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ORIGINAL PAPER

Soil type mediates indirect interactions between *Centaurea solstitialis* and its biocontrol agents

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Abstract Many invasive plants are attacked by more than one biocontrol agent. Attack by multiple enemies may give rise to indirect interactions, the nature of which may be influenced by the abiotic environment. We conducted a field experiment to determine (1) whether indirect interactions arose between *Centaurea solstitialis*, a foliar pathogen and three insect seed predators and (2) how the outcome was influenced by soil type (serpentine and non-serpentine). Because serpentine soils support high numbers of endemic species they are a priority for conservation. They also have very low calcium concentrations and Ca^{++} regulates plants' ability to defend against pathogen infection. *C. solstitialis* growing on serpentine soil may therefore be more vulnerable to the pathogen and this may in turn affect the plant's subsequent interactions with seed predators. We found that pathogen infection had a direct, negative impact on plant performance but its impact was not greater on serpentine plants. When attacked by the seed predators, inflorescences produced more viable seed when they were on plants infected with the pathogen than when they were on uninfected plants and the data suggest that this reflects reductions in larval seed-feeding. On the non-serpentine soil, the pathogen's direct, negative impact was entirely canceled out by its indirect, positive effect via reduced seed predation. On the serpentine soil, plants attacked by the pathogen and the insect seed predators produced half as many seeds than plants attacked only by the seed predators. Our results demonstrate that biocontrol agent interactions may be modified by the plant and by the abiotic environment in a way that fundamentally alters their net impact on the weed.

Keywords Calcium · *Chaetorellia* · *Eustenopus villosus* · Nitrogen · *Puccinia jaceae solstitialis* · Serpentine · Systemic acquired resistance

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Introduction

Biological control, the practice of using enemies from an invader's native range to control its abundance in the introduced range, is often cited as our best hope for controlling the most widespread and well-established

exotics invaders (van Driesche et al. 2008). Historically, biocontrol practitioners espoused the use of multiple agents that attack different parts of the plant simultaneously or sequentially. The reasoning behind this approach is that one agent attacking the plant in isolation may not significantly reduce plant performance but the cumulative impact of multiple agents might (van Driesche et al. 2008). In such a scenario, the agents' impact on the invader ought to be additive or, ideally, super-additive, as is the case for *Senecio jacobaea* and its two biocontrol agents, the leaf herbivore *Longitarsus jacobaeae* and the florivore *Tyria jacobaeae*. When exposed only to the florivore the plant is able to partially compensate for its losses but when the plant is subjected to leaf herbivory before it is attacked by the florivore, it is unable to compensate and the impact of the two agents is super-additive (James et al. 1992).

Although this multi-agent approach has been largely abandoned in favor of releasing a single agent of high impact, most weeds in the USA are nevertheless subject to attack by multiple agents (Coombs et al. 2004). This is the result of either the historic use of the multi-agent approach or because new agents are released when established agents have failed to control the weed. In either case, additive or synergistic interactions among agents is possible and desirable.

However, it is also possible for agents to interfere with one another, even when they never interact directly (Denno et al. 1995, Swope and Parker 2010a). In previous work, we found that infection by the foliar pathogen *Puccinia jaceae* f.s. *solstitialis*, a newly released biocontrol agent, had a direct, negative impact on its host, the invasive plant *Centaurea solstitialis*, but infection also significantly reduced seed predation by the seed predator *Eustenopus villosus*, a well-established biocontrol agent (Swope and Parker 2010a). We hypothesized two possible underlying mechanisms. Pathogen infection can cause plants to reallocate resources, especially nitrogen, to the seeds (Chapin 1980, Mattson 1980) and in response, seed predators may have been able to complete metamorphosis while consuming fewer seeds. Alternatively, *C. solstitialis* plants may have responded to initial infection with a biochemical defense (systemic acquired resistance; SAR) that was also effective against the seed predator (Karban et al. 1987). Ultimately the pathogen's direct negative effect on its invasive host was canceled out by its indirect, positive impact via reduced seed predation (Swope and Parker 2010a). Regardless of whether the net impact of the direct and indirect interactions was super-additive (as in James et al. 1992) or sub-additive (as in Swope and Parker 2010a), it is clear that the impact of one species may be influenced, sometimes quite strongly, by the presence or absence of another species.

It is also possible for abiotic conditions to influence interactions among species. A common example from the ecological literature is that of nurse plant relationships in high versus low stress environments. In a geographically wide-ranging demonstration of this, Callaway et al. (2002) showed that for plants growing below treeline (relatively low stress conditions), neighboring plants generally reduced survival, growth and reproduction of the focal plant via competition. But above treeline, where conditions were considerably more stressful, the same plant species benefited from their neighbors which acted as facilitators by ameliorating abiotic stresses such as low temperatures, evapotranspirational water loss and soil instability. By changing the strength and even the nature of direct interactions, the abiotic environment has the potential to change the outcome of subsequent indirect interactions as well, but this remains largely unstudied.

How direct and indirect interactions are affected by the abiotic environment is especially relevant to biocontrol of invasive species. Invasive species often occupy large geographic areas in the introduced range that tend to span numerous environmental gradients. It is possible that both direct and indirect interactions will be affected by the various abiotic conditions the plant and its biocontrol agents encounter throughout the range, meaning that the same suite of agents may have one impact on the plant in one place and a different impact elsewhere.

In this study, we expand on previous work (Swope and Parker 2010a) to explore how abiotic conditions affect direct and indirect interactions between the recently released biocontrol pathogen *Puccinia jaceae* f.s. *solstitialis* and a suite of well-established insect seed predators (also biocontrol agents) via their shared host, *Centaurea solstitialis*. We focused on the influence of soil type, specifically serpentine versus non-serpentine soils. Serpentine soils occur throughout the world in areas of tectonic activity and are of high conservation value in California because they support an assemblage of rare and endemic plants and animals

(Roberts and Proctor 1992). Further, *C. solstitialis* invasion of serpentine sites across the state is increasing (Gelbard and Harrison 2003; Batten et al. 2006). Serpentine soils have characteristics that create stressful conditions for most plants including high concentrations of heavy metals, low water holding capacity and low Ca/Mg ratios (Proctor and Woodell 1975; Kruckeberg 1984; Alexander et al. 2007) and occur in discrete patches in a matrix of more benign soil types.

