production via pollinators but that experiment was performed only on a south-facing slope, i.e., those results are consistent with the results from the south-facing slopes reported here and different from the results from the adjacent north-facing slopes. Similarly, it has been shown that indirect interactions between *Puccinia* and the insect biocontrol agents *E. villosus* and *C. succinea* are dependent on soil type, which can vary over a few meters (Swope and Stein 2012). It is possible that the net impact of interactions with pollinators on plant fitness might be similarly affected by soil type or other factors that affect the plant's access to essential resources.

The outcome may also vary in time. Precipitation totals were well above average in the year in which this experiment was conducted, and this led to higher levels of pathogen infection than is seen in drought years, as well as larger direct effects of the infection on plant performance (Swope, unpublished data). It is possible that, in drier years, when infection levels are lower and the pathogen's direct impact on the plant is also less pronounced, the indirect effect via the pollinators will also be less pronounced or even disappear. It is also possible that, in drought years, reproduction will be resource-limited even on the more benign north-facing slopes (as it was on the south-facing slopes), eliminating the indirect effect altogether.

Finally, the outcome may vary not only in response to fine-scale spatial variation or short-term temporal variation in abiotic factors but also in response to differences in the biotic context, specifically the pollinator community. The majority of floral visitors in both study populations were *A. mellifera*, and this is consistent with other work done at Mt. Diablo State Park (Swope and Parker 2010a, 2012) and elsewhere in California (Barthell et al. 2001; Swope

and Parker 2012). Although there appears to be relatively little variation in the pollinator community for *C. solstitialis*, other invaders may depend on a variety of native pollinators which may show species-specific responses to biocontrol damage, which may in turn translate into indirect effects of a greater or lesser magnitude.

Individual- vs. population- level impacts

Whether reduced seed set due to attack by biocontrol agents, pollen limitation, or an interaction between the two, leads to population-level control of the invader will depend on a number of factors. Reduced seed set is not likely to lead to control of the invader if the population growth rate has a low sensitivity to seed set (as is likely to occur in long-lived, iteroparous species; e.g., Parker 2000), when microsites rather than seed input limits recruitment, or when survival is strongly density-dependent (Myers and Bazely 2003; Garren and Strauss 2009). In the particular case of *C. solstitialis*, recruitment is frequently seed-limited at this site as well as at sites elsewhere across California (Swope and Parker 2010b), meaning that an agent that reduces per capita seed production, directly or indirectly, by deterring pollinators, has the potential to contribute to control of *C. solstitialis*. However, *Puccinia* itself is not likely to contribute to the control of *C. solstitialis* because, while it may increase pollen limitation under some circumstances, it also reduces seed predation by the well-established, higher impact insect biocontrol agents (Swope and Parker 2010a; Swope and Stein 2012). This study excluded those insect agents in order to examine biocontrol–pollinator interactions, so it remains unknown how their inclusion in the interaction web might alter the net impact on the plant. As the number of interacting species increases so does the complexity of the interactions and the potential for indirect interactions, making it very difficult to predict the outcome. This makes a strong argument for testing prospective agents in the presence of other key interacting species, including other biocontrol agents and pollinators. Whether agents that deter pollinators exert a meaningful measure of control in other invasive plant species will depend not only on the degree to which this indirect interaction reduces seed production but also on how strongly seed input influences plant density and population growth rate.

Acknowledgments Sandra Li assisted with seed counting. Dale Woods, California Department of Food and Agriculture, provided *Puccinia* spores. Cynthia Hays provided helpful comments during the data interpretation and the USDA Agricultural Research Service provided funding. Elizabeth Elle and two anonymous reviewers provided thoughtful suggestions that improved an earlier version of this manuscript. The experiments comply with the current laws of the country (USA) in which they were conducted.

