Variable effects of a generalist parasitoid on a biocontrol seed predator and its target weed

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Abstract. Biological control (the importation of enemies from an invader's native range) is often considered our best chance of controlling the most widespread invaders. Ideally, the agent reduces invader abundance to some acceptably low level, and the two coexist at low density with the agent providing continuous control over the long term. But the outcome may be complicated when the agent is attacked by native predators and parasites. We used a spatially explicit, discrete-time, individual-based, coupled plant–seed predator–parasitoid model to estimate the impact of the biocontrol agent *Eustenopus villosus* (a seed predator) on the invasive, annual weed *Centaurea solstitialis*, both with and without the generalist parasitoid *Pyemotes tritici*. We estimated the agent's ability to reduce plant density, spread rate, and population growth rate over 50 years. We used long-term demographic data from two sites in central California, USA, to parameterize the model and assess how populations in different climatic zones might respond differently to the agent and the parasitoid. We found that the biocontrol agent reduced plant density (relative to predictions for an uncontrolled invasion), but its impact on the invader's spread rate was modest and inconsistent. The agent had no long-term impact on population growth rate (λ). Parasitism caused a trophic cascade, the strength of which varied between sites. At our coastal site, the parasitoid entirely eliminated the impact of the agent on the plant. At our Central Valley site, even when parasitized, the agent significantly reduced plant density and spread rate over several decades (although to a lesser degree than when it was not parasitized), but not invader λ. Surprisingly, we also found that the length of time the invader was allowed to spread across the landscape prior to introducing the agent (5, 25, or 50 years) had little influence over its ability to control the weed in the long term. This is encouraging news for land managers attempting to control invasive plants that have already established widespread, high-density populations. Unfortunately, our results also show that attack by the native generalist parasitoid had a larger influence over how effectively the agent reduced invader performance.

Key words: Centaurea solstitialis; Eustenopus villosus; individual-based model; invasive; seed predation; spatially explicit; trophic cascade.

Introduction

Invasion by exotic species is both a cause and a consequence of biotic change worldwide (Vitousek et al. 1997, Wilcove et al. 1998). Invasions themselves are essentially a population-level problem in that both invader density and the rate at which their populations are growing are measures of their success (Sakai et al. 2001) and impact (Parker et al. 1999). Once established, density can be so high within a population and populations can be spread over such large geographic areas that chemical and mechanical means of control become impractical, are prohibitively expensive, and/or have undesirable impacts on nontarget species and/or the soil. Biological control is often touted as an effective and affordable strategy for controlling such widespread and well-established invaders. In classical biological control, enemies from the invader's native range are released in the introduced range with the goal of suppressing the invader. For invasive plants, agents are typically herbivorous insects that are highly host specific so as to minimize the likelihood that they will attack native or agricultural species. The expectation is not necessarily that the agent will eradicate the plant, but more realistically, that it will reduce the invader's abundance and maintain it at some acceptably low level over the long term (van Driesche et al. 2008). Invader success can be partitioned into different aspects of its population performance: its density, the rate at which it is spreading into uninvaded areas, and/or its population growth rate (Shea et al. 2010). It is possible for an agent to reduce one aspect of invasion success, e.g., spread rate or density, but not others, e.g., growth rate (Shea et al.

2010, Swope and Parker 2010). Managers tasked with protecting various resources may have a greater need to control one aspect of an invasion in some areas and other aspects in other areas. For example, it may be more important to reduce density where an invader has already come to occupy the entirety of the locally available habitat, but reducing the spread rate may be more important where the invader is adjacent to habitat occupied by an endangered species.

A number of factors may influence how effectively an agent controls its target weed, including the age and size of the invasion at the time the agent is released and top-down control of the agent by generalist predators and parasitoids. In many cases, the invasion is already well established by the time a biocontrol agent is introduced. An invasion that has already reached high densities and spread across large areas by the time the agent is released may be less sensitive to biocontrol than a very small invasion that is in the earliest stages of establishment (Crawley 1989). We also know that generalist predators and parasitoids readily attack novel insects (Cornell and Hawkins 1993), including those introduced as biocontrol agents (Goeden and Louda 1976, Paynter et al. 2010), and that may reduce the impact of the agent on the weed.

How invasive plant populations interact with their biocontrol agents over long time spans is the ultimate measure of a biocontrol program's success. However, given the difficulty of collecting the necessary data, it is not surprising that there is a dearth of such studies (but see Buckley et al. 2005), and we know of none that include a third trophic level (predator or parasitoid). Further, we know of no studies that explore how the outcome is influenced by the age of the invasion at the time a biocontrol program is initiated. We took advantage of a long-term, multisite set of demographic data for the invasive plant *Centuarea solstitialis* L. (Asteraceae; yellow starthistle) and constructed a simulation model to make just such long-term projections.

C. solstitialis has successfully invaded large areas of California, USA, and the seed predator *Eustenopus villosus* (Coleoptera: Curculionidae) is its primary biocontrol agent. *C. solstitialis* has an annual life cycle, and recruitment each year is heavily dependent on current seed production. These traits ought to make *C. solstitialis* (and other invaders that share these traits) particularly vulnerable to population regulation by seed predators (Crawley 1989, Louda and Potvin 1995). A combination of field experiments and observational studies have shown that *C. solstitialis* recruitment is frequently seed limited at our study sites in California (Swope and Parker 2010), meaning that the seed predator *E. villosus* may reduce plant density, spread rate (c^*), and growth rate (λ) in theory and in practice. However, *E. villosus* is heavily attacked by the native, generalist parasitoid *Pyemotes tritici* (Acari: Pyemotidae), which kills weevil larvae before they consume

seeds, potentially reducing its effectiveness as a biocontrol agent.