The low Ca/Mg ratio of most serpentine soils has the potential to change the outcome of *C. solstitialis*-*Puccinia*-seed predator interactions. Calcium plays a critical role in allowing plants to detect and respond to pathogen infection because calcium-binding sensor molecules must be activated to initiate defensive responses (Lamb et al. 1989; Blumwald et al. 1998; Scheel 1998, Grant and Mansfield 1999). Numerous examples from the agricultural literature show that a deficiency of Ca^{++} in the soil leads to higher rates of disease in crops and that the addition of Ca^{++} reduces disease prevalence (reviewed by Engelhard 1989). Serpentine soils' low Ca/Mg ratio makes the selective uptake of calcium ions difficult and so serpentine-dwelling plants may be highly susceptible to pathogen infection. We know of only one set of experiments that test how the low Ca^{++} concentration in serpentine soils affects pathogen infection in a serpentine endemic. Springer and colleagues used a naturally occurring gradient of Ca^{++} concentration in serpentine soils and found that *Hesperolinon californicum* plants in the higher Ca^{++} soils had lower rates of infection by the rust pathogen *Melampsora lini* (Springer et al. 2007). They also found that experimentally increasing soil Ca^{++} concentration reduced infection levels (Springer 2009a; but see also Springer 2009b). In addition, the initiation of this Ca^{++}-based pathway appears to contribute to the induction of systemic acquired resistance (SAR) (Mishina and Zeier 2007), a whole-plant response that increases resistance to a broad spectrum of enemies including insects. The low Ca^{++} of the serpentine soils may therefore also change *C. solstitialis*' interactions with the seed predator biocontrol agents.

We wanted to know if soil type changes the direct interaction between the plant and the pathogen and if this in turn affects the plant's later interactions with its seed predators. Specifically, we asked the following questions: (1) Does pathogen infection have a larger direct effect on plant performance on serpentine soils than on non-serpentine soils? (2) Does pathogen infection interfere with seed predation by all of the insect species (or just *E. villosus* as previously documented) and (3) is the degree of interference among agents reduced on serpentine plants compared to non-serpentine plants? Finally we wanted to know if (4) the net impact of the pathogen and the seed predators on whole-plant seed production was greater on serpentine soils than on non-serpentine soils.

Methods

Study system

Centaurea solstitialis L. (Asteraceae) is an annual thistle native to Eurasia. The first record of it in California dates to 1869 when a population was found near San Francisco (DiTomaso and Gerlach 2000). It has spread rapidly since and now infests over 6 million ha in the state (Pitcairn et al. 2006). *C. solstitialis* seeds germinate with the onset of the autumn rains, plants overwinter as a basal rosette of leaves and flower during the summer drought. By late summer all plants have dispersed seed and died.

Between 1985 and 1992, the USDA released three species of tephritid fly and three species of weevil as biocontrol agents intended to control *C. solstitialis* invasions in California. Four of the six agents were found at both of our study sites. *Eustenopus villosus* (Coleoptera: Curculionidae) was the most common, *Chaetorellia succinea* (Diptera: Tephritidae) was also present in high numbers and *C. australis* was encountered at very low frequency. The dominance of *E. villosus* and the presence of one or both *Chaetorellia* species is typical of *C. solstitialis* invasions in California (Pitcairn et al. 1998). A fourth agent, *Urophora sirunaseva* (Diptera: Tephritidae), was also present in our study populations but in very low numbers. All four species are predispersal seed predators and each leaves species-specific evidence of seed predation making it easy to identify which species was feeding in each inflorescence even when the agent itself is not present (however, feeding by the two *Chaetorellia* species cannot be distinguished from one another).

In 2003, the USDA approved the pathogen *Puccinia jaceae* f.s. *solstitialis* (Uredinales: Pucciniaceae)

as a seventh biocontrol agent for *C. solstitialis*. (To avoid confusion with *C. solstitialis*, we will refer to the pathogen as *Puccinia*.) *Puccinia* is a non-systemic, biotrophic pathogen. Infection is confined to the leaves of the plant during the winter rosette stage. When the summer drought begins, the plant sheds its leaves (and along with them, the pathogen) and begins to produce inflorescences which are attacked by the insect seed predators. Because the pathogen and the insects are spatially and temporally separated from one another, any effect of the pathogen on the seed predators must be indirect.

Field sites

We located two established *C. solstitialis* invasions on adjacent patches of serpentine and non-serpentine (residuum weathered from sandstone) soils at the McLaughlin Natural Reserve (University of California Reserve System, Lake County, California, USA). We located our serpentine site in an area of the reserve known as "The Grid" (38°49′29″N, 122°20′38″W, 428 m elev). Using existing soils data from The Grid (Wright et al. 2006), we selected an area where Ca/Mg ratios ranged from 0.19 to 0.42 for our experiment.

Our non-serpentine site was located 0.64 km southeast of the serpentine site (38°49′36.07″N, 122°20′20.88″W, 395 m elev). The soil at our non-serpentine site has not been previously described so we collected seven soil samples from this site using a 5 cm wide × 15 cm deep soil core. Samples were analyzed separately for N, P, K, Ca, Mg and pH. Ca/Mg ratios ranged from 1.86 to 4.75 (ESM Table 1). Both sites were flat with no shading from trees and dominated by exotic grasses (*Avena barbata*, *A. fatua*, *Bromus diandrus*, *B. hordeaceus*, and *Lolium multiflorum*), as is typical of central California grasslands. Foliar cover by the co-occurring plants was high and not different on the two soil types (serpentine: mean cover in a 35 cm-diameter plot around each experimental plant was 78 ± 13% SD; Non-serpentine: 71 ± 18% SD).

Experimental pathogen infection

We randomly selected 200 naturally recruiting seedlings on the serpentine soil and 100 seedlings on the non-serpentine soil (January 2010). We started with a larger sample size on the serpentine soil because we expected the stressful nature of those soils might lead to higher seedling mortality and we wanted to maximize the likelihood that enough plants survived to flowering to permit analysis. Half of the plants on each soil type were randomly assigned to the *+Puccinia* group and the other half to the uninfected group. To be sure that plants in the two groups were, on average, the same size at the start of the experiment, we measured the length of the longest leaf and counted the total number of leaves at the time of inoculation. In an attempt to make the competitive environment uniform, we removed all conspecific neighbors within a 25 cm radius of each experimental plant. We did not remove the annual grasses because they appear to buffer vulnerable seedlings from desiccation and removing them increases *C. solstitialis* mortality (Swope, *unpublished data*).

Plants in the *+Puccinia* group were sprayed to runoff with 300 mg of urideniospores in a solution of 300 mL of DI water and five drops of the wetting agent Tween20 (polyoxyethylene sorbitan monolaurate; Acros Organics, Morris Plains, New Jersey, USA). Plants in the uninfected control group were sprayed with DI water and Tween20. All plants were inoculated on 18 February, 2010 and again on 12 March, 2010.

Successful pathogen infection can be nondestructively assessed in the field by the development of pustules on the leaves. Pustules tend to develop first on the ventral side of the leaf and spread from the tip to the base and then develop on the dorsal side of the leaf, again spreading from tip to base; peak pustule development is reached 6–9 weeks after inoculation (Dale Woods, CDFA, *personal communication*). Plants were inspected for pustule development on April 19, 2010, eight and a half weeks after the first round of inoculations and five and half weeks after the second. We used pustule development as a proxy for the degree of infection and we quantified it in two ways. First, we counted the total number of leaves on the plant and noted how many leaves had pustules, i.e., the proportion of leaves that appeared to be infected. Second, we selected the most infected leaf on each plant and visually divided both the dorsal and ventral surface into three equally-sized regions (tip, mid, and base). The leaf was given one point for each region in which pustules were found. The highest score a leaf could receive was six (pustules evident the entire length of both surfaces of the leaf). Both of these measures are

imperfect assessments of the degree of infection but have the advantage of being nondestructive.

