

Complex interactions among biocontrol agents, pollinators, and an invasive weed: a structural equation modeling approach

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Abstract. Herbivores, seed predators, and pollinators can exert strong impacts on their host plants. They can also affect the strength of each other's impact by modifying traits in their shared host, producing super- or sub-additive outcomes. This phenomenon is especially relevant to biological control of invasive plants because most invaders are attacked by multiple agents. Unfortunately, complex interactions among agents are rarely studied. We used structural equation modeling (SEM) to quantify the effect of two biocontrol agents and generalist pollinators on the invasive weed *Centaurea solstitialis*, and to identify and quantify the direct and indirect interaction pathways among them. The weevil *Eustenopus villosus* is both a bud herbivore and a predispersal seed predator; the fly *Chaetorellia succinea* is also a predispersal seed predator; *Apis mellifera* is the primary pollinator. We conducted this work at three sites spanning the longitudinal range of *C. solstitialis* in California (USA) from the coast to the Sierra Nevada Mountains. SEM revealed that bud herbivory had the largest total effect on the weed's fecundity. The direct effect of bud herbivory on final seed set was 2–4 times larger in magnitude than the direct effect of seed predation by both agents combined. SEM also revealed important indirect interactions; by reducing the number of inflorescences plants produced, bud herbivory indirectly reduced the plant's attractiveness to ovipositing seed predators. This indirect, positive pathway reduced bud herbivory's direct negative effect by 11–25%. In the same way, bud herbivory also reduced pollinator visitation, although the magnitude of this pathway was relatively small. *E. villosus* oviposition deterred *C. succinea* oviposition, which is unfortunate because *C. succinea* is the more voracious of the seed predators. Finally, *C. succinea* oviposition indirectly deterred pollinator visitation, thereby enhancing its net effect on the plant. This study demonstrates the powerful insights that can be gained from the SEM approach in understanding the multiple direct and indirect interactions among agents and pollinators and their effects on an invasive weed. Such an approach may improve our ability to manage weeds with biocontrol agents by identifying pathways that could be exploited by future agents and minimizing the possibility of interference with established agents.

Key words: California, USA; *Centaurea solstitialis*; *Chaetorellia succinea*; *Eustenopus villosus*; herbivory; pollination; seed predation; structural equation modeling (SEM); yellow starthistle.

INTRODUCTION

All organisms interact with numerous other species over the course of their lifetimes. Some of these interactions will have a positive impact on performance (e.g., pollinators), while others will have a negative impact (e.g., seed predators and herbivores). Interactions among numerous enemies and mutualists over a lifetime may function in a synergistic or an antagonistic manner to produce nonadditive outcomes. For example, herbivory can have a direct, negative impact on plant performance but it may also indirectly reduce plant performance if damaged plants receive fewer pollinator visits because they are less rewarding, less attractive, or

less conspicuous to pollinators (Euler and Baldwin 1996, Strauss et al. 1996, Lehtilä and Strauss 1997, Mothershead and Marquis 2000). Such indirect interactions are likely to be ubiquitous in nature, but their outcomes are inherently difficult to predict and quantify.

How multiple species interact with one another via their shared host is especially relevant to the use of biological control agents to manage invasive plants. Most invasive plants that have been the target of biocontrol efforts are subject to attack by more than one agent. On average, seven agents have successfully established for each weed species in North America (Coombs et al. 2004). But how these agents interact with one another, directly and indirectly, is often unknown. At one point, the “cumulative stress” approach to biocontrol was favored (van Driesche et al. 2008). This approach is based on the idea that multiple agents ought to collectively reduce plant fitness even when each agent

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acting independently has only small effects. Although this multi-agent approach has largely been abandoned in favor of selecting a single agent of large impact, the reality is that many of the most problematic weeds are already subject to attack by more than one agent, either as the result of the historical use of the multi-agent approach, or because practitioners continue to release agents when previous ones prove ineffective (Denoth et al. 2002). Given the potential for direct and indirect interactions among biocontrol agents, we need quantitative approaches that allow us to identify these interactions and estimate their relative strengths with the goal of reducing the potential for interference among agents and possibly even exploiting previously unappreciated avenues of impact.

Both temporal and spatial variation in abiotic factors that are important to the plant may drive variation in the frequency and intensity of the plant's interactions with its enemies and its mutualists. As a generic example, in wetter years or sites, plants ought to be larger than they are in drier years or sites. If pollinators prefer to visit larger plants, then pollinator visitation may be more frequent under these more favorable circumstances. But so may attack by enemies if they too are more likely to attack larger plants. Almost by definition, invasive plants can be found across large geographic areas that span numerous environmental gradients in the introduced range. Understanding how spatiotemporal variation affects the intensity and frequency of the plant's interactions with its biocontrol agents and pollinators is therefore important to weed control.

Here we use structural equation modeling (SEM) to assess how two biocontrol insects interact with their host plant, the invasive weed *Centaurea solstitialis*, with one another and with pollinators, and how these direct and indirect interactions affect the weed's lifetime fitness. SEM uses the regression coefficients from multiple linear regressions to examine the effect of several direct and indirect pathways on a response variable, in this case, *C. solstitialis* seed production (Schemske and Horvitz 1988, Mitchell 1993). Because it quantifies both direct and indirect pathways, SEM allows us to explore complex networks in which multiple processes operate simultaneously (Grace 2006, Grace et al. 2010) and so is well suited to revealing the relative importance of multiple interacting species on plant performance. This sort of insight cannot be gained from comparing the mean response within an analysis of variance framework.

Structural equation modeling has only recently been applied to the problem of invasive species and their biocontrol agents. Larson et al. (2008) used SEM to understand how biocontrol and abiotic factors (soil texture and site) affect the density of the invasive plant leafy spurge (*Euphorbia esula*). Others have applied SEM in a community ecology framework to explore how plant invasion affects native plant communities (Harrison et al. 2006, Van Riper and Larson 2009), or

how a biological control agent can change community composition through its effects on the abundance of a dominant invader (Larson and Larson 2010). To our knowledge, our study is the first to use SEM to explore how multiple biocontrol agents interact with one another directly and indirectly to affect plant fitness.

C. solstitialis is an annual thistle that is highly invasive in California, where it is attacked by two biocontrol insects that are both well-established across the state. The weevil *Eustenopus villosus* (Coleoptera: Curculionidae) attacks the plant in both its adult and larval stages. Adult weevils feed on the youngest buds prior to egg-laying. This kills the bud and prevents it from maturing into an inflorescence. Larval *E. villosus* act as predispersal seed predators and consume on average 67% of the pollinated achenes in each attacked inflorescence (S. M. Swope, unpublished data). The second biocontrol agent, *Chaetorellia succinea* (Diptera: Tephritidae), is also a predispersal seed predator, and on average reduces seed production by >95% per inflorescence (S. M. Swope, unpublished data). In addition to these insect enemies, insect pollinators play an important role because *C. solstitialis* is self-incompatible and dependent on generalist insect pollinators for reproduction (Sun and Ritland 1998, Barthell et al. 2001).