Our study had three main goals. First, we wanted to estimate the impact of the seed predator on *C. solstitialis* density, c^*, and λ over 50 years of attack. Secondly, we wanted to know how attack by the parasitoid changed the agent's impact on the plant. Finally, we wanted to explore two temporal aspects of the weed–biocontrol interaction: (1) How the agent's impact might be influenced by the age (size) of the invasion at the time the agent was released and (2) how the agent's impact might change over the course of a half century of control. We constructed a spatially explicit, individual-based simulation model (IBM) parameterized from four consecutive years of demographic data collected from established invasions in California to make projections over a half century of biocontrol. Because *C. solstitialis* can be found across the state in very different climatic zones, we ran separate simulations using demographic data from invasions in coastal areas of California and from hotter, drier inland areas and compared the results to explore how invasions in these different areas might respond differently to both seed predation and parasitism.

Methods

Study species

Centaurea solstitialis is an annual thistle native to Eurasia and invasive in California, USA. The first vouchered specimen from the invaded range is from the San Francisco Bay Area and was collected in 1869 (DiTomaso and Gerlach 2000). *C. solstitialis* has since spread rapidly and now covers six million hectares in the state and has successfully invaded areas with climates as different as the cool wet coastal regions, the hot dry Central Valley and the high elevations of the Sierra Nevada Mountains (Pitcairn et al. 2006).

C. solstitialis seeds germinate with the onset of autumn rains, seedlings overwinter as basal rosettes and plants flower during the summer drought. By late summer, all plants have dispersed seed and died. Germination is dependent on fall and winter precipitation, and the seed bank appears to be short lived and contributes little to recruitment relative to current seed rain (Garren and Strauss 2009). In a separate study, we confirmed the very limited contribution of the seed bank to recruitment at these two sites (the methods and results of which are detailed in the Appendix). Seed dispersal is limited. Roché (1992) found that nearly half (48%) of all achenes fall within 0.3 m of the maternal plant and that nearly all (96%) fall within 0.6 m.

Eustenopus villosus is a weevil native to Greece and was released in California as a biological control agent in 1990. It has since become the most widespread and locally abundant of all the *C. solstitialis* biocontrol agents in the state (Wilson et al. 2003). *E. villosus* is univoltine, and female weevils lay an average of 35 eggs in their lifetime (Fornasari and Sobhian 1993). Eggs are

laid singly in the inflorescences and the larvae act as predispersal seed predators. *E. villosus* adults are sluggish movers. They tend to walk up and down branches of flowering plants in search of oviposition sites rather than flying between plants (S. M. Swope, *personal observation*). They engage in short, infrequent bouts of powered flight only during the hottest times of day and may not fly at all on cool, overcast days (Fornasari and Sobhian 1993; S. M. Swope, *personal observation*).

Pyemotes tritici is a generalist parasitoid mite that attacks *E. villosus* larvae as they mature and pupate inside the inflorescence (see Plate 1). Because attack by the parasitoid kills the larvae, parasitized larvae consume very few if any seeds. *P. tritici* is cosmopolitan in distribution, although it is unclear whether the weevil was brought to the United States already infested with the parasitoid or if it acquired it once here (R. Carruthers, *personal communication*). Either way, larval weevil mortality due to parasitoid attack at our sites is high (25–38%) and has been documented from the coast all the way to the high elevations of the Sierra in California (S. M. Swope, *unpublished data*).

Study sites

We wanted to understand how *C. solstitialis* invasions in different parts of the invaded range would respond to seed predation by parasitized and unparasitized weevils so we selected two study sites in areas of California with very different climates: the coast and the Central Valley in the interior part of the state. Although *C. solstitialis* has persisted in coastal areas since its introduction, it remains at lower densities there than in the Central Valley, where it is a very successful invader. We located two naturally occurring invasion at each of the two sites. Our coastal populations are located in the Loma Alta Open Space Preserve (Marin County, 38°00′ N, 122°36′ W, 268 m elevation), ~20 km from the first vouchered *C. solstitialis* specimen in California. This site has a coastal climate characterized by mild temperatures year-round (January mean temperature is 13°C; July mean is 28°C) and the long-term (1943–2010) mean annual precipitation is 1238 mm (Western Region Climate Center, data *available online*).[5] Our Central Valley populations are located in Mount Diablo State Park (Contra Costa County, 37°51′ N, 121°55′ W, 432 m elevation), which has a classically mediterranean climate with cool wet winters (January mean is 11°C) and hot dry summers (July mean is 31°C), and mean annual precipitation is about half (609 mm) that of our coastal site (Western Region Climate Center, *available online*, see footnote 5). The coast and Central Valley sites are separated from one another by ~65 km, and the two populations at each site are 1.5 km (coast) and 3.5 km (Central Valley) apart.

Demographic data collection

We used data collected from the first four years of our ongoing demography study to construct a spatially explicit IBM. At the time of seed dispersal (late summer) in 2006, we established twenty randomly located 0.5 × 0.5 m plots in each of the four populations. The permanent boundaries of each plot were defined by wire frames secured to the ground at each corner with garden staples and marked with rebar. We censused every plot at three-week intervals year-round using small wooden stakes to mark every seedling that emerged in each plot for four years (equivalent to four nonoverlapping generations; 2006–2010). We followed the fate of each plant for its entire life (thus far, a total of 2413 seedlings in the two coastal populations and 6004 seedlings in the two Central Valley populations). We recorded pre-flowering mortality and, for those plants that survived to flowering, we estimated each plant's fecundity by counting both the number of inflorescences and the proportion of inflorescences used as oviposition sites by *E. villosus*. (Inflorescences that have been used as oviposition sites by *E. villosus* are easy to nondestructively indentify in the field as described in *Fecundity, seed predation, and parasitism*.)