Direct effect of pathogen on plant performance

Plants were allowed to flower and senesce in the field. We inspected every plant in the study once a week during flowering and collected every inflorescence just prior to seed dispersal to measure reproductive output. Because we removed inflorescences only after the pedicel and receptacle had senesced and the petal cap had loosened but not fallen off, removing them is very unlikely to have caused a compensatory response in the plant. Each inflorescence was stored in a separate coin envelop and dissected in the lab. When plants died we harvested them by clipping them at ground level, dried them at 60°C for 48 h and weighed them.

Interactions with seed predators

A factorial design in which we also experimentally reduced attack by the seed predators would have been ideal but unfortunately, this was not possible. In other work spanning three sites and 2 years, we have attempted to reduce attack by the insect agents by spraying plants with the insecticide Ortho Systemic Insect Killer (Scotts, Marysville, Ohio, USA) (Swope, *unpublished data*). This yielded only modest success against *Chaetorellia* spp while concentrations high enough to reduce attack by *E. villosus* were also phytotoxic. We therefore made no attempt to reduce insect attack here.

Because each of the insect agents (*E. villosus*, *Chaetorellia* spp, and *U. sirunaseva*) leaves species-specific evidence of seed predation, we were able to determine if an inflorescence had escaped attack or, if it had been attacked, which species was responsible. Viable seeds are easy to distinguish from non-viable ones under a dissecting scope based on size, shape and color (on the rare occasion we were unsure about viability we germinated seeds in a Petri dish). We dissected every inflorescence produced by the experimental plants and categorized the seeds as viable (filled and undamaged by seed predators), damaged (filled but partially eaten and so no longer capable of germinating), or non-viable (unfilled). Less than 1% of the 1,224 inflorescences we dissected were attacked by more than one seed predator of the same or different species.

We quantified the impact of the larval seed feeding on seed production in two ways. First, we estimated the effect size for each insect species. To do this, we paired two inflorescences on the same plant and that had matured in the same week, one of which had escaped attack and the other of which had been attacked by one of the seed predators. We estimated the proportion of seeds consumed as

$$\frac{U_{VS} - SP_{VS}}{U_{VS}}$$

in which U_{VS} refers to the number of viable seeds produced by the unattacked inflorescence and SP_{VS} refers to the number of viable seeds produced by the inflorescence attacked by a seed predator. By pairing inflorescences in this way we controlled for variation among plants as well as any temporal variation driven by changes in the pollinator community and/or the resources available to mature pollinated ovules.

For the second assessment of the seed predators' impact, we focused on the direct measure of its consequences to plant fitness: total viable seeds per inflorescence. This measurement assumes that agents are selecting inflorescences randomly, i.e., that there is no difference in the number of ovules per inflorescence between those that were selected as oviposition sites and those that were not. We were able to confirm this in a small-scale experiment (detailed in ESM Table 2) and found that there was no difference in the number of ovules produced by the inflorescences used as oviposition sites and those that were not. By tracking every inflorescence and counting the number of viable seeds each produced, we were able to calculate total seed output per plant.

Eustenopus villosus survival

It is possible to determine larval mortality for *E. villosus* (but not for the other insects in this study). When laying an egg, an adult female chews a hole in the wall of the capitulum and inserts a single egg. She then seals the hole with frass, leaving a distinctive oviposition wound that is visible on the outside of the inflorescence. When the larva (or egg) dies, there is a small amount of damaged plant tissue on the inside of the capitulum wall directly behind the oviposition wound that is easy to detect when dissecting the inflorescence; when the larva survived there is a well-developed pupal chamber lined with frass and partially

eaten seeds. This makes identifying inflorescences used as oviposition sites by *E. villosus* easy and calculating larval survival is straightforward.

Nutrient content of plants

Dried plants (described above) were analyzed for Ca^{++} using the wet ash digestion method (Jones 2001) to determine if the difference in Ca/Mg ratio of the two soil types was reflected in the plant's uptake of Ca^{++}. To determine if either soil type or pathogen infection affected the nitrogen content of the seeds (quality of seeds as a food source), we ground seeds to ensure complete combustion and measured total N using a TrueSpec Elemental Analyzer (Leco, St. Joseph, Michigan, USA). The instrument was calibrated with EDTA (9.57% N) and the sample size was 0.15 g.

Statistical analyses

For all analyses, we used General Linear Models (GLMs; Systat 12.0 SPSS, Chicago, Illinois, USA) to determine the effect of soil type and pathogen infection on plant performance. Soil type and pathogen infection were treated as fixed independent variables. Response variables were transformed when necessary to meet the assumptions of the test (noted in the figure legends). To determine how soil type alone affected plant uptake of Ca^{++} and the degree to which plants were infected, the response variables were plant tissue Ca^{++} content (mg g^{-1}), infection score (described above) and the percentage of leaves with pustules on them. To determine the direct effects of both soil type and pathogen infection on plant performance, the response variables were plant biomass, number of inflorescences per plant, the number of viable seeds per (unattacked) inflorescence and the nitrogen content of the seeds. To determine if soil type and pathogen infection affected the plant's interactions with its seed predators (an indirect interaction), the response variables were the proportion of seeds consumed per inflorescence and the number of viable seeds produced by individual inflorescences. Each species of seed predator was analyzed separately. We used a χ^2 test of independence to determine if either soil type or pathogen infection influenced *E. villosus* larval survival. To assess how soil type and pathogen infection affected lifetime fitness of *C. solstitialis* in the presence of the seed predators, the response variable was whole-plant seed production.

To determine (a) if the magnitude of the pathogen's direct, negative impact was larger on serpentine-dwelling plants than on non-serpentine plants and (b) if the pathogen's indirect interaction with the seed predators was affected by soil type, we looked for a significant interaction between soil type and pathogen infection.

Results

Degree of disease development

Of the 150 plants that were sprayed with *Puccinia*, all but two showed a detectable degree of pustule development on their leaves. The two plants that did not display any evidence of infection (one on each soil type) were dropped from the study. No plants assigned to the uninfected group became infected with *Puccinia*.

As expected, the low Ca/Mg ratio of the serpentine soil significantly reduced the concentration of Ca^{++} in plant tissue (ESM Table 3). Serpentine plants also had significantly higher levels of pustule development on their leaves than non-serpentine plants (ESM Figure 1). On average, non-serpentine plants had an infection score of 4.05 (equivalent to pustules covering 67.5% of the most infected leaf) and serpentine plants had an infection score of 5.33 (pustules covering 88.9% of the most infected leaf). A significantly higher percentage of the serpentine-dwelling plants' leaves had pustules on them compared to the non-serpentine plants (ESM Table 4) but the actual difference was small (non-serpentine: 26.7% $\pm$ 1.1 SE; serpentine: 30.9% $\pm$ 1.0 SE).