Numerous indirect interactions among these insects are possible. For example, bud herbivory by *E. villosus* is expected to have a direct negative impact on the plant by reducing the number of inflorescences the plant produces. It may also have an indirect positive impact if plants subjected to bud herbivory produce fewer inflorescences and this in turn makes the plant less attractive to the adult agents as they seek oviposition sites. Similarly, plants with fewer inflorescences may receive fewer pollinator visits, which may in turn reduce seed set. In this way, the agent's direct negative impact on the plant may be enhanced via this indirect pathway.

Our goals were to (1) compare the magnitude of the impact of *E. villosus* bud herbivory to that of seed predation by each of the insect biocontrol agents on the lifetime fitness of their host plant *C. solstitialis*, (2) determine if the agents' damage deterred pollinators and if this indirectly enhanced their impact on the plant, (3) determine if the insect agents enhanced each other's impact or interfered with one another, and (4) assess whether these impacts changed through time or across the longitudinal range of *C. solstitialis* in California.

METHODS

Study species

Centaurea solstitialis seeds germinate throughout the fall and winter in response to precipitation. Over winter, plants persist as rosettes and invest primarily in taproot growth (DiTomaso and Healy 2007). Plants begin to produce buds and flowers with the onset of the summer drought (typically June) and complete their life cycle in mid- to late summer.

Eustenopus villosus emerges and starts to feed on the plant before *Chaetorellia succinea*. As described in the *Introduction*, *E. villosus* adults destroy the youngest buds by feeding on them and the plant is subject to 1–3 weeks of bud herbivory before either of the agents begins to lay eggs. Bud herbivory leaves a distinctive scar at the base of the bud, making it possible to determine if the bud died from herbivory or other causes. The larvae of both *E. villosus* and *C. succinea* are predispersal seed predators and both leave species-specific signs of attack that are apparent upon nondestructive observation. When laying eggs, female *E. villosus* chew a hole through the capitulum wall, insert a single egg, and then seal the hole with frass, leaving an oviposition wound that is visible on the external wall of the inflorescence (a few flowers immediately above the wound also die). Internally, *E. villosus* larvae will excavate a well-defined, frass-lined pupal chamber filled with partially eaten seeds. In contrast, *C. succinea* oviposits on older, larger buds, typically after the peak egg-laying phase for *E. villosus* (S. M. Swope, *personal observation*). Inflorescences used as oviposition sites by *C. succinea* never reach full petal expansion and petals appear desiccated. An inflorescence that has been fed on by *C. succinea* larva is filled with a fibrous, often sticky mass of plant tissue and partially eaten achenes.

C. solstitialis is an obligate outcrosser dependent on generalist pollinators for reproduction (Sun and Ritland 1998). The majority of the inflorescence visitors in this study were *Apis mellifera*, which is typical of *C. solstitialis* throughout California (Barthell et al. 2001, Swope and Parker 2010a). Because oviposition by both insect agents visibly damages inflorescences, pollinators may be able to identify and avoid attacked inflorescences.

Study sites

C. solstitialis is native to Eurasia and was first recorded in California in coastal areas near San Francisco in 1869 (DiTomaso and Gerlach 2000). In the 1930s, *C. solstitialis* began to spread into wildlands areas in the interior part of the state (Maddox 1981, Maddox and Mayfield 1985), where it currently forms dense and persistent populations (Pitcairn et al. 2006). Since the late 1990s, *C. solstitialis* has been expanding its range into the mid- and high elevations along the crest of the Sierra Nevada Mountains in the eastern part of the state (Pitcairn et al. 2006).

We conducted two consecutive years of data collection in 2007 and 2008 in three established invasions that were located longitudinally across the invaded range in California. The “Coast” population is located in the Loma Alta Open Space Preserve (268 m elevation) ~20 km from the first vouchered *C. solstitialis* specimen in California. This site is characterized by mild temperatures year-round and relatively high precipitation, which falls entirely in the form of rain. The “Interior” population is located in Mount Diablo State Park (432

m elevation) where *C. solstitialis* was described as “rare” in 1944 (Ertter and Bowerman 2002) but is now considered to be the most problematic invasive plant in the Park (M. Hastings, *personal communication*). This site has a Mediterranean climate with hot dry summers and cool wet winters. It has the lowest mean annual precipitation of the three sites. Precipitation principally falls as rain but will occasionally fall as snow. The “Sierra” population is in the El Dorado National Forest (1219 m elevation) where the plant was first reported in 2000 (M. Taylor, *personal communication*). This site has a montane climate, and most of the precipitation falls as snow in the winter months with some light summer rainfall. Details about the study sites can be found in Appendix A.

Interactions with insect biocontrol agents

We randomly selected 120 flowering plants at each site in each year. When plants senesced in the field, we counted the total number of buds on each plant and noted whether they were damaged by *E. villosus* bud herbivory. We also counted the number of inflorescences, again noting whether they were unattacked or showed signs (described in *Methods: Study species*) of having been used as an oviposition site by either *E. villosus* or *C. succinea*. We clipped plants at ground level, dried them at 60°C for 48 hours, and weighed them.

To quantify fecundity and assess the impact of seed predation, we removed infructescences as they matured in the field, stored each in a separate coin envelope, and carefully dissected them in the laboratory. We collected infructescences immediately prior to seed dispersal, once the pedicel had senesced. At this stage, seeds were germinable and it is highly unlikely that removing the infructescences caused the plant to respond in a compensatory manner.

Filled achenes can be distinguished from unfilled achenes with a dissecting scope based on size, shape, and color. *E. villosus* larvae feed exclusively on filled achenes. *C. succinea* will occasionally feed on unfilled achenes. Neither species consumes the entire achene and both leave partially eaten achenes in the capitulum. This makes it possible to quantify the proportion of all achenes that were pollinated, and the fraction of those that were damaged by larval feeding and thus are no longer viable (referred to as “damaged achenes” in the structural equation model). We calculated the total number of pollinated achenes as the sum of undamaged filled achenes and filled achenes that had been damaged by seed predators; the number of viable seeds is equivalent to the number of undamaged, filled achenes.

Occasionally seeds began to disperse before we could collect the infructescences. In those cases, we were still able to determine whether the inflorescence had been used as an oviposition site and by which species based on both external and internal signs of oviposition. We estimated seed production using the mean number of seeds produced

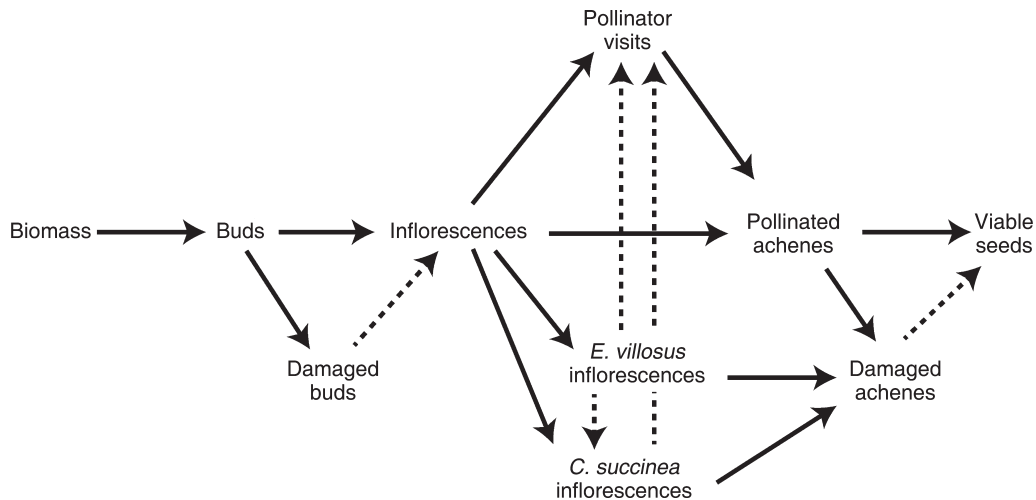


FIG. 1. Structural equation model of the hypothesized effects of *Eustenopus villosus* bud herbivory, *E. villosus* and *Chaetorellia succinea* seed predation, and pollination on lifetime fitness of the invasive plant *Centaurea solstitialis* in three established invasions in California, USA. Arrows point from the independent variable to the dependent variable. The expected nature of the impact of the independent variable on the dependent variable is indicated by a solid (positive) or dashed (negative) line. The full model also includes arrows directly from biomass to inflorescences and from biomass to pollinated achenes (see *Methods: Structural equation model* for details), which are not shown for the sake of clarity.