To keep the IBM model relatively simple and interpretable, the IBM does not attempt to partition total variation in fecundity into effects of temporal vs. fine-scale spatial heterogeneity, nor does it attempt to model the effects of temporal variability on the form and intensity of density dependence. Thus, to generate a set of average conditions along with an appropriate estimate of total variability, we pooled all four years of data from the two populations at each site to create an average set of conditions for the coastal site and another for the Central Valley site suitable for prospective analyses (Caswell 2000). We modeled density-dependent inflorescence production using a negative binomial distribution whose mean tracked density as

$$\ln(\mu) = \alpha + \beta\ln(N) \qquad (1)$$

where μ is mean fecundity and N is the number of plants per plot. Thus, $\exp(\alpha)$ is the mean number of inflorescences per plant when there is only one plant per plot, and β is a measure of the strength of density dependence. Negative β corresponds to decreased fecundity at higher density. The slopes (β) were significantly different from 0 at both sites and although their values were very small, the highest fecundities were only observed at low density, suggesting that density may nevertheless be biologically important. We found no significant effect of density on germination or seedling survival to flowering. We expected to see some degree of annual variability in germination and survival (in response to annual precipitation), but we did not, so we used a constant probability for each. See Table 1 for all parameters used in the model. Because the seed bank contributes so little to recruitment in *C. solstitialis* invasions in California (Garren and

TABLE 1. Parameters and functional forms used for demographic processes in the individual-based simulation model (IBM) from each site in California, USA.

Parameter	Site		How modeled	Source
	Coast	Central Valley		
Plant (*Centaurea solistitialis*)				
Germination probability	0.34	0.18	binomial with constant probability	field data
Probability of seedling survival to flowering†	0.355 ± 0.168	0.336 ± 0.168	binomial with constant probability	field data
No. inflorescences per plant (mean)	$e^{0.66-0.0098\times d}$	$e^{1.060-0.015\times d}$	negative binomial	field data
No. inflorescences per plant (dispersion)‡	1.55	2.503	negative binomial	field data
No. viable seeds per unattacked inflorescence†	17 ± 1.75	14 ± 2.03	constant	field data
Percentage of seeds consumed by seed predator (per inflorescence)†	66.8% ± 5.9%	67.2% ± 4.0%	constant fraction, surviving seeds rounded to nearest integer	field data
Seed dispersal distance (mean)	0.3 m	0.3 m	negative exponential	Roché (1992)
Seed predator (*Eustenopus villosus*)				
Larval mortality due to parasitoid	25%	38%	binomial with constant probability	field data
Larval mortality due to other causes	41%	4%	binomial with constant probability	field data
No. eggs per female	35 (range: 9–75)	35 (range: 9–75)	constant	Fornasari and Sobhian (1993)
Overwinter survival	80%	80%	binomial with constant probability	estimate
Maximum detection range§	20 m	20 m	constant	estimate
Powered flight distance¶	1 m	1 m	constant	field data
No. lifetime flights#	105	105	constant	estimate
Preference for nearby plants (β)	5	5	constant	estimate

Note: When estimates of on field-derived parameter values are available, their ranges are given in parentheses. Field data were collected by S. M. Swope from 2006 to 2010.

† Site data are shown as means ± SD.

‡ Equations refer to the site-specific density-dependent functions for fecundity; *d* is the natural log of density.

§ Detection range is the distance over which a weevil is able to detect a plant.

¶ Powered flight distance is the maximum length of a single powered, directed flight.

Lifetime flights are the total number of powered flights a weevil may take in its lifetime.

Strauss 2009), including at these two sites (Appendix), we did not include a seed bank in the model.

Fecundity, seed predation, and parasitism

To quantify viable seed production and losses to seed predators, we collected >2700 inflorescences from the coast populations and >4300 inflorescences from the Central Valley populations over the four years of the field component of this study. Inflorescences were collected from off-plot plants in our study populations so as not to affect seed rain into our long-term demography plots. Each inflorescence was collected after it was fully mature as indicated by the desiccation and loosening of the petal cap, but immediately before seed dispersal, stored in an individual coin envelope, and carefully dissected in the laboratory. Pollinated achenes are easy to distinguish from unpollinated ones under a dissecting scope based on size, color, and shape. Weevils feed on pollinated achenes (ignoring the unpollinated ones) and leave partially eaten achenes inside the capitulum. It is therefore easy to count the total number of achenes in each inflorescence and categorize them as (1) pollinated and undamaged, (2) pollinated but no

longer viable because they have been partially eaten, or (3) unpollinated. This makes calculating seed predation straightforward (the percentage of all pollinated achenes that have been damaged by seed predators). On average, *E. villosus* seed predation reduced viable seed production by two-thirds per inflorescence at both sites (Table 1).