Plant performance

Plants growing on the non-serpentine soil were, on average, three times larger than plants growing on serpentine soil (Fig. 1a). Pathogen infection reduced mean plant biomass by 37% on non-serpentine soil and by 41% on serpentine soils, but the interaction between soil type and pathogen infection was not significant. Similarly, both serpentine soil and pathogen infection reduced the total number of inflorescences a plant produced in its lifetime (Fig. 1b). Non-serpentine plants produced more than twice as

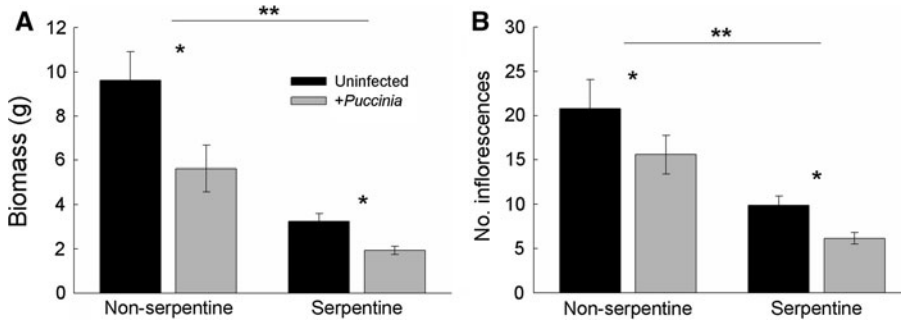

Fig. 1 The effect of soil type and pathogen (*Puccinia jaceae solstitialis*) infection on *Centaurea solstitialis* **a** biomass (soil: $F_{1,253} = 65.524$, MS = 59.569, $P = 0.0001$; pathogen: $F_{1,253} = 26.269$, MS = 24.209, $P = 0.0001$; interaction: $F_{1,253} = 0.332$, MS = 0.302, $P = 0.57$) and **b** total inflorescences per plant (soil: $F_{1,253} = 38.712$, MS = 26.298, $P = 0.0001$; pathogen: $F_{1,253} = 8.725$, MS = 5.927, $P = 0.003$; interaction: $F_{1,253} = 0.766$, MS = 0.520, $P = 0.38$). Biomass was Ln-transformed for analysis; inflorescences was Ln($y + 1$) transformed for analysis; untransformed data are shown. *Error bars* represent ±1SE. *Double asterisks* indicated significant difference between soil types; *single asterisks* indicate significant difference between infected and uninfected plants

many inflorescences as did serpentine plants. On non-serpentine soils, pathogen infection reduced the number of inflorescences per plant by 25% and by 37% on serpentine soil but again the interaction between soil type and pathogen infection was not significant.

Despite clear effects on plant size and the number of inflorescences plants produced, neither soil type nor pathogen infection affected the total number of ovules per inflorescence (ESM Table 2) or the number of viable seeds per inflorescence in the absence of seed predation (non-serpentine, uninfected: 25.78 ± 10.56 SD; non-serpentine, +*Puccinia*: 26.24 ± 14.05 SD; serpentine, uninfected: 26.80 ± 8.89 SD; serpentine, +*Puccinia*: 26.66 ± 10.30 SD; ESM Table 5).

Interactions with seed predators

Eustenopus villosus larvae consumed a significantly higher proportion of seeds when they matured in the inflorescence of a serpentine plant than a non-serpentine plant; they also consumed a higher proportion of seeds when the plant was uninfected than when it was infected with *Puccinia* (ESM Figure 2A). The interaction between soil type and pathogen infection was not significant. Because this method for estimating seed consumption required not only an unattacked inflorescence for each plant (and for many plants, the percentage of attacked inflorescences was high), but also one that had matured within a week of the attacked inflorescence to which it was paired, we were unable to make seed consumption estimates for the less common agents. We had 136 independent estimates of seed consumption for *Chaetorellia* spp and 62 estimates (all but two of which were on serpentine plants) for *U. sirunaseva*. A post hoc power analysis indicates that these sample sizes are insufficient for analysis, even for *Chaetorellia* spp. However, the overall pattern was the same as that for *E. villosus* seed consumption (ESM Figure 2B&C).

We did not have the same problem with sample size when comparing the number of viable seeds per inflorescence because this measurement did not require a comparison to an unattacked inflorescence. When subjected to seed predation by *E. villosus* and *Chaetorellia* spp, inflorescences produced significantly more viable seeds when plants were growing on non-serpentine than on serpentine soil (Fig. 2a, b). (As noted above, *U. sirunaseva* attacked so few inflorescences on non-serpentine plants that we were unable to make a comparison across soil types.) When attacked by seed predators (*E. villosus*, *Chaetorellia* spp and *U. sirunaseva*), inflorescences produced more viable seed when the plant was infected with *Puccinia* than when it was uninfected (Fig. 2a–c). The interaction between soil type and pathogen infection was not significant for *E. villosus* but it was for *Chaetorellia* spp. More specifically, the difference in the number of viable seeds produced by infected and uninfected plants was smaller on serpentine soils than it was on non-serpentine soils.

Eustenopus villosus larval survival was lowest on the non-serpentine soil and highest on the serpentine