by that infructescences type (unattacked, attacked by *E. villosus*, attacked by *C. succinea*) for that plant. Ultimately, we collected 3021 infructescences and had to rely on estimates of seed production for 82 (2.7%) of these. We counted a total of 56 290 filled and unfilled achenes.

Pollinator observations

A team of field personnel conducted a single 60-min pollinator observation for three consecutive days during peak flowering for each plant. We defined peak flowering as when the maximum number (but not necessarily the majority) of inflorescences were simultaneously receptive to pollinators (the onset of petal expansion through full bloom). Peak flowering was determined by counting the total number of buds each plant produced, how many inflorescences were open, and how many had senesced. Because plants in the different populations and even plants within a single population reached peak flowering at different times, sites were visited as often as necessary to conduct the observations at peak flowering for each plant.

Each plant was observed individually in random order and the number of pollinator visits and the pollinator species was recorded. A visit was defined as a pollinator landing on an inflorescence for ≥ 2 s. When a pollinator sequentially visited two inflorescences on the same plant (or the same inflorescence more than once) we counted it as a single pollinator visit. Because we do not have information on pollen carryover, we felt this was the most appropriate approach for quantifying visitation to this self-incompatible plant. Pollinator activity peaked between 07:30 and 09:30 hours at the Interior and Sierra sites but later at the Coast site (09:30–11:30). We

conducted our observations at the time of peak pollinator activity at each site.

Structural equation modeling

We developed eight models based on the sequence of the plant's interactions with the biocontrol agents and pollinators in the field and on a priori knowledge about potential interactions between the insects and the plant. The model with the best fit (see *Methods: Model fit*) is presented in Fig. 1; the alternative models are presented in Appendix B. Larger plants are expected to produce more buds, which in turn produce more inflorescences and seeds. Plants with more buds are also expected to attract more *E. villosus* in the bud-feeding stage, and this will presumably lead to a greater number of damaged buds. Bud herbivory will have a direct negative effect on seed production if it reduces the number of inflorescences the plant produces. Plants with more inflorescences are expected to attract both more pollinators and more egg-laying *E. villosus* and *C. succinea*, with opposing effects on plant fitness. We hypothesized that *E. villosus* oviposition would deter egg-laying by *C. succinea* and that oviposition by both agents would deter pollinator visitation. Because oviposition necessarily precedes seed predation, we included arrows from oviposition to damaged seeds, and of course, achenes that are damaged by seed predators will reduce the number of viable seeds the plant produces. We also included an arrow from pollinated achenes to damaged achenes because both species prefer to feed on pollinated achenes. We included direct arrows from plant biomass to the number of inflorescences and pollinated achenes to standardize each of these variables for plant size (Adler

et al. 2001; there is not an arrow from biomass to viable seeds because in the absence of seed predators, pollinated achenes and viable seeds are the same thing). Biomass was ln-transformed and all other variables were $\ln(y + 1)$ transformed for analysis.

We use the term “direct effect” to refer to the effect of one interactor (*E. villosus*, *C. succinea*, or pollinators) on the plant. In SEM parlance, the term “indirect effect” refers to an interaction between two species that is mediated by a third species, but we will also refer to plant-mediated interactions between *E. villosus* bud herbivory and *E. villosus* seed predation as an indirect effect. Even though the damage is caused by the same species, the modes of attack are distinct. Further, weevils move readily among plants as they feed and lay eggs, and these two activities require buds at different phenological stages and so bud herbivory and seed predation ought to be largely independent of one another.

Several indirect interactions are possible. The negative effect of bud herbivory on seed production may be enhanced if bud herbivory leads to fewer inflorescences and a plant with fewer inflorescences attracts fewer pollinators. Similarly, bud herbivory may also have an indirect positive effect on seed production if plants with fewer inflorescences are less likely to be selected as oviposition sites by either agent. It is also possible that pollinators will avoid plants that are damaged by the insect agents, which could enhance the agents’ negative effect on the plant.

C. succinea emerged 3–4 weeks later than *E. villosus* at our study sites (S. M. Swope, *personal observation*) and began laying eggs after *E. villosus* oviposition was past its peak. *E. villosus* may reduce *C. succinea* egg-laying simply by using many of the inflorescences first or if *C. succinea* avoids plants with *E. villosus* damage even when there are inflorescences suitable for oviposition. For this reason, our structural equation model includes an arrow from *E. villosus* inflorescences to *C. succinea* inflorescences to indicate that attack by *E. villosus* can affect attack by *C. succinea* but not vice versa. Eggs of both species are laid singly in each inflorescence and so larvae do not compete for seeds. Of the 3021 inflorescences we dissected, <1% contained more than one larva of either or both species.

We chose to use absolute rather than proportional variables (e.g., total number of buds lost to *E. villosus* herbivory rather than the proportion of buds) because *C. solstitialis* is an annual plant with only one opportunity to produce seed, so the total number of buds killed is more relevant to fitness than the proportion killed. This is consistent with the SEM approach used in other studies where fitness or reproductive success was the response variable (Mitchell 1994, Adler et al. 2001, Albert et al. 2001, Iriando et al. 2003). Additionally, weed managers trying to control invasive plants will be most concerned with

reducing absolute rather than proportional seed output.

Model fit

We relied on two assessments of model fit. We used the root mean square error of approximation (RMSEA) to test the adequacy of several nested models and, because this is a complex model, we also used the Browne-Cudeck criterion (Browne and Cudeck 1989). The B-C criterion is similar to the Akaike information criterion (Akaike 1973) but imposes a greater penalty for additional parameters. Eight biologically plausible models (Appendix B) were compared against two null models, one in which there were no relationships among variables and a second, saturated model in which all variables were linked to one another. The model presented in Fig. 1 has an RMSEA score of 0.034 (an RMSEA score <0.05 indicates a good fit of the model to the data) and the lowest B-C score. All analyses (including the assessment of spatiotemporal variation, discussed in *Methods: Spatiotemporal variation*) were conducted with AMOS 16.0 (SPSS, Chicago, Illinois, USA).