References

- Ågren J (1996) Population size, pollinator limitation, and seed set in the self-incompatible herb *Lythrum salicaria*. *Ecology* 77:1779–1790
- Baker HG (1965) Characteristics and modes of origin of weeds. In: Baker HG, Stebbins GL (eds) *The genetics of colonizing species*. Academic, New York, pp 147–172
- Baker HG (1974) The evolution of weeds. *Annu Rev Ecol Syst* 5:1–24
- Barber NA, Adler L, Bernardo HL (2011) Effects of above- and belowground herbivory on growth, pollination and reproduction in cucumber. *Oecologia* 165:186–377
- Barthell JF, Randall JM, Thorp RW, Robbin W, Wenner AM (2001) Promotion of seed set in yellow starthistle by honey bees: evidence of an invasive mutualism. *Ecol Appl* 11:1870–1883
- Biere A, Honders SC (2006) Coping with third parties in an nursery pollination mutualism: *Hadena bicurvis* avoids oviposition on pathogen-infected, less rewarding *Silenelatifolia*. *New Phytol* 169:719–727
- Cavers PB, Heagy MI, Kokron RF (1979) The biology of Canadian weeds. 35. *Alliaria petiolata* (M. Bieb.) Cavara and Grande. *Can J Plant Sci* 59:217–229
- Crompton CH, Hall IV, Jensen KIN, Hildebrand PD (1988) The biology of Canadian weeds. 83. *Hypericum perforatum* L. *Can J Plant Sci* 68:149–162
- DiTomaso JM, Gerlach JD (2000) *Centaurea solstitialis*. In: Bossard CC, Randall JM, Hoshovsky MC (eds) *Invasive plants of California's wildlands*. University of California Press, Berkeley, pp 101–106
- DiTomaso J, Healy EA (2007) *Weeds of California and other western states*. University of California Press, Oakland
- Erterter B, Bowerman ML (2002) *The flowering plants and ferns of Mount Diablo*. California Native Plant Society, California
- Euler M, Baldwin I (1996) The chemistry of defense and apparency in the corollas of *Nicotiana attenuata*. *Oecologia* 107:102–112
- Garren JM, Strauss SY (2009) Population-level compensation by an invasive thistle thwarts biological control from seed predators. *Ecol Appl* 19:709–721
- Harrod RJ, Taylor RJ (1995) Reproduction and pollination biology of *Centaurea* and *Acroptilon* species, with emphasis on *C. diffusa*. *Northwest Sci* 69:97–105
- Hladun KR, Adler LS (2009) Influence of leaf herbivory, root herbivory, and pollination on plant performance in *Cucurbita moschata*. *Ecol Entomol* 34:144–152
- Juenger T, Bergelson J (1997) Pollen and resource limitation of compensation to herbivory in scarlet gilia, *Ipomopsis aggregata*. *Ecology* 78:1684–1695
- Karban R, Strauss SY (1993) Effects of herbivores on growth and reproduction of their perennial host, *Erigeron glaucus*. *Ecology* 74:39–46
- Kessler A, Halitschke R, Poveda K (2011) Herbivory-mediated pollinator limitation: negative impacts of induced volatiles on plant–pollinator interactions. *Ecology* 92:1769–1780
- Knight TM, Steets JA, Vamosi JC, Mazer SJ, Burd M, Campbell DR, Dudash MR, Johnston MO, Mitchell RJ, Ashman T-L (2005) Pollen limitation of plant reproduction: pattern and process. *Annu Rev Ecol Syst* 36:467–497
- Knight TM, Steets JA, Ashman TL (2006) A quantitative synthesis of pollen supplementation experiments highlights the contribution of resource reallocation to estimates of pollen limitation. *Am J Bot* 93:271–277
- Lehtilä K, Strauss SY (1997) Leaf damage by herbivores affects attractiveness to pollinators in wild radish *Raphanus raphanistrum*. *Oecologia* 111:396–403
- Levin DA (1970) Assortative pollination in *Lythrum*. *Am J Bot* 57:1–5