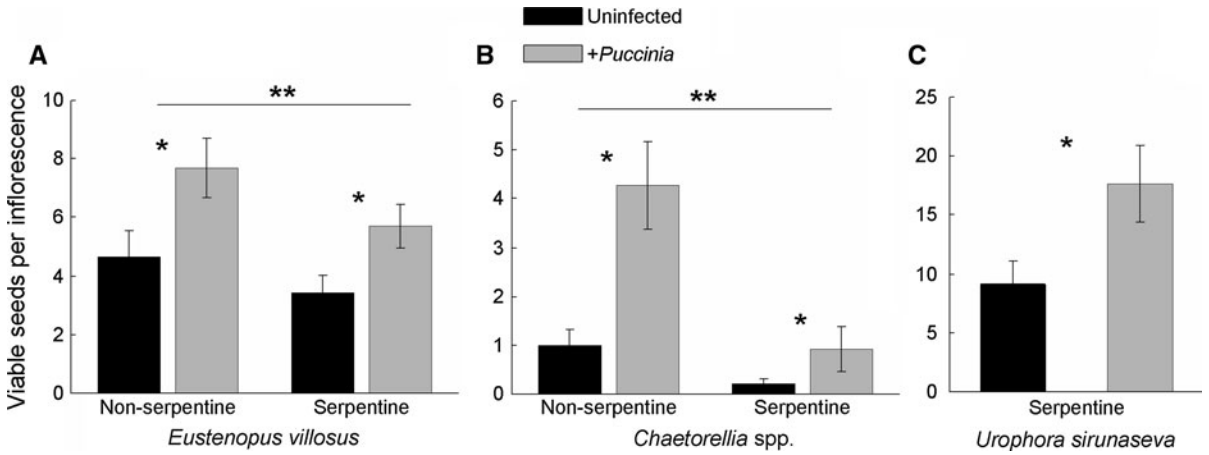

Fig. 2 The effect of soil type and pathogen infection on the number of viable seeds produced by inflorescences attacked by the biocontrol seed predators **a** *Eustenopus villosus* (soil: $F_{1,386} = 3.942$, MS = 5.129, $P = 0.04$; pathogen: $F_{1,386} = 17.404$, MS = 22.640, $P = 0.0001$; interaction: $F_{1,386} = 0.077$, MS = 0.100, $P = 0.78$); **b** *Chaetorellia australis* and *C. succinea* (soil: $F_{1,188} = 26.100$, MS = 13.273, $P = 0.0001$; pathogen: $F_{1,188} = 16.683$, MS = 8.484, $P = 0.0001$; interaction: $F_{1,188} = 6.785$, MS = 3.450, $P = 0.01$); and **c** *Urophora sirunaseva* (pathogen: $F_{1,78} = 6.532$, MS = 13.014, $P = 0.013$). There were too few *U. sirunaseva*-attacked inflorescences on the non-serpentine soil to permit the inclusion soil type in the analysis. Data were Ln($y + 1$) transformed for analysis; untransformed data are shown. *Error bars* represent ±1SE

soil and pathogen infection marginally increased larval survival but only on the serpentine soil (Table 1). On non-serpentine plants, *E. villosus* larval survival was 38.30 and 40.50% on uninfected and infected plants, respectively. On the serpentine soil, *E. villosus* larval survival was 59.70% when the plants were uninfected and 69.20% when the plants were infected.

Total N in the soil at our serpentine site was twice as high on average as it was in the non-serpentine soil (ESM Table 1) and seeds from the serpentine plants had significantly higher nitrogen content than seeds from non-serpentine plants. Infected plants had higher seed N when they were on the non-serpentine soil but not when they were on the serpentine soil; the interaction between soil type and pathogen infection was significant (Fig. 3).

Table 1 The effect of soil type and pathogen infection on survival of *E. villosus* larvae

Source	Wald χ^2	*df*	*P*
Soil type	41.011	1	0.0001
Pathogen	2.516	1	0.113
Soil × pathogen	1.005	1	0.316

Lifetime fitness of *C. solstitialis*

Uninfected plants on the serpentine soils produced about two-thirds as many seeds in their lifetime as uninfected plants on the non-serpentine soils (Fig. 4). The addition of the pathogen to the suite of biocontrol agents had no net impact on lifetime fitness of the non-serpentine plants. In fact, infected and uninfected plants on the non-serpentine soil produced nearly identical numbers of seeds. But the addition of the pathogen reduced lifetime fitness of the serpentine plants by half. The interaction between soil type and pathogen infection was significant.

Discussion

Given the stressful nature of serpentine soils, it is not surprising that plants were smaller and produced fewer inflorescences when they grew on these soils than when they grew on non-serpentine soils (Proctor and Woodell 1975; Kruckeberg 1984; Alexander et al. 2007). It is also not surprising that *Puccinia* infection reduced plant performance on both soil types given that it is a biocontrol agent and was released precisely because of its negative impact on the plant. We hypothesized that the pathogen would have a greater

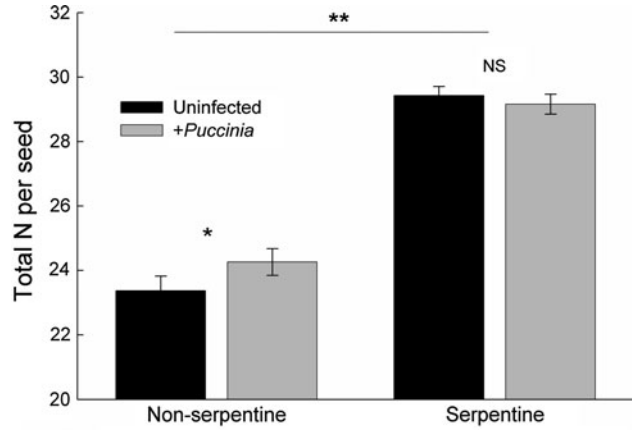

Fig. 3 The effect of soil type and pathogen infection on N concentration in *Centaurea solstitialis* seeds (soil: $F_{1,426} = 229.595$, MS = 3226.524, $P = 0.0001$; pathogen: $F_{1,426} = 0.169$, MS = 2.374, $P = 0.68$; interaction: $F_{1,426} = 3.923$, MS = 55.126, $P = 0.048$). *Error bars* represent ±1SE

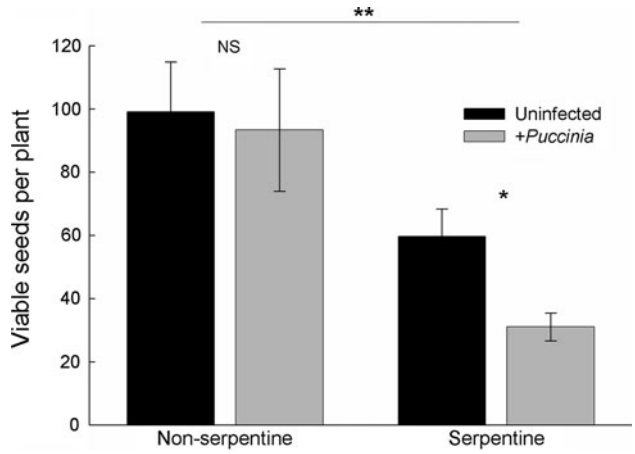

Fig. 4 The effect of soil type and pathogen infection on whole-plant (viable) seed production in the presence of the biocontrol seed predators (soil: $F_{1,253} = 44.991$, MS = 95.177, $P = 0.0001$; pathogen: $F_{1,253} = 3.795$, MS = 8.029, $P = 0.005$; interaction: $F_{1,253} = 1.392$, MS = 2.945, $P = 0.08$). Data were Ln($y + 1$) transformed for analysis; untransformed data are shown. *Error bars* represent ±1SE

direct impact on the plant when it was growing on serpentine soils than when it was growing on non-serpentine soils. Although serpentine plants took up considerably less Ca^{++} and had greater pustule development on their leaves, the magnitude of the pathogen's direct impact on biomass and inflorescence production was not larger than it was on non-serpentine plants.