As we dissected inflorescences in the laboratory, we also quantified mortality of weevil larvae and its cause. To lay an egg, an adult female weevil chews a small hole through the wall of a mature bud (just prior to petal emergence) into which she inserts a single egg and then seals with frass. This leaves a distinctive wound that allows us to determine if an inflorescence has been used as an oviposition site even when the larvae died. When the larvae survived and fed on the seeds, we found partially eaten seeds, frass, and a well-developed pupal chamber (often with an adult weevil inside). When the larvae died, we found only a small amount of damaged tissue on the interior of the capitulum wall immediately behind the oviposition wound that is visible on the exterior of the capitulum. As with seed predation, larval mortality was a straightforward calculation.

TABLE 2. Details of biocontrol agent (*Eustenopus villosus*) release for each site.

Parameter: agent release	Site	
	Coast	Central Valley
5-year-old invasion		
No. of weevils released	100	100
Size of release area	1 × 1 m	1 × 1 m
No. plants at time of release	27 (2−74)	26 (2−78)
25-year-old invasion		
No. of weevils released	1000	1000
Size of release area	4 × 4 m	4 × 4 m
No. plants at time of release	492 (219−768)	1014 (345−1762)
50-year-old invasion		
No. of weevils released	1000	1000
Size of release area	4 × 4 m	4 × 4 m
No. plants at time of release	2163 (1418−3006)	5415 (3578−6537)

Note: Values are medians, with ranges in parentheses, and are derived from the model output.

In general, quantifying predation on arthropods can be difficult, but we were fortunate in that *P. tritici* leaves clear evidence of attack. When larvae (or, less frequently, an adult weevil that had managed to pupate before dying) were killed by the parasitoid *P. tritici*, we found the dead larva (or pupa) with larvae or physogastric *P. tritici* visibly emerging from its body (see Plate 1). In these cases, mortality was attributed to the parasitoid. When an inflorescence had an oviposition wound but no larvae and no evidence of parasitism, we attributed larval mortality to causes other than parasitism, e.g., unfavorable local climatic conditions, stochastic causes, or poor host plant quality.

Overall, mortality of larval weevils was high in both populations. At the coastal site, 25% of weevil larvae died due to parasitism and 41% died due to other, undetermined causes; at the Central Valley site, 38% of larvae died due to parasitism, but only 4% died due to undetermined causes. The high weevil mortality in the coastal populations due to causes other than parasitism may reflect a poor match between the weevil and the climate at this site.

Individual-based model

We constructed a spatially explicit, individual-based simulation model to examine the effects of seed predation by the biocontrol agent *E. villosus* on *C. solstitialis* density, c^*, and λ and how this might be altered by the generalist parasitoid *P. tritici*. We modeled the coast and Central Valley sites independently using the parameters in Table 1 to examine whether these populations were behaving differently. We modeled continuous space with each plant assigned x,y coordinates on an infinite plane. We modeled the germination of each seed and the survival to flowering of each seedling as individual realizations of a binomial trial with constant probabilities at each site (Table 1). Each plant was allowed to have any whole, non-negative number of inflorescences drawn from a negative binomial distribution with a constant

dispersion specific to each site and means determined by site-specific density dependent functions (Table 1). Each plant could host any whole, non-negative number of weevils. The weevils are pre-dispersal seed predators that reduce plant fecundity but not survival or growth, and the weevil's population dynamics are coupled to the plant's because they depend on the inflorescences as oviposition sites. The output was a landscape of plants with varying numbers of inflorescences, some of which were attacked by weevils and some of which had escaped attack by chance. At each time step (equal to one generation or year), we quantified total population size for both the plant and the weevil, as well as the number of plants in the central 1 × 1 m area from which we calculated density and λ. We also estimated the diameter of the invasion at each time step as the square root of the area of a rectangle enclosing the outermost individuals in the x and y directions. This provides a geometric mean rate of spread in the x and y directions that is less sensitive to outliers than the distance of the single furthest plant. We used the rate of change in diameter to calculate the linear spread rate (m/yr; c^*).

We initialized the model with a single adult plant in the center of the grid. At each time step in the simulation, recruitment, survival, and fecundity were determined according the site-specific data (Table 1). Seed dispersal occurred randomly in space in a randomly chosen direction, and dispersal distance was drawn from a negative exponential distribution with the mean = 0.30 m (Roché 1992).

To release the agent into the invasion, we randomly dispersed weevils (with or without the parasitoid) over an area at the center of the grid (see Table 2 for details). We located our release area at the center of the invasion because plant density was highest there and this would expose the majority of established plants to the agent while minimizing initial intraspecific competition among weevils. Any plants beyond the boundaries of this

release area escaped initial attack, but the virtual weevils had the ability to detect and move to those plants.

Modeling weevil movement, intraspecific competition, and survival

The weevils were introduced to each plant population at the overwintering stage. Weevils were able to move up to 1 m at a time (a single powered, directed flight) and were allowed to make up to 105 individual trips in a lifetime. We do not know the distances over which *E. villosus* can detect its host, so we allowed it to be able to detect a plant across the entire landscape. This is in agreement with other weevil species for which we have good estimates of detection ranges (e.g., Buckley et al. 2005). We set the total number of ovipositing trips in a lifetime at 105 to allow each weevil to move to and explore three plants for each of the 35 eggs it may lay in its lifetime, bearing in mind that *E. villosus* only flies during a restricted period each day and has a short lifespan as an ovipositing adult (15.7 ± 9.0 d [mean ± SD]; Fornasari and Sobhian 1993).