Spatiotemporal variation

Because we were interested in assessing whether interactions varied across our three study sites and across the two years, we conducted a separate SEM analysis for each population in each year ($n = 6$ populations). To determine if there were any significant differences across sites or years, we began with two models, one in which all parameters were free (i.e., we imposed no equality constraints across sites and years) and one in which all parameters were constrained to be equal. The constrained model had a very poor fit to the data. To determine which parameters were different across the sites and years, we began with the model in which all parameters were free, and then constrained individual pairs of parameters in a stepwise fashion, assessing the change in model fit using a single degree of freedom χ^2 test ($P < 0.05$; Grace 2003).

We report unstandardized regression coefficients in the results because, unlike standardized regression coefficients, they represent the effect of x on y in absolute terms (i.e., per capita effects) and are insensitive to any potential differences in variances among groups (Grace 2003).

RESULTS

There was no significant temporal variation in the strength of the interactions in the two years of this study. Therefore, for the sake of simplicity, we combine the results from both years at each of the three sites and report only variation among sites.

Plants at the Sierra site were on average 2.5–3.5 times larger than Coast and Interior plants (Table 1) and plant size had a large, positive effect on bud, inflorescence, and seed production (Fig. 2). Sierra plants produced

TABLE 1. General statistics (mean per plant \pm SD) for *Centaurea solstitialis* at the three study sites in California, USA.

Trait	Coast	Interior	Sierra
Biomass, dry (g)	1.203 \pm 1.216	1.115 \pm 1.193	3.763 \pm 4.520
Buds (no.)	10.40 \pm 10.099	12.35 \pm 16.805	25.280 \pm 25.672
Damaged buds (no.)	6.26 \pm 6.645	5.95 \pm 8.488	11.800 \pm 13.281
Inflorescences (no.)	3.33 \pm 3.117	4.57 \pm 5.211	10.780 \pm 11.415
<i>E. villosus</i> inflorescences (no.)	11.61 \pm 1.608	2.44 \pm 3.160	5.830 \pm 6.298
<i>C. succinea</i> inflorescences (no.)	0.83 \pm 1.670	0.74 \pm 1.692	2.290 \pm 3.472
Pollinator visits (no.)	2.08 \pm 4.009	0.82 \pm 1.832	3.530 \pm 4.927
Pollinated achenes (no.)	62.52 \pm 58.302	105.12 \pm 117.860	330.74 \pm 377.147
Damaged achenes (no.)	26.39 \pm 36.325	51.530 \pm 58.544	180.62 \pm 233.815
Viable seeds (no.)	36.13 \pm 32.173	53.59 \pm 78.691	150.12 \pm 186.788

4.5–5.5 times more viable seeds on average than Coast and Interior plants (Table 1). Despite these large differences in absolute size and fecundity, the strength of the pathway from biomass to buds to inflorescences and seeds did not differ among sites (Fig. 2).

Bud herbivory and seed predation

Bud herbivory strongly reduced the number of inflorescences plants produced, indicating that plants were not able to compensate for this herbivory. This path coefficient ranged from -0.526 to -0.708 at the three sites (Table 2, Fig. 2). When we calculated the total direct effect of *E. villosus* bud herbivory on *C. solstitialis* fecundity, we found that the magnitude of this pathway ranged from -1.4368 to -1.7465 (Table 3). Somewhat unexpectedly, when we calculated the total direct effect of *E. villosus* seed predation on *C. solstitialis* fecundity, we found that its magnitude was much smaller than that of bud herbivory. The magnitude of this pathway ranged from -0.0711 to -0.3892 (Table 3). The total direct effect of *E. villosus* bud herbivory on final seed output was 5.9 (Coast), 4.0 (Interior), and more than 20 times (Sierra) greater than its total effect via seed predation in the three populations (Table 3).

The correlation (path coefficient) between *C. succinea* oviposition and the number of damaged achenes was stronger than the correlation between *E. villosus* oviposition and the number of damaged achenes at all three sites (Table 2). When we calculated the total direct effect of seed predation by both species, their relative strengths showed the same pattern (Table 3). At the Coast, the magnitude of the pathway (total direct effect) of *C. succinea* seed predation on *C. solstitialis* fecundity was $\sim 70\%$ greater than *E. villosus* seed predation (-0.4898 compared to -0.2928 , respectively); at the Interior, it was 23% larger (-0.4805 and -0.3892 , respectively); and at the Sierra it was 2.7 times larger in magnitude (-0.1919 compared to -0.0711 , respectively; Table 3).

E. villosus attack also had indirect, positive effects on *C. solstitialis* seed output. First, by reducing the number of inflorescences, bud herbivory reduced the plant's attractiveness to ovipositing seed predators. The magnitude of the pathway from *E. villosus* bud herbivory to

C. solstitialis fecundity via *E. villosus* oviposition was 0.1399 (Coast), 0.1694 (Interior), and 0.0309 (Sierra; Table 3). The magnitude of this pathway via *C. succinea* oviposition was larger at each of the sites (Coast = 0.2979; Interior = 0.2114; Sierra = 0.1278; Table 3). When we considered these two indirect pathways together, we found that the combined magnitude of the indirect pathways via *E. villosus* and *C. succinea* oviposition reduced the total effect of bud herbivory by 25% at the Coast and Interior and by 11% at the Sierra (Table 3). It is important to note that in the same way, bud herbivory also indirectly reduced pollinator visitation, and this had an indirect, negative effect on the plant, a desirable outcome in a weed–biocontrol system, but the magnitude of this pathway was very small (Coast = -0.0457 ; Interior = -0.0630 ; Sierra = -0.0002 ; Table 3) relative to the magnitude of the indirect, positive effect via the seed predators.

E. villosus had a second indirect, positive effect on *C. solstitialis* fecundity because it deterred *C. succinea* oviposition (Table 3, Fig. 2). The path coefficients from *E. villosus* oviposition to *C. succinea* oviposition were -0.453 (Coast), -0.531 (Interior), and -0.733 (Sierra; Table 2). The net effect of this indirect pathway from *E. villosus* oviposition to *C. solstitialis* fecundity via *C. succinea* oviposition was positive at all three sites (Coast = 0.2219; Interior = 0.2552; Sierra = 0.1407). This indirect, positive effect reduced *E. villosus*' direct, negative effect by 75% at the Coast and by 66% at the Interior. At the Sierra, *E. villosus*' indirect, positive effect on final seed output was nearly twice as large in magnitude as its direct negative effect. As a result, the total effect of *E. villosus* oviposition on plant fecundity at this site was actually positive (0.2118; Table 3).