Soil type altered the pathogen's impact on the plant in the presence of the seed predators. Inflorescences attacked by any one of the seed predators produced more viable seed on average when the plant was infected with the pathogen than when it was uninfected on both soils types. Although we were able to estimate actual seed consumption only for *E. villosus*, we think the differences we measured in seed production (per inflorescence) are likely attributable to a reduction in seed feeding by all insects. We arrive at this interpretation because neither soil type nor pathogen infection affected the mean number of viable seeds produced by unattacked inflorescences and because insects appeared to be choosing randomly between available inflorescences when ovipositing. Additionally, in our estimates of seed consumption, which are a more robust estimate of the purported interference, *Chaetorellia* and *U. sirunaseva* showed a similar pattern to *E. villosus* although we were unable to analyze these data due to small sample size. If we are correct that the differences in seed production reflect differences in seed consumption, then we interpret the significant interaction term for *Chaetorellia* spp. to mean that the pathogen interfered with *Chaetorellia* seed predation to a lesser degree when the plant was on the serpentine soil than when it was on the non-serpentine soil.

The net impact of the pathogen and the seed predators' numerous direct and indirect interactions on lifetime fitness of the plant was contingent upon soil type. On the non-serpentine soil, the direct negative effects of the pathogen on the plant were

canceled out by its indirect positive effects via reduced seed predation. The pathogen had an unambiguously negative, direct effect on the plant (lower biomass and fewer inflorescences) but the apparent interference with the seed predators means that on average, plants attacked by both the pathogen and the seed predators produced the same amount of seed as plants attacked only by the seed predators. This is consistent with previous work that examined the interaction between *Puccinia* and *E. villosus* on non-serpentine soils at a different site (Swope and Parker 2010a) and found that *Puccinia* had a direct negative impact on *C. solstitialis* fitness that was offset by its indirect positive impact via reduced larval seed feeding. Other work has shown that endophytic fungi can have a similar effect on seed-feeding biocontrol agents. In a lab setting, Newcombe et al. (2009) demonstrated reduced seed feeding by the biocontrol seed predator *Larinus minutus* (Coleoptera: Curculionidae) on the invasive plant *Centaurea stoebe* (Asteraceae) inoculated with two endophytic fungi. In general however, the potential for pathogen and insect biocontrol agents to interfere with each other is largely unstudied.

In contrast, on the serpentine soil, the addition of the pathogen to the suite of biocontrol agents reduced mean whole-plant seed production by half. This is initially counterintuitive because viable seed production was higher in attacked inflorescences from infected plants than from uninfected plants. Two factors appear to be especially important in producing this final result. First, the direct impact of the pathogen on the plant (reduced biomass and number of inflorescences) was measured on a whole-plant basis and was quite large in magnitude while the interference between the pathogen and the insects (reduced larval seed-feeding) was measured on a per-inflorescence basis and the impact was smaller in magnitude. The net impact on whole-plant seed production integrates all of these direct and indirect impacts measured at these different scales. Second, *E. villosus* larval survival was highest on infected, serpentine plants meaning that these plants effectively experienced a higher level of attack by the dominant agent than plants with lower *E. villosus* survival.

Mechanisms underlying interference

Our data provide some circumstantial evidence that may help tease apart the contribution of two possible mechanisms underlying the purported reduced larval seed feeding in infected plants: a change in seed quality and SAR.

Biotrophic pathogen infection can have wide-ranging effects on plant nutrient status, including changing the concentration of nitrogen, structural elements or water in infected and uninfected tissue. All of these changes can occur even in the absence of measurable changes in plant morphology, size or fitness (reviewed in Stout et al. 2006). Larval seed predators tend to be especially sensitive to relatively small changes in host plant chemistry, particularly nutrient concentration (Hare and Dodds 1987; Tamura and Hiura 1998). If Puccinia infection caused *C. solstitialis* plants to reallocate N to the seeds (Chapin 1980; Mattson 1980), the agents' larvae may be able to complete metamorphosis while consuming fewer seeds. larvae may be able to complete metamorphosis while consuming fewer seeds.

While we did find that non-serpentine plants (but not serpentine plants) produced seed with higher N content when the plant was infected than when it was not, suggesting reallocation, this does not appear to account for the differences in seed production and presumably reduced larval seed feeding. In fact, we found the opposite. When comparing the uninfected plants on the two soil types, inflorescences attacked by *E. villosus* and *Chaetorellia* spp. produced fewer seeds when they were on serpentine plants (higher seed N), suggesting that both insects ate more of the N-rich seeds, not fewer. Yet the data suggest that all of the seed predators ate fewer seeds when the plant was infected even when infection increased the seed's N content, as was the case on the non-serpentine soil. (We have no explanation for why infected serpentine plants did not reallocate N to the seeds.) If we assume that the differences in seed production reflect actual differences in seed feeding, we conclude that the reduced larval seed-feeding on infected plants is not attributable to an increase in the quality of the seeds as a food resource.

Alternatively, it is possible that pathogen infection induced SAR. Numerous studies have shown that induced defenses such as SAR can be systemic even when damage is localized, as is the case here (McIntyre et al. 1981; Stout et al. 1999; Cardoza et al. 2003) and that SAR can be induced by both pathogens and insects and be broadly effective against both groups of enemies (Karban et al. 1987; Baldwin

and Schmelz 1996; Conrath et al. 2002; Rojo et al. 2003). The reduced larval seed feeding on infected plants that we observed may be the result of SAR on both soil types. Because Ca^{++} plays a critical role in plants' ability to respond to infection (Blumwald et al. 1998), we hypothesized that serpentine plants infected by *Puccinia* would be less defended against the seed predators, i.e., the degree of interference between the agents will be less in serpentine plants than in non-serpentine plants.

Our data provide conflicting circumstantial support for the hypothesized SAR response and the potential role of soil Ca^{++} in mediating it. Consistent with this hypothesis is the fact that serpentine plants suffered higher infection intensities than non-serpentine plants. But contrary to our Ca^{++}-SAR hypothesis, this did not lead to larger reductions in fitness for serpentine plants compared to non-serpentine plants. Additionally, if the initial pathogen infection primed the plant so that the seeds are defended when the larvae begin feeding and if serpentine plants are less defended, then our hypothesis would predict that *E. villosus* larvae should have higher survival rates when maturing on serpentine plants, but lower rates when maturing on infected plants on both soil types. Consistent with this hypothesis, we found that *E. villosus* larvae had higher survival rates on serpentine plants but contrary to it, we found that the larval survival rate was highest on infected, serpentine plants. In one final example of conflicting evidence for this hypothesis, we found that *Puccinia* interfered with seed feeding by *Chaetorellia* spp. to a lesser degree on serpentine soil but we did not find evidence of reduced interference between *Puccinia* and *E. villosus*, although this may simply reflect differences between the two agents in their sensitivity to SAR.

Two key questions remain unanswered. First, is SAR responsible for the reduced larval seed feeding that we see by the insects on infected plants compared to uninfected plants? Secondly, what is the relationship between soil Ca^{++} and the plant's ability to respond to infection with SAR? Many plant species respond to infection and other forms of attack with SAR, and there is no a priori reason to think that *C. solstitialis* is incapable of doing so. However, we cannot determine whether SAR is the mechanism without identifying and quantifying the defensive compounds in the seeds and experimentally determining whether soil Ca^{++} affects the concentration of defensive chemicals, something we are currently working on.