- Mal TK, Lovett-Doust J, Lovett-Doust L, Mulligan GA (1992) The biology of Canadian weeds. *Lythrum salicaria*. Can J Plant Sci 72:1305–1330
- Marquis RJ (1984) Leaf herbivores decrease fitness of a tropical plant. Science 226:537–539
- Montalvo AM, Ackerman JD (1990) Short- and long-term limitations to fruit production in a tropical orchid. Ecology 71:263–272
- Morris W et al (2007) Direct and interactive effects of enemies and mutualists on plant performance: a meta-analysis. Ecology 88:1021–1029
- Mothershead K, Marquis J (2000) Fitness impacts of herbivory through indirect effects on plant–pollinator interactions in *Oenothera macrocarpa*. Ecology 81:30–40
- Myers JH, Bazely DR (2003) Ecology and control of introduced plants. Cambridge University Press, Cambridge
- Parker IM (1997) Pollinator limitation of *Cytisus scoparius* (Scotch broom), an invasive exotic shrub. Ecology 78:1457–1470
- Parker IM (2000) Invasion dynamics of *Cytisus scoparius*: a matrix model approach. Ecol Appl 10:726–743
- Pitcairn MJ, Schoenig S, Yacoub R, Gendron J (2006) Yellow starthistle continues its spread in California. Calif Agric 60:83–90
- Primack RB, Hall P (1990) Costs of reproduction in the pink lady's slipper orchid: a 4 year experimental study. Am Nat 136:638–655
- Reichard SE (1994) Assessing the potential of invasiveness in woody plants introduced in North America. University of Washington, Seattle
- Roy BA (1996) A plant pathogen influences pollinator behavior and may influence reproduction of nonhosts. Ecology 77:2445–2457
- Shishkoff N, Bruckart WL (1996) Water stress and damage caused by *Puccinia jaceae* on two *Centaurea* species. Biol Control 6:57–63
- Stout MJ, Thaler JS, Thomma BPHJ (2006) Plant-mediated interactions between pathogenic microorganisms and herbivorous arthropods. Annu Rev Entomol 51:663–689
- Strauss SY, Murch P (2004) Towards and understanding of the mechanism of tolerance: compensating for herbivore damage by enhancing a mutualism. Ecol Entomol 29:234–239
- Strauss SY, Conner JK, Rush SL (1996) Foliar herbivory affects floral characters and plant attractiveness to pollinators: implications for male and female plant fitness. Am Nat 147:1098–1107
- Sun M, Ritland K (1998) Mating system of yellow starthistle (*Centaurea solstitialis*), a successful colonizer in North America. Heredity 80:225–232
- Swope SM, Parker IM (2010a) Trait-mediated interactions and lifetime fitness of the invasive plant *Centaurea solstitialis*. Ecology 91:2284–2293
- Swope SM, Parker IM (2010b) Widespread seed limitation affects plant density but not population trajectory in the invasive plant *Centaurea solstitialis*. Oecologia 164:117–128
- Swope SM, Parker IM (2012) Complex interactions among biocontrol agents, pollinators, and an invasive weed: a structural equation modeling approach. Ecol Appl 22:2122–2134
- Swope SM, Stein IR (2012) Soil type mediates indirect interactions between *Centaurea solstitialis* and its biocontrol agents. Biol Invasions 14:1697–1710
- Upadhyaya MK, Turkington R, McIlvride D (1986) The biology of Canadian weeds. 75. *Bromus tectorum* L. Can J Plant Sci 66:689–710
- van Driesche, Hoddle M, Center T (2008) Control of pests and weeds by natural enemies. Blackwell, Malden
- Vilá M, Weber E, D'Antonio CM (1998) Pollination and seed set in hybrid and parental morphotypes of *Carpobrotus* in California. Can J Bot 76:1165–1169
- Zimmerman M, Pyke GH (1988) Reproduction in *Polemonium*: assessing the factors limiting seed set. Am Nat 131:723–738