Pollinators

Nearly all (91%) of the inflorescence visitors were *Apis mellifera*. The other visitors were two *Bombus* species found at all three sites and one unidentified beetle morphotype found at the Coast and Interior. Plants with more inflorescences attracted more pollinators at the Coast (path coefficient = 0.911) and the Interior (path coefficient = 0.390) but not at the Sierra (Table 2, Fig. 2). The path coefficient between the number of

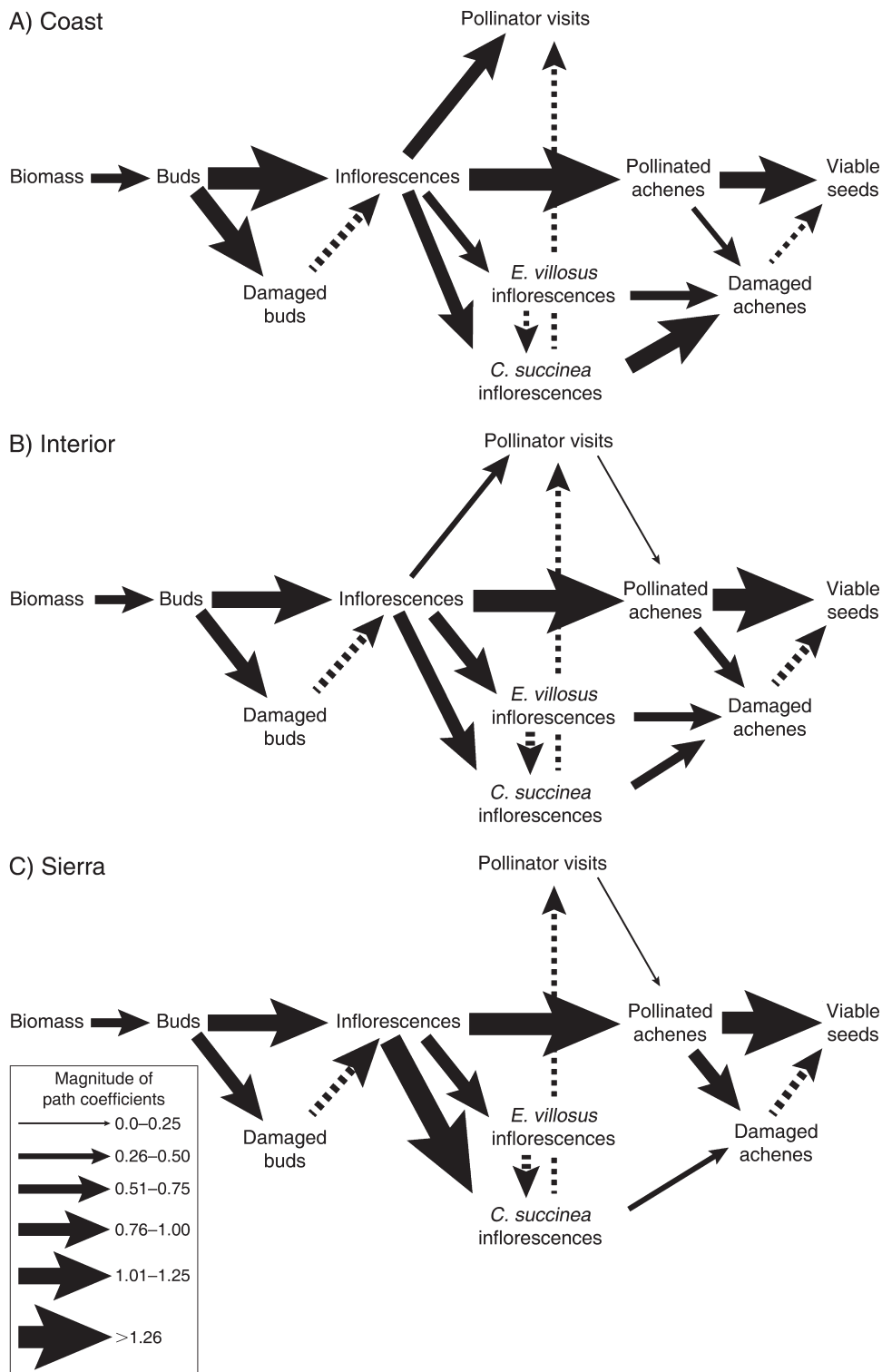


FIG. 2. Results from the structural equation model for the effects of bud herbivory, seed predation, and pollinator visitation on lifetime fitness of *C. solstitialis*. The Coast, Interior, and Sierra sites are shown separately. Only significant paths ($P < 0.10$) are shown. The thickness of the arrows represents the magnitude of the unstandardized path coefficient, and negative effects are shown as dashed arrows. See Table 2 for actual values, levels of significance, and the magnitude of unanalyzed causes.

TABLE 2. Unstandardized path coefficients and the proportion of variance explained (r^2_{adj}) by each regression model used to build the path model.

Dependent variable	Independent variable(s)	Coast			Interior			Sierra		
		Path coefficient	R^2	$p_{u,variable}$	Path coefficient	R^2	$p_{u,variable}$	Path coefficient	R^2	$p_{u,variable}$
No. buds	<i>C. solstitialis</i> biomass	0.574**	0.51	0.62	0.656**	0.51	0.62	0.702**	0.84	0.40
No. damaged buds	no. buds	1.068**	0.83	0.41	0.980**	0.84	0.40	0.987**	0.89	0.33
No. inflorescences	no. buds	1.336**	0.72	0.53	1.243**	0.87	0.36	1.103**	0.96	0.20
	no. damaged buds	-0.708**			-0.569**			-0.526**		
No. <i>E. villosus</i> inflorescences	<i>C. solstitialis</i> biomass	0.050 ^{NS}			0.068†			0.236**		
	no. inflorescences	0.675	0.58	0.65	0.765**	0.72	0.53	0.826**	0.81	0.44
No. <i>C. succinea</i> inflorescences	no. inflorescences	0.859**	0.48	0.72	0.773**	0.40	0.78	1.266**	0.69	0.56
	no. <i>E. villosus</i> inflorescences	-0.453*			-0.531**			-0.733**		
No. pollinator visits	no. inflorescences	0.911**	0.21	0.89	0.390**	0.27	0.85	0.002 ^{NS}	0.30	0.84
	no. <i>E. villosus</i> inflorescences	-0.205 ^{NS}			0.110 ^{NS}			0.294 ^{NS}		
	no. <i>C. succinea</i> inflorescences	-0.476**			-0.291**			-0.3468*		
No. pollinated achenes	no. inflorescences	1.983**	0.84	0.40	1.866**	0.85	0.39	1.857**	0.89	0.33
	no. pollinator visits	0.057 ^{NS}			0.194†			0.104†		
	<i>C. solstitialis</i> biomass	-0.059 ^{NS}			-0.016 ^{NS}			-0.282 ^{NS}		
No. damaged achenes	no. pollinated achenes	0.381**	0.66	0.58	0.548**	0.78	0.47	0.860**	0.71	0.54
	no. <i>E. villosus</i> inflorescences	0.681**			0.597**			-0.142 ^{NS}		
	no. <i>C. succinea</i> inflorescences	1.139**			0.737**			0.383*		
No. viable seeds	no. pollinated achenes	1.244**	0.84	0.40	1.463**	0.80	0.45	1.471**	0.80	0.45
	no. damaged achenes	-0.430**			-0.652**			-0.501**		

Note: The $p_{u,variable}$ is the magnitude of the unmeasured factors affecting the dependent variable and is calculated as $\sqrt{1 - r^2_{adj}}$. * $P < 0.05$; ** $P < 0.01$; † $P = 0.05-0.10$; NS, $P > 0.10$.

pollinator visits and the number of pollinated achenes was not significant at the Coast and marginally significant at the Interior and the Sierra but the strength of the relationship was not particularly strong (0.194 and 0.104, respectively; Table 2). This suggests that plants were, for the most part, close to fully pollinated. The positive effect of pollination on the plant was slightly offset by its positive effect on seed predators, which feed almost exclusively on pollinated achenes (Table 2). *C. succinea* oviposition reduced pollinator visitation at all three sites (Coast = -0.476; Interior = -0.291; Sierra = -0.3468; Table 2, Fig. 2). While this indirect effect is desirable, the total effect of *C. succinea* oviposition to final seed set via the pollinators was small (Coast = -0.0338; Interior = -0.0826; Sierra = -0.0529), especially relative to its direct effect via seed predation (Table 3). *E. villosus* oviposition had no effect on pollinator visitation (Table 2).