Implications for biocontrol

One concern often raised about the multi-agent approach to biocontrol is that agents might interfere with one another directly by competing for access to the same plant parts (e.g., Denno et al. 1995). In previous work we have argued that indirect, plant-mediated interference between agents may also be a risk of the multi-agent approach to biocontrol (Swope and Parker 2010a) and results from this study also support this concern, at least under some circumstances. As more species (agents) are added to the interaction web, it becomes more difficult to predict how they will interact with one another, in part because there is the potential for a greater number of direct and indirect interactions and in part because the outcome may be species-specific (e.g., Kluth et al. 2001; Van Zandt and Agrawal 2004) and even life stage-specific (Swope and Parker 2010a). Adding another complication is the fact that the outcome may be influenced by abiotic conditions that can vary in space and time. Ultimately, the impact of attack by multiple enemies on the plant may be highly idiosyncratic, dependent on the particular agents, how their interactions are modified by the plant and how the abiotic environment changes the plant's response to each agent individually and all agents collectively. Regardless of the mechanism responsible for the reduced seed feeding, our data show that *E. villosus* responded to pathogen infection by consuming fewer seeds and the evidence suggests that the other seed predators (*Chaetorellia* spp. and *U. sirunaseva*) did as well. We conclude from this that the pathogen interferes with a range of insect taxa and we caution that the potential exists for interference between this pathogen and prospective biocontrol agents that have yet to be identified or released.

In addition to the spatial variability that we documented across soil types, our results may also arise only under certain climatic conditions that vary temporally. *Puccinia* requires a cool, wet environment to establish and reproduce (Woods et al. 2010; Fisher et al. 2011) and the year in which we conducted this study was atypically wet late into the spring. This means that plants remained infected for a longer period of time than they do in dry years perhaps

allowing for larger direct and indirect pathogen impacts on the plant. In their review of cross-effects of induced plant responses to herbivory and infection, Rostás et al. (2003) concluded that a shorter time between induction by one species and attack by a second, may lead to larger effects. The wet spring also meant that because the plants stayed infected for a longer period of time, the length of time between infection and attack by seed predators was shorter than it would be in a drier year, thus potentially enhancing interactions between agents. It is worth noting that our previous work in which we documented similar interactions between the pathogen and *E. villosus* (Swope and Parker 2010a) was also conducted in a year with an atypically wet spring.

Puccinia's sensitivity to dry conditions may also explain why others have found only modest effects of infection on *C. solstitialis*. Fisher et al. (2007) found no effect of *Puccinia* infection on plant mortality, biomass or inflorescence production at a site in the hot, dry Central Valley (CA, USA) and O'Brien et al. (2010) found that total inflorescence production was not different between plots inoculated with *Puccinia* and control plots in the interior of the state where the pathogen is poorly adapted to local climatic conditions (Woods et al. 2010; Fisher et al. 2011).

Despite the fact that *Puccinia* and the insects together reduced *C. solstitialis* lifetime fitness by half at the serpentine site, it is not yet clear whether this is a desirable combination of agents to control *C. solstitialis* on serpentine soils in general for three reasons. First, our study was conducted at a single site but serpentine soils vary in their characteristics, including the Ca/Mg ratio (e.g., Springer et al. 2007), in ways that might affect the magnitude of the agents' net impact on the plant. Second, any form of management, including biocontrol, that reduces seed production has the potential to reduce the density of this invasive, annual thistle when and where recruitment is seed limited (Louda 1983; Crawley 1989; Sheppard et al. 2002, Parker 2001). Other work has shown that seed limitation occurs frequently even in long-established, high density *C. solstitialis* invasions in California (Swope and Parker 2010b) and that seed predators have the ability to reduce *C. solstitialis* density and spread rate (Swope and Satterthwaite 2012). But no research has been conducted on *C. solstitialis* recruitment limitation at this site or on serpentine soils in general so it is not yet clear if reductions in whole plant seed production will translate into population level control. Finally, while serpentine sites are of particular conservation concern and our data show that this combination of agents can have large effects on individual plants, releasing the new agent may be undesirable because reducing the abundance of *C. solstitialis* at non-serpentine sites is also a high conservation priority if only because non-serpentine soil types occur over a much larger area in the state. Our data show that this combination of agents does not interact in a synergistic manner to reduce plant performance on non-serpentine sites. Once released, we cannot keep Puccinia from attacking plants on the non-serpentine soils that surround the serpentine patches. It is generally agreed that the established agents have not satisfactorily controlled *C. solstitialis* in California (DiTomaso and Healy 2007) and the release of additional agents in the future is likely. Given what we think is evidence of interference between the pathogen and the three species seed-feeding insects, we caution against releasing an agent that is only modestly effective even though it is host-specific due to the potential for interference with prospective agents we may wish to release in the future.

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References

Alexander EB, Coleman RG, Keeler-Wolfe T (2007) Serpentine geoecology of western North America: geology, soils, and vegetation. Oxford University Press, New York

Baldwin IT, Schmelz EA (1996) Immunological "memory" in the induced accumulation of nicotine in wild tobacco. Ecology 77:236–246

Batten KM, Scow KM, Davies KF, Harrison SP (2006) Two invasive plants alter soil microbial community composition in serpentine grasslands. Biol Invas 8:217–230

Blumwald E, Aharon GS, Lamb BCH (1998) Early signal transduction pathways in plant–pathogen interactions. Trends Plant Sci 3:342–346

Callaway RC, Brooker RW, Choler P, Kikvidze Z, Lortie C, Michalet R, Paolini L, Pugnaire FI, Newingham B, Aschehoug ET, Armas C, Kikodze D, Cook BJ (2002) Positive interactions among alpine plants increase with stress. Nature 417:844–848

Cardoza YJ, Lait CG, Schmelz EA, Huang J, Tumlinson JH (2003) Fungus-induced biochemical changes in peanut plants and their effect on development of beet armyworm, *Spodoptera exigua* Hubner (Lepidoptera: Noctuidae) larvae. Environ Entomol 32:220–228

Chapin FS III (1980) The mineral nutrition of wild plants. Ann Rev Ecol Syst 11:233–260

Conrath U, Pieterse CMJ, Mauch-Mani B (2002) Priming in plant-pathogen interactions. Trends Plant Sci 7:210–216

Coombs EM, Clark JK, Piper GL, Cofrancesco AF (2004) Biological control of invasive plants in the United States. Oregon State University Press, Corvallis

Crawley MJ (1989) Insect herbivores and plant population dynamics. Annu Rev Entomol 34:531–564

Denno R, McClure M, Ott J (1995) Interspecific interactions in phytophagous insects: competition revisited and resurrected. Annu Rev Entomol 40:297–331

DiTomaso JM, Gerlach JD (2000) *Centaurea solstitialis*. In: Bossard CC, Randall JM, Hoshovsky MC (eds) Invasive plants of California's Wildlands. University of California, Berkeley, pp 101–106