Because eggs are laid singly, there is no competition among larvae, but there is competition among adult weevils for oviposition sites. In our model, weevils would not oviposit on an inflorescence that had already been used by another weevil (or previously used by itself), but instead would move to any "available" inflorescence on the host plant and if there were none, would move to another plant. In our model, weevils "knew" which plants they themselves had already visited and would not return to those plants as they searched for new oviposition sites, but they were unable to determine if a plant had already been used by another weevil from a distance and so had to move to each plant to make that determination, potentially using up one or more of their lifetime trips visiting a plant on which they could not oviposit.

We established the probably that any individual weevil would move to any plant that it had not already visited according to the following equation in which a plant had a probability of being selected by the weevil proportional to

$$P_i = \frac{\alpha}{d_i^\beta}$$

where d_i is the distance from the weevil's current position to plant i, β describes how rapidly the probability of moving to a plant falls off with increasing distance, and α is chosen so that the sum of the probabilities P_i of picking plant i sum to 1 for all plants not previously attacked within the maximum detection distance. We set $\beta = 5$, making the weevil more likely to select a nearby plant rather than a distant plant (reducing this number will have the reverse effect), based on their tendency to move from one plant to a neighboring plant (S. M. Swope, *personal observation*) and their limited flying abilities (Fornasari and Sobhian 1993). We scheduled weevil movement by randomizing the order of weevils with remaining trips and eggs and allowing each weevil to

make one trip (if needed) and lay one egg (if possible) before the next weevil was allowed to move. This process was repeated, re-ordering the weevils randomly after each complete cycle through the weevils with remaining trips and eggs, until all the weevils had used the year's allotment of eggs and/or trips at each time step.

Weevils require buds at a particular phenological stage for oviposition (large buds, a few days prior to petal emergence), and our field data indicate that up to 50% of the inflorescences on each plant are vulnerable to oviposition at any given time. Weevils could lay eggs in as many inflorescences on a single plant as were present and were phenologically suitable for oviposition (50%).

Weevils suffer two bouts of mortality each year: overwintering mortality as adults (prior to egg-laying) and as larva during the summer. We modeled survival through each of these two time periods as a binomial process similar to germination and seedling survival in plants. We set overwintering mortality at 20%, although this may be low based on the few published estimates of overwintering mortality for other weevil species (e.g., Greenberg et al. 2004, Haye et al. 2010). In the two-trophic-level scenario (plant and biocontrol agent), we set weevil larval mortality equal to mortality attributable to causes other than parasitism (coast 41%; Central Valley 4%). In the three-trophic-level scenario (plant, biocontrol agent, and parasitoid), we set larval mortality equal to total mortality (mortality due to other causes plus mortality due to parasitism) because both operate simultaneously (coast 66%; Central Valley 42%). *Pyemotes tritici* is wind dispersed, i.e., has no directed dispersal, and our field data show no evidence of a spatial pattern in its attack. There was also no spatial pattern in mortality due to undetermined (nonparasitism) causes. Because the parasitoid is a generalist, we assumed it was present and fully capable of attacking its host when the agent is first introduced.

Trophic and temporal scenarios

Our model reproduced naturally occurring plant and weevil densities and spatial patterns at both field invasions. This allowed us to proceed by perturbing the model to explore how different control scenarios affected different aspects of invader success (density, c^*, and λ). First, we ran a separate plant-only scenario for each site in which the plant was allowed to invade the landscape for 100 generations with no top-down control. Results from these simulations served as our reference populations for each site against which we measured agent impact. We had two trophic scenarios: The agent without its parasitoid and the agent with its parasitoid to explore how the inclusion of the parasitoid altered the outcome of the plant–seed predator interactions at the two sites.

To assess whether very small, young invasions are more sensitive to control by the seed predator than are large, well-established invasions, we had three starting conditions in which we allowed the plant to invade the landscape for different lengths of time (5, 25, or 50 years)

Table 3. Summary data derived from the model for each trophic and temporal scenario.

| | Centaurea solstitialis | | |
Site and trophic scenario	Abundance	Density (plants/m²)	λ
Coast			
5-year invasion + 50 years of biocontrol			
Plant only	2603 (1509–3504)	103 (70–146)	1.181 (1.090–1.255)
Plant + seed predator	1941 (1231–2545)	82 (39–120)	1.184 (1.072–1.269)
Plant + seed predator + parasite	2654 (1642–3265)	99 (41–162)	1.175 (1.071–1.349)
25-year invasion + 50 years of biocontrol			
Plant only	5181 (3452–6198)	132 (83–176)	1.122 (0.743–1.886)
Plant + seed predator	3983 (2955–5125)	115 (80–160)	1.121 (1.033–1.207)
Plant + seed predator + parasite	5037 (3428–7047)	136 (87–177)	1.110 (1.075–1.187)
50-year invasion + 50 years of biocontrol			
Plant only	9622 (7396–11 120)	185 (131–232)	1.095 (0.865–1.594)
Plant + seed predator	7799 (6535–8563)	140 (116–189)	1.067 (0.947–1.257)
Plant + seed predator + parasite	9341 (7718–10 999)	178 (137–221)	1.097 (1.045–1.139)
Central Valley			
5-year invasion + 50 years of biocontrol			
Plant only	6204 (4168–8185)	250 (194–344)	1.186 (1.131–1.252)
Plant + seed predator	764 (205–1425)	58 (9–109)	1.090 (0.276–3.687)
Plant + seed predator + parasite	3725 (2590–5251)	181 (99–309)	1.209 (0.596–2.014)
25-year invasion + 50 years of biocontrol			
Plant only	12 459 (9717–14 899)	359 (279–447)	1.133 (0.847–1.426)
Plant + seed predator	2496 (1567–3487)	101 (52–171)	1.117 (0.991–1.290)
Plant + seed predator + parasite	8637 (6731–10 808)	275 (186–362)	1.122 (1.056–1.171)
50-year invasion + 50 years of biocontrol			
Plant only	23 472 (19 071–28 243)	497 (414–572)	1.012 (0.849–1.307)
Plant + seed predator	7085 (5513–7736)	162 (106–197)	1.004 (0.814–1.519)
Plant + seed predator + parasite	17 465 (14 547–19 543)	332 (301–437)	1.008 (0.864–1.096)