Comparisons among biocontrol agents

When the direct and indirect effects were summed for each of the two biocontrol agents, we found that *E. villosus* bud herbivory had the largest net effect on *C. solstitialis* fecundity (-1.2355 to -1.3545; Table 3). The total effect of bud herbivory was larger than the total effect of either *E. villosus* seed predation (-0.1341 to 0.2118) or *C. succinea* seed predation (-0.1545 to -0.5429) and larger even than the total effect of seed predation by both agents combined. The combined effects of *E. villosus* bud herbivory and seed predation by both biocontrol agents on *C. solstitialis* seed output was largest at the Coast (-1.9445) and the Interior (-1.9126) and weaker (but still large) at the Sierra

(-1.2828; Table 3). When the positive effects of the pollinators were included, the total effect of all species on lifetime fitness of the plant was greatest at the Coast (-1.883), intermediate at the Interior (-1.6981), and smaller at the Sierra (-1.1746; Table 3).

Spatial variation

There were nine cases in which there was significant variation in the strength of the pathways among the sites (Table 4). At the Sierra, there was a stronger relationship between the number of inflorescences on the plant and the number of inflorescences used by both *C. succinea* and *E. villosus* for oviposition. In contrast, the number of inflorescences was more strongly associated with the number of pollinator visits at the Coast than at the Interior, and the relationship was not significant at the Sierra. At all three sites, the number of inflorescences attacked by *C. succinea* reduced the number of pollinator visits, but the effect was weaker at the Interior than it was at the Coast and the Sierra. The strength of the relationship between the number of pollinated achenes and the number of achenes damaged by seed predators was strongest at the Sierra (the Coast and Interior were not different from each other). Interestingly, the relationship between the number of inflorescences attacked by either *C. succinea* or *E. villosus* and the number of damaged achenes was weakest at the Sierra. In fact, we were surprised that the relationship between *E. villosus* inflorescences and damaged achenes was not significant at the Sierra. The positive relationship between the number of pollinated achenes and the number of viable seeds was weaker at the Coast than it was at the other two sites, while the

TABLE 3. Magnitude of direct and indirect effects of bud herbivory, seed predation by *Eustenopus villosus* and *Chaetorellia* spp., and pollination on seed production in *Centaurea solstitialis*.

Site, type of effect	Pathway	Magnitude
Coast		
Bud herbivory		
DE	$P_{\text{Inflor,DamBuds}} \times P_{\text{PollAch,Inflor}} \times P_{\text{ViabSeeds,PollAch}}$	-1.7465
IE via <i>E. villosus</i> seed predation	$P_{\text{Inflor,DamBuds}} \times P_{\text{EustInflor,Inflor}} \times P_{\text{DamAch,EustInflor}} \times P_{\text{ViabSeeds,DamAch}}$	0.1399
IE via <i>C. succinea</i> seed predation	$P_{\text{Inflor,DamBuds}} \times P_{\text{ChaetInflor,Inflor}} \times P_{\text{DamAch,ChaetInflor}} \times P_{\text{ViabSeeds,DamAch}}$	0.2979
IE via pollination	$P_{\text{Inflor,DamBuds}} \times P_{\text{PollVis,Inflor}} \times P_{\text{PollAch,PollVis}} \times P_{\text{ViabSeeds,PollAch}}$	-0.0457
Total effect of bud herbivory		-1.3545
<i>Eustenopus villosus</i> seed predation		
DE	$P_{\text{DamAch,EustInflor}} \times P_{\text{ViabSeeds,DamAch}}$	-0.2928
IE via <i>C. succinea</i>	$P_{\text{ChaetInflor,EustInflor}} \times P_{\text{DamAch,ChaetInflor}} \times P_{\text{ViabSeeds,DamAch}}$	0.2219
Total effect of <i>E. villosus</i> seed predation		-0.0710
Total effect of <i>E. villosus</i> (bud herbivory + seed predation)		-1.4254
<i>Chaetorellia succinea</i> seed predation		
DE	$P_{\text{DamAch,ChaetInflor}} \times P_{\text{ViabSeeds,DamAch}}$	-0.4898
IE via pollinators	$P_{\text{PollVis,ChaetInflor}} \times P_{\text{PollAch,PollVis}} \times P_{\text{ViabSeeds,PollAch}}$	-0.0338
IE via pollen and seed pred	$P_{\text{PollVis,ChaetInflor}} \times P_{\text{PollAch,PollVis}} \times P_{\text{DamAch,PollAch}} \times P_{\text{ViabSeeds,DamAch}}$	0.0044
Total effect of <i>C. succinea</i> seed predation		-0.5191
Total of both biocontrol agents		-1.9445
Pollination		
DE	$P_{\text{PollAch,PollVis}} \times P_{\text{ViabSeeds,PollAch}}$	0.0709
IE via seed pred	$P_{\text{PollAch,PollVis}} \times P_{\text{DamAch,PollAch}} \times P_{\text{ViabSeeds,DamAch}}$	-0.0093
Total effect of pollination		0.0616
Total effect of bud herbivory, seed predation and pollination		-1.8830
Interior		
Bud herbivory		
DE	$P_{\text{Inflor,DamBuds}} \times P_{\text{PollAch,Inflor}} \times P_{\text{ViabSeeds,PollAch}}$	-1.5533
IE via <i>E. villosus</i> seed predation	$P_{\text{Inflor,DamBuds}} \times P_{\text{EustInflor,Inflor}} \times P_{\text{DamAch,EustInflor}} \times P_{\text{ViabSeeds,DamAch}}$	0.1694
IE via <i>C. succinea</i> seed predation	$P_{\text{Inflor,DamBuds}} \times P_{\text{ChaetInflor,Inflor}} \times P_{\text{DamAch,ChaetInflor}} \times P_{\text{ViabSeeds,DamAch}}$	0.2114
IE via pollination	$P_{\text{Inflor,DamBuds}} \times P_{\text{PollVis,Inflor}} \times P_{\text{PollAch,PollVis}} \times P_{\text{ViabSeeds,PollAch}}$	-0.0630
Total effect of bud herbivory		-1.2355
<i>Eustenopus villosus</i> seed predation		
DE	$P_{\text{DamAch,EustInflor}} \times P_{\text{ViabSeeds,DamAch}}$	-0.3892
IE via <i>C. succinea</i>	$P_{\text{ChaetInflor,EustInflor}} \times P_{\text{DamAch,ChaetInflor}} \times P_{\text{ViabSeeds,DamAch}}$	0.2552
Total effect of <i>E. villosus</i> seed predation		-0.1341
Total effect of <i>E. villosus</i> (bud herbivory + seed predation)		-1.3697
<i>Chaetorellia succinea</i> seed predation		
DE	$P_{\text{DamAch,ChaetInflor}} \times P_{\text{ViabSeeds,DamAch}}$	-0.4805
IE via pollinators	$P_{\text{PollVis,ChaetInflor}} \times P_{\text{PollAch,PollVis}} \times P_{\text{ViabSeeds,PollAch}}$	-0.0826
IE via pollen and seed pred	$P_{\text{PollVis,ChaetInflor}} \times P_{\text{PollAch,PollVis}} \times P_{\text{DamAch,PollAch}} \times P_{\text{ViabSeeds,DamAch}}$	0.0202
Total effect of <i>C. succinea</i> seed predation		-0.5429
Total of both biocontrol agents		-1.9126
Pollination		
DE	$P_{\text{PollAch,PollVis}} \times P_{\text{ViabSeeds,PollAch}}$	0.2838
IE via seed pred	$P_{\text{PollAch,PollVis}} \times P_{\text{DamAch,PollAch}} \times P_{\text{ViabSeeds,DamAch}}$	-0.0693
Total effect of pollination		0.2145
Total effect of bud herbivory, seed predation and pollination		-1.6981
Sierra		
Bud herbivory		
DE	$P_{\text{Inflor,DamBuds}} \times P_{\text{PollAch,Inflor}} \times P_{\text{ViabSeeds,PollAch}}$	-1.4368
IE via <i>E. villosus</i> seed predation	$P_{\text{Inflor,DamBuds}} \times P_{\text{EustInflor,Inflor}} \times P_{\text{DamAch,EustInflor}} \times P_{\text{ViabSeeds,DamAch}}$	0.0309
IE via <i>C. succinea</i> seed predation	$P_{\text{Inflor,DamBuds}} \times P_{\text{ChaetInflor,Inflor}} \times P_{\text{DamAch,ChaetInflor}} \times P_{\text{ViabSeeds,DamAch}}$	0.1278
IE via pollination	$P_{\text{Inflor,DamBuds}} \times P_{\text{PollVis,Inflor}} \times P_{\text{PollAch,PollVis}} \times P_{\text{ViabSeeds,PollAch}}$	-0.0002
Total effect of bud herbivory		-1.3401
<i>Eustenopus villosus</i> seed predation		
DE	$P_{\text{DamAch,EustInflor}} \times P_{\text{ViabSeeds,DamAch}}$	-0.0711
IE via <i>C. succinea</i>	$P_{\text{ChaetInflor,EustInflor}} \times P_{\text{DamAch,ChaetInflor}} \times P_{\text{ViabSeeds,DamAch}}$	0.1407
Total effect of <i>E. villosus</i> seed predation		0.2118