DiTomaso J, Healy EA (2007) Weeds of California and other western states. University of California Press, Oakland

Engelhard AW (1989) Soilborne plant pathogens; management of diseases with macro- and microelements. APS, St Paul

Fisher AJ, Smith L, Woods DM, Bruckart W III (2007) Developing an optimal release strategy for the rust fungus *Puccinia jaceae* var. *solstitialis* for biological control of *Centaurea solstitialis* (yellow starthistle). Biol Control 42:161–171

Fisher AJ, Smith L, Woods DM (2011) Climatic analysis to determine where to collect and release *Puccinia jaceae* var. *solstitialis* for biological control of yellow starthistle. Biocontrol Sci Tech 21:333–351

Gelbard JL, Harrison S (2003) Roadless habitats as refuges for native grasslands: interactions with soil, aspect, and grazing. Ecol Appl 13:404–414

Grant M, Mansfield J (1999) Early events in host–pathogen interactions. Curr Opin Plant Biol 2:312–319

Hare DJ, Dodds JA (1987) Survival of the Colorado potato beetle on virus-infected tomato in relation to plant nitrogen and alkaloid content. Entom Exp Appl 44:31–35

James RR, McEvoy PB, Cox CS (1992) Combining the cinnabar moth (*Tyria jacobaeae*) and the ragwort flea beetle (*Longitarsus jacobaeae*) for control of ragwort (*Senecio jacobaea*): an experimental analysis. J Appl Ecol 29:589–596

Jones J (2001) Laboratory guide for conducting soil tests and plant analysis. CRC Press, Boca Raton

Karban R, Adamchek R, Schnathorst WC (1987) Induced resistance and interspecific competition between spider mites and a vascular wilt fungus. Science 235:678–680

Kluth S, Kruess A, Tscharntke T (2001) Interactions between the rust fungus *Puccinia punctiformis* and ectophagous and endophagous insects on creeping thistle. J Appl Ecol 38:548–556

Kruckeberg A (1984) California serpentines: flora, vegetation, geology, soils, and management problems. University of California Press, Berkeley

Lamb CJ, Lawton MA, Dron M, Dixon RA (1989) Signals and transduction mechanisms for activation of plant defenses against microbial attack. Cell 56:215–224

Louda SM (1983) Seed predations and seedling mortality in the recruitment of a shrub *Haplopappus venetus* (Asteraceae) along a climatic gradient. Ecology 64:511–521

Mattson WJ Jr (1980) Herbivory in relation to plant nitrogen content. Ann Rev Ecol Syst 11:119–161

McIntyre JL, Dodds JA, Hare JD (1981) Effects of localized infections of *Nicotiana tabacum* by tobacco mosaic virus on systemic resistance against diverse pathogens and an insect. Phytopathology 71:297–301

Mishina TE, Zeier J (2007) Pathogen-associated molecular pattern recognition rather than development of tissue necrosis contributes to bacterial induction of systemic acquired resistance in Arabidopsis. Plant J 50:500–513

Newcombe G, Shipunov A, Eigenbrode SD, Raghavendra AKH, Ding H, Anderson CL, Menjivar R, Crawford M, Schwarzländer M (2009) Endophytes influence protection and growth of an invasive plant. Commun Integr Biol 2:29–31

O'Brien J, Kyser GB, Woods DM, DiTomaso JM (2010) The effect of *Puccinia jaceae* var. *solstitialis* on the yellow starthistle biological control insects *Eustenopus villosus* and *Chaetorellia succinea*. Biol Control 52:182–187

Parker IM (2001) Safe site and seed limitation in *Cytisus scoparius* (Scotch broom): invasibility, disturbance, and the role of cryptogams in a glacial outwash prairie. Biol Invasions 3:323–332

Pitcairn MJ, O'Connell RA, Gendron JM (1998) Yellow starthistle: survey of statewide distribution. In: Woods DM (ed) Biological control program annual summary 1997. California Department of Food and Agriculture, Plant Health and Pest Prevention Services, Sacramento, pp 64–66

Pitcairn MJ, Schoenig S, Yacoub R, Gendron J (2006) Yellow starthistle continues its spread in California. Calif Agric 60:83–90

Proctor J, Woodell S (1975) The ecology of serpentine soils. In: Macfadyen A (ed) Advances in ecological research. Academic Press, London, vol 9, pp 255–366

Roberts BA, Proctor J (1992) The ecology of areas with serpentinized rocks. Kluwer, Dordrecht

Rojo E, Solano R, Sanchez-Serrano J (2003) Interactions between signaling compounds involved in plant defense. J Plant Growth Regul 22:82–98

Rostás M, Simon M, Hilker M (2003) Ecological cross-effects of induced plant responses towards herbivores and phytopathogenic fungi. Basic Appl Ecol 4:43–62

Scheel D (1998) Resistance response physiology and signal transduction. Curr Opin Plant Biol 1:305–310

Sheppard AW, Paynter PQ, Rees M (2002) Factors affecting invasion and persistence of broom *Cytisus scoparius* in Australia. J Appl Ecol 39:721–734

Springer YP (2009a) Edaphic quality and plant–pathogen interactions: effects of soil calcium on fungal infection of a serpentine flax. Ecology 90:1852–1862

Springer YP (2009b) Do extreme environments provide a refuge from pathogens? A phylogenetic test using serpentine flax. Am J Bot 96:2010–2021

Springer YP, Hardcastle BA, Gilbert GS (2007) Soil calcium and plant disease in serpentine ecosystems: a test of the pathogen refuge hypothesis. Oecologia 151:10–21

Stout MJ, Fidanstef AL, Duffey SS, Bostock RM (1999) Signal interaction in pathogen and insect attack: systemic plant-mediated interactions between pathogens and herbivores of the tomato, *Lycopersicon esculentum*. Physiol Mol Plant Pathol 54:115–130

Stout MJ, Thaler JS, Thomma BPHJ (2006) Plant-mediated interactions between pathogenic microorganisms and herbivorous arthropods. Ann Rev Entom 51:663–689

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, Satterthwaite WH (2012) Variable effects of a generalist parasitoid on a biocontrol seed predator and its target weed. Ecol Appl. doi:10.1890/10-2120.1

Tamura S, Hiura T (1998) Proximate factors affecting fruit set and seed mass of *Styrax obassia* in a masting year. Ecoscience 5:100–107

Van Driesche R, Hoddle M, Center T (2008) Control of pests and weeds by natural enemies. Blackwell, Malden

Van Zandt PA, Agrawal AA (2004) Community-wide impacts of herbivore induced plant responses in milkweed (*Asclepias syriaca*). Ecology 85:2616–2629

Woods DM, Fisher AJ, Villegas B (2010) Establishment of the Yellow Starthistle Rust in California: release, recovery, and spread. Plant Dis 94:174–178

Wright JW, Davies KF, Lau JA, McCall AC, McKay JK (2006) Experimental verification of ecological niche modeling in a heterogeneous environment. Ecology 87:2433–2439