Notes: Abundance (final population size) and density (central 1 m²) were measured after 50 years of biocontrol and from an uncontrolled invasion of the same age (a 55-year-old invasion in the 5-year scenario, 75-year-old invasion in the 25-year scenario, and 100-year-old invasion in the 50-year scenario). Population growth rate (λ) and spread rate ($c*$) were calculated over the last five years of each scenario run to minimize the influence of year to year variability. Values are medians with ranges in parentheses. "Ext." refers to the percentage of *Centaurea solstitialis* invasions that went extinct after the biocontrol agent was released or to the percentage of *Eustenopus villosus* populations that went extinct after establishing.

before we introduced the agent. Establishment and spread ought to be highly seed limited in the earliest stages of an invasion and so a seed predator is predicted to have a large impact when introduced to such young invasions. At the other end of the spectrum, it is possible that an invasion may escape meaningful control when it establishes large, high-density populations it before being subjected to agent attack (alternatively, such large populations are able to support larger agent populations and this might facilitate top-down regulation). In one scenario, we allowed the plant to invade the landscape for only five years (generations) before we released the agent. This was meant to mimic a satellite invasion such as one might find along the leading edge of an invasion. At the other extreme, we allowed the plant to invade the landscape for 50 years before the agent was introduced. These plant populations were very large and spread over an extensive area by the time they were subjected to biocontrol. We also ran an intermediate scenario in which we allowed the plant to invade for 25 years prior to agent release. Both of the latter two scenarios mimic many real-world invasions in which the weed establishes for several years before the population is detected or before a biocontrol solution is sought. In all three scenarios (5-, 25-, or 50-year-old invasions), we allowed the invader and agent to co-exist for an additional 50 years and assessed the agent's impact on the plant at five-year intervals by comparing it to an uncontrolled plant population of the same age (the plant-only scenario).

Measures of agent impact

We measured the agent's impact on three aspects of invasion success (plant density, $c*$, and λ) by comparing it to an uncontrolled invasion of the same age. We subjected the plant to 50 years of biocontrol, and we report the proportional reduction (relative to an uncontrolled invasion) in each element (density, $c*$, and λ) at five-year intervals. In addition, we report final plant and weevil abundance after 50 years of biocontrol. As with real-world invasions, our simulated plant invasion occasionally failed due to chance, so we ran as many simulations as necessary to achieve a sample size of 50 replicate invasions for each of the scenarios at each site. Because our model is stochastic, we used median values (density, $c*$, λ, and final abundance) to minimize the influence of any outliers. Density and λ were calculated from the central 1 m² of the landscape, and $c*$ and λ were calculated over five-year time spans to minimize year-to-year variation. We used Matlab R2011a (MathWorks 2011) to create and run the simulation model.

TABLE 3. Extended.

Centaurea solstitialis		Eustenopus villosus	
$c*$	Ext. (%)	Abundance	Ext. (%)
0.312 (0.126–0.511)	1		
0.307 (0.119–0.444)	2	698 (425–980)	2
0.297 (0.156–0.403)	0	105 (100–300)	50
0.273 (0.121–0.431)	0		
0.272 (0.049–0.574)	0	1602 (1151–2048)	0
0.277 (0.073–0.503)	0	455 (238–657)	0
0.290 (0.090–0.470)	0		
0.294 (0.124–0.369)	0	3200 (2606–3554)	0
0.297 (0.087–0.505)	0	810 (576–1062)	0
0.263 (0.097–0.399)	0		
0.165 (0.018–0.336)	12	899 (217–1452)	0
0.261 (0.098–0.489)	4	2173 (1454–2975)	2
0.271 (0.148–0.503)	0		
0.192 (0.001–0.357)	0	2806 (1571–3649)	0
0.238 (0.175–0.413)	0	5297 (3965–6678)	0
0.294 (0.158–0.493)	0		
0.225 (0.089–0.406)	0	7580 (5940–8966)	0
0.293 (0.246–0.480)	0	10 908 (9008–12 507)	0

RESULTS

Differences among sites

In the absence of any top-down control by the seed predator, both plant density and final population size were approximately two and a half times greater in the Central Valley invasions than in the coastal invasions after a century of uncontrolled expansion (Table 3). The impact of the agent on the plant was greater at the Central Valley site than it was at the coast site in all of the scenarios presumably due to the higher weevil survival rate, regardless of the presence or absence of the parasitoid, at this site (Table 1). The parasitoid substantially reduced weevil survival at both sites and this, in turn, significantly reduced the agent's impact on the plant by inducing a classic trophic cascade.

Agent impact on invader success

Seed predation by the biocontrol agent affected different aspects of invasion success differently. Overall, the seed predator had its largest and most persistent impact on plant density, a modest impact on $c*$ that was persistent in some cases and transient in others, and the least impact on plant λ. At the coast, the parasitoid-free agent reduced plant density by 13–24% at the end of 50 years of attack (Fig. 1A–C, Table 3). Spread rate was highly variable even in the uncontrolled populations (plant-only scenario). At the coast, the agent reduced $c*$ by 90% when introduced to the youngest invasions (five years), but this impact was fleeting and the plant was soon spreading at nearly the same rate as an uncontrolled invasion (Fig. 1A–C). The agent's impact on $c*$ was largely nonexistent when introduced to the 25- and 50-year-old invasions. The unparasitized agent had no effect on λ.

At the Central Valley site, the parasitoid-free agent reduced plant density by an impressive 67–80% (Fig. 2A–C) after 50 years of attack (Table 3). It also reduced $c*$ by 29–37% (Fig. 2A–C) by the end of the scenario runs. The agent substantially reduced λ immediately following release, but the plant fully recovered over a period of several generations and after half a century of biocontrol, the invasions were growing as quickly as uncontrolled ones (Fig. 2A–C).

Influence of the parasitoid

The parasitoid P. tritici reduced the agent's impact on the plant at both sites. But because the parasitoid had very different effects on the seed predator's populations at the two sites (see Weevil population performance below), it also had very different effects on the plant populations as well. At the coast, where the agent's impact was relatively modest even in the best-case scenario (no parasitoid), the parasitoid largely eliminated the agent's impact regardless of which aspect of control (density, $c*$, or λ) was measured (Fig. 1D–F). The one exception was the agent's initial impact on $c*$ in the youngest invasions (Fig. 1D). Here the initial impact on $c*$ was nearly identical in magnitude to the parasitoid-free model (Fig. 1A), but it also disappeared after only 15 years of biocontrol. The parasitized weevil caused a very small reduction in coast plant density, which was detectable in all cases (Fig. 1D–F), but it
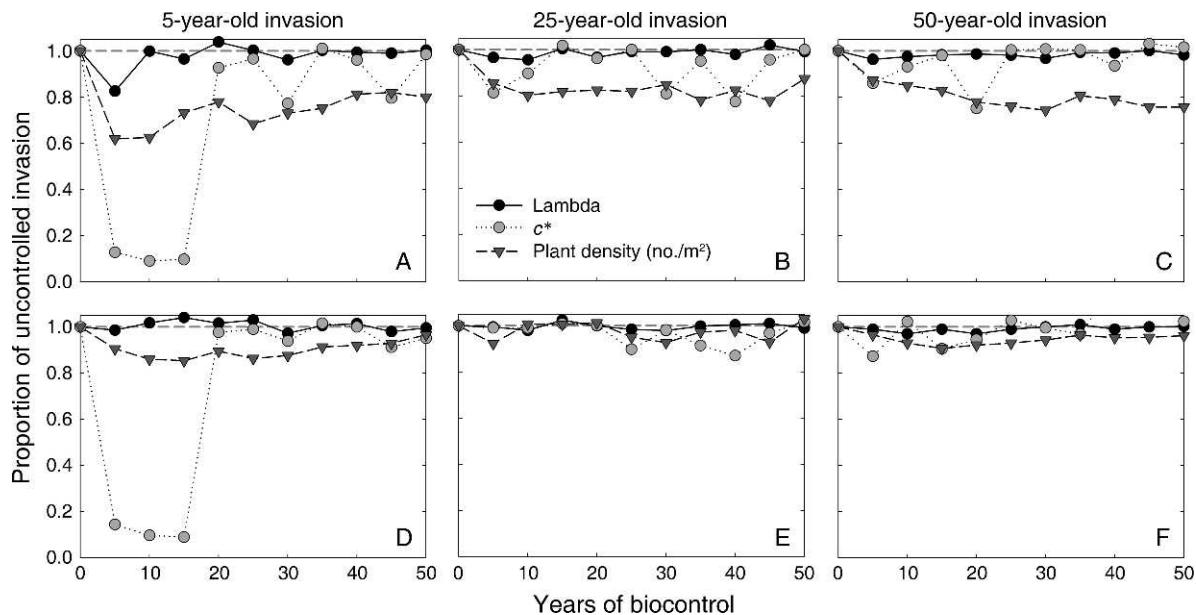
FIG. 1. California (USA) coastal plant (*Centaurea solstitialis*) population performance (growth rate [λ], spread rate [*c**], and plant density), as a proportion of an uncontrolled population of the same age, when attacked by the seed predator *Pyemotes tritici* in the (A–C) absence and the (D–F) presence of the parasitoid. The biocontrol agent *Eustenopus villosus* was released into invasions of different ages: (A, D) a 5-year-old invasion, (B, E) a 25-year-old invasion, and (C, F) a 50-year-old invasion. Values are medians.

would be difficult to argue that this represents meaningful control.

At the Central Valley, the parasitoid eliminated more than half of the agent's impact on plant density. But even when parasitized, the weevil reduced density by 24–33% after 50 years of attack (Fig. 2D–F, Table 3).

In the absence of the parasitoid, the seed predator reduced both *c** and λ for several generations, but the inclusion of the parasitoid largely eliminated the agent's impact on these two aspects of invader performance except briefly immediately following release (Fig. 2D–F).
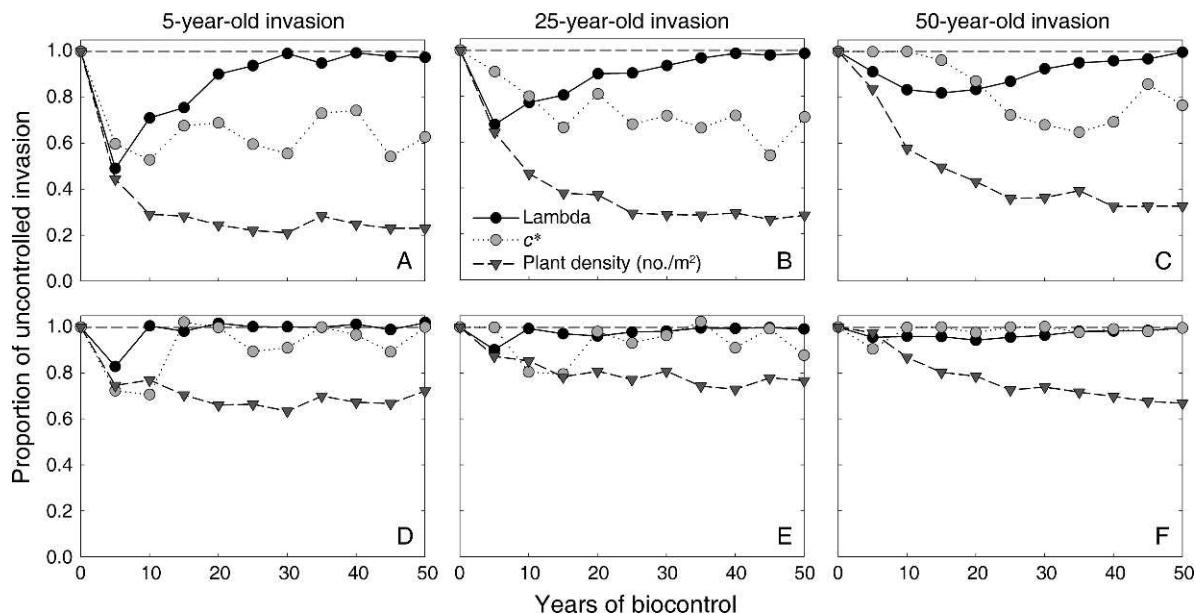
FIG. 2. Central Valley plant population performance, as a proportion of an uncontrolled population of the same age, when attacked by the seed predator in the (A–C) absence and the (D–F) presence of the parasitoid. The agent was released into invasions of different ages: (A, D) a 5-year-old invasion, (B, E) a 25-year-old invasion, and (C, F) a 50-year-old invasion. Values are medians. See the Fig. 1 legend for clarification of variables.
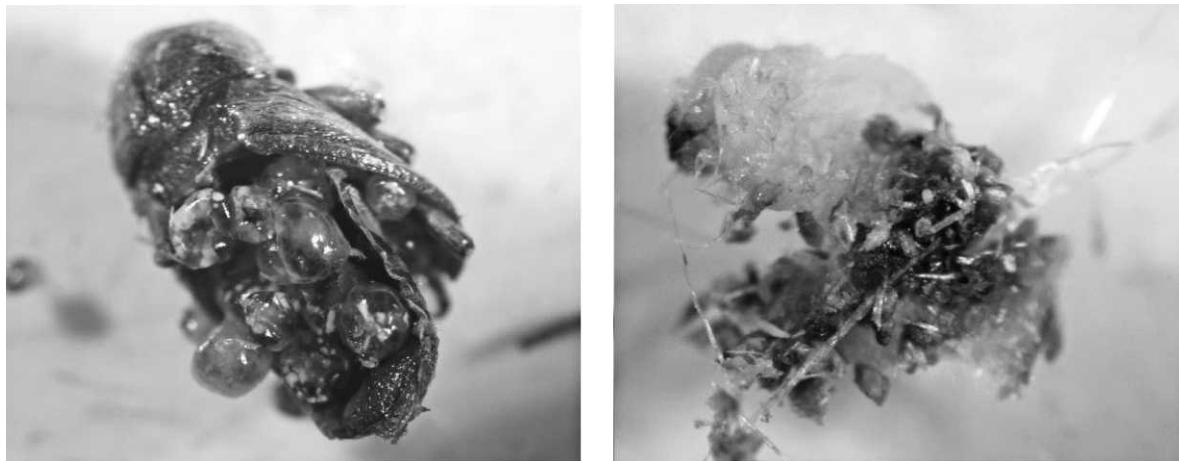
PLATE 1. (Left) *Eustenopus villosus* pupa with evidence typical of attack by physogastric *Pyemotes tritici*; (right) attacked larva. Photo credit: Joe Braasch.

Measuring agent impact through time

The impact of the agent on the plant generally changed over the course of the half century of biocontrol. In some cases, measuring the agent's impact in the years immediately following its introduction would have underestimated its long-term impact, and in other cases, it would have overestimated it. At both sites, with and without the parasitoid, the agent's short-term impact on plant density was a good predictor of its long-term impact. In some cases (Fig. 1D–F), the agent had little or no impact on plant density, and this was still true after 50 years of attack. In other cases (Fig. 1A–C, Fig. 2A–F), the agent caused an initial decline in density that persisted over the long term. The agent's impact on λ in the short term was an overestimation of its long-term impact in every case, even when the initial reduction was substantial (e.g., Fig. 2A).

At the coast, with and without the parasitoid, the agent either had an appreciable but short-lived impact on c^*, i.e., its short-term impact on c^* was an overestimation of its long-term impact (Fig. 1A–D) or it had no impact on c^*, in which case, short-term impact was an accurate predictor of long-term impact. At the Central Valley, the parasitoid-free agent's impact on c^* either persisted (Fig. 2A and B) or increased thorough time (Fig. 2C). The parasitoid largely eliminated the agent's impact on c^* at the Central Valley, so its short-term impact was a