TABLE 3. Continued.

Site, type of effect	Pathway	Magnitude
Total effect of <i>E. villosus</i> (bud herbivory + seed predation)		-1.1283
<i>Chaetorellia succinea</i> seed predation		
DE	$P_{\text{DamAch,ChaetInflor}} \times P_{\text{ViabSeeds,DamAch}}$	-0.1919
IE via pollinators	$P_{\text{PollVis,ChaetInflor}} \times P_{\text{PollAch,PollVis}} \times P_{\text{ViabSeeds,PollAch}}$	-0.0529
IE via pollin and seed pred	$P_{\text{PollVis,ChaetInflor}} \times P_{\text{PollAch,PollVis}} \times P_{\text{DamAch,PollAch}} \times P_{\text{ViabSeeds,DamAch}}$	0.0155
Total effect of <i>C. succinea</i> seed predation		-0.1545
Total of both biocontrol agents		-1.2828
Pollination		
DE	$P_{\text{PollAch,PollVis}} \times P_{\text{ViabSeeds,PollAch}}$	0.1530
IE via seed pred	$P_{\text{PollAch,PollVis}} \times P_{\text{DamAch,PollAch}} \times P_{\text{ViabSeeds,DamAch}}$	-0.0448
Total effect of pollination		0.1082
Total effect of bud herbivory, seed predation, and pollination		-1.1746

negative relationship between the number of damaged achenes and the number of viable seeds was weakest at the Interior, intermediate at the Sierra, and strongest at the Coast.

DISCUSSION

Multiple biocontrol agents are often released for each target weed species, either by design as in the cumulative stress approach to biocontrol (sensu Harris 1981, van Driesche et al. 2008) or when one agent fails to control the weed so additional agents are released. Several studies have shown that multiple biocontrol agents can have an additive or even a synergistic impact on plant performance (e.g., James et al. 1992, Masters et al. 2001, Swope and Stein 2012) or that agents can interfere with one another such that their combined impacts are less than expected (Ehler and Hall 1982, Denno et al. 1995, Swope and Parker 2010a). But the specific direct and indirect pathways by which these effects arise and the relative strength of the different pathways has rarely been quantified. In this study, we used SEM to quantify the strength of several pathways by which agents can influence plant performance both directly and indirectly via their interactions with each other and with pollinators. We found that bud herbivory and seed predation by two insect biocontrol agents reduced plant perfor-

mance directly, and that attack by one of the agents also deterred pollinators from visiting the plants. We also found that one of the agents interfered with the other.

Interactions between biocontrol agents and pollinators

In this study, the total effect of bud herbivory on *C. solstitialis* fecundity was greater than the total effect of seed predation by either agent independently or by both agents combined. We think that bud herbivory had a larger effect on final seed output than did seed predation for two reasons. First, bud herbivory killed the bud, invariably preventing it from flowering whereas the seed predators typically did not consume all of the seeds in the inflorescence in which they were developing. Second, the fact that bud herbivory reduced the number of inflorescences plants produced indicates that plants were unable to compensate for losses to the herbivore.

The magnitude of the bud herbivore’s direct effect was somewhat offset by its indirect effect via the seed predators. Bud herbivory reduced the number of inflorescences produced, which in turn reduced the plant’s attractiveness to the biocontrol agents as they searched for oviposition sites. Bud herbivory and the consequent reduction in inflorescences also meant that plants were less attractive to pollinators at two of the three sites, a desirable outcome in a weed-biocontrol

TABLE 4. Variation among the three sites in the strength of the regression coefficients.

Dependent variable	Independent variable(s)	Spatial variation
No. <i>E. villosus</i> inflor.	no. inflorescences	S > I = C
No. <i>C. succinea</i> inflor.	no. inflorescences	S > I > C
No. pollinator visits	no. inflorescences	C > I > S ^{NS}
	no. <i>C. succinea</i> inflorescences	C = S > I
No. damaged achenes	no. pollinated achenes	S > I = C
	no. <i>E. villosus</i> inflor.	C = I > S ^{NS}
	no. <i>C. succinea</i> inflor.	C > I > S
No. viable seeds	no. pollinated achenes	S = I > C
	no. damaged achenes	I > S > C

Notes: The nature of the differences ($P < 0.05$) between the Coast (C), Interior (I), and Sierra (S) populations is indicated <, >, or =. “NS” indicates that the path was not significant at a particular site but significant at the others.

system in which the plant is dependent on insect pollinators, but the magnitude of this pathway was quite small.

C. succinea seed predation had a larger total effect on final seed set in the weed than did *E. villosus* seed predation at all three sites. Unfortunately, *E. villosus* oviposition deterred oviposition *C. succinea*. This is problematic from a biocontrol perspective because *C. succinea* is the more voracious of the two seed predators, typically consuming nearly all of the achenes in an inflorescence compared to *E. villosus* which consumes, on average, two-thirds of the achenes. Further, the magnitude of *E. villosus*' indirect effect on seed output via *C. succinea* was large relative to the magnitude of its direct effect. In fact, at the Sierra, the indirect, positive effect of *E. villosus* on final seed output via *C. succinea* oviposition was nearly twice as large in magnitude as its direct, negative effect. This meant that at the Sierra, *E. villosus* oviposition actually had a net positive effect on final seed output by deterring *C. succinea*. Leaving aside *E. villosus*' effect on the plant via bud herbivory and considering only seed predation, this is an example of one of the primary concerns raised about using multiple agents to control invasive plants, namely that the lower impact agent (*E. villosus*) may reduce the effect of the higher impact agent (*C. succinea*).

A second indirect effect also arose between one agent and the pollinators. Inflorescences attacked by *C. succinea* never reach full petal expansion, and petals appear desiccated. We suspected that this visible damage may deter pollinators from visiting those inflorescences or even the whole plant, and our data support this hypothesis. However, the magnitude of this indirect path on final seed output was quite small, presumably because the plants in this study experienced either no (Coast) or very mild (Interior and Sierra) pollen limitation as indicated by the small path coefficients between pollinator visits and pollinated achenes. Nevertheless, we think this highlights an underappreciated and potentially important impact of biocontrol agents on their target weeds. Several studies have shown that herbivory can reduce a plant's ability to attract and reward its pollinators (Euler and Baldwin 1996, Strauss et al. 1996, Lehtilä and Strauss 1997, Mothershead and Marquis 2000). To date, we know of no work other than our own that has explicitly addressed whether or to what degree attack by a biocontrol agent deters pollinators. Many invasive plants are dependent on insect pollinators for reproduction (Richardson et al. 2000), including *C. solstitialis*. Exploiting this dependence by selecting an agent that not only inflicts direct damage but also deters pollinators by making the plant less rewarding or less attractive may be a productive new avenue to explore as practitioners search for prospective agents.

Ultimately, although *C. succinea* seed predation had a greater net effect on seed production in the weed than did *E. villosus* seed predation, *E. villosus* had the larger

net effect on final seed output, due almost entirely to its bud herbivory.

Spatiotemporal variation.—There was no significant temporal variation in our study. It is possible that there is little temporal variation in these interactions in general but it may also be a feature of the particular years in which we conducted this study. All three of our study sites experienced below-average precipitation in 2007 and 2008, which may account for the lack of temporal variation. More favorable climatic conditions may change the strength of the interactions between the plant and the insects. For example, *C. solstitialis* flowers during the hot, dry summers, and soil moisture is thought to be the primary limiting resource at this time. In wet years, when the summer drought does not begin in earnest until the flowering period is well underway, seed set appears to be primarily pollen-limited, while in drought years (when soil moisture can be very scarce) seed set appears to be primarily resource-limited (S. M. Swope, *unpublished manuscript*). This may mean that when seed set is pollen-limited, e.g., at wetter sites or in years with higher precipitation, biocontrol attack that deters pollinators may reduce seed set to a greater degree than we observed in these two years.

There were several cases of differences among sites. The relationship between the number of inflorescences and oviposition by both insects was stronger at the Sierra than at the other two sites. Ovipositing insects require buds at a particular phenological stage and they appear to be seeking out individual buds rather than individual plants, particularly at the Sierra site. More inflorescences per plant also led to more pollinator visits at the Coast and the Interior but not at the Sierra. We expected pollinators to consistently visit bigger plants, so we were surprised by the degree of variation we saw across sites. One factor that could influence pollinator behavior is plant density, which was six times higher at the Sierra (277.56 ± 146.23 plants/m², mean \pm SD) than at the Interior (43.80 ± 51.032 plants/m²), which was another five times more dense than at the Coast (8.668 ± 10.47 plants/m²). Pollinators at sites with lower plant density may be better able to perceive individual plants and preferentially visit larger plants.

Impact vs. effective control

The agents and their three modes of attack (*E. villosus* bud herbivory, *E. villosus* seed predation, and *C. succinea* seed predation) all reduced the final seed output of the target weed *C. solstitialis* independently and collectively. But it can be difficult to predict how effectively agents will control populations of their target weed based on their impacts on individual plants. Reducing seed output of individuals may not lead to effective population-level control (e.g., reduce plant density or population growth rate) if recruitment is limited by a scarcity of germination microsites rather than by seed. Elsewhere we have shown that recruitment at the Coast and the Interior will vary between being seed-limited and microsite-limited at

spatial scales relevant to dispersal, which for this species is a matter of meters or less (Swope and Parker 2010b). These agents are reducing the seed output of individual *C. solstitialis* plants at these two sites and so are likely providing some level of population-level control by reducing recruitment of *C. solstitialis* in patches where microsites are not saturated.

The agents' total effect on final seed output at the Sierra was large although it was smaller than at the other two sites. This is somewhat unfortunate given that in our previous work we detected the strongest seed-limited recruitment and no microsite limitation at this site compared to the others (Swope and Parker 2010b). In other words, any reduction in final seed output ought to have a larger impact on recruitment and subsequently on plant density at this site than at the Coast or the Interior. Further, seedling and flowering plant density were substantially higher here than at the other two sites, and to the extent that the invader's density is a measure of its impact and success (Parker et al. 1999, Sakai et al. 2001), we might conclude that controlling *C. solstitialis* at the Sierra site is a higher priority.

This work shows that seed predators may not be the best means of reducing a plant's seed output in some cases. Bud herbivory had a much larger effect on final seed output than did seed predation by either species independently and even both species combined. This result appears to be at least partly contingent upon the plant's inability to compensate for losses to herbivory. Further, both agents indirectly reduced pollinator visitation. *E. villosus* bud herbivory reduced pollinator visits by reducing the number of inflorescences plants produced, and *C. succinea* oviposition reduced visitation because pollinators appear to avoid visibly damaged inflorescences. One of the often-cited strengths of a multi-agent approach to biocontrol is that the agents will have a synergistic impact on the weed. It is also possible that interactions between an agent and pollinators could work in a similarly synergistic fashion to reduce seed output. This possibility is supported by the fact that both *E. villosus* bud herbivory and *C. succinea* oviposition deterred pollinators and thereby indirectly reduced seed output, despite the very mild degree of pollen limitation of plants in these years. However it is also possible for one agent to reduce the impact of another. Here we found that *E. villosus* oviposition reduced the frequency of *C. succinea* attack but SEM also reveals that the magnitude of this interference is small relative to the total effect of *E. villosus* attack (bud herbivory and seed predation).

All invasive plants interact with numerous other species, including biocontrol agents and native herbivores and pollinators. It is important that we understand the response of the plant to this complex biotic environment across a range of environmental conditions. Because multispecies interactions are likely to give rise to indirect interactions and nonadditive outcomes that are inherently difficult to detect and quantify in an

ANOVA framework, SEM offers biocontrol practitioners a powerful tool to gain insight into how agents interact with one another and with other species such as pollinators. SEM is also means of suggesting interaction pathways that might be exploited to maximize control but might otherwise be overlooked (e.g., via pollinators).

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SUPPLEMENTAL MATERIAL

Appendix A

General description of the three study sites (*Ecological Archives* A022-115-A1).

Appendix B

Alternative structural equation models of the plant's interactions with the biocontrol agents and pollinators (*Ecological Archives* A022-115-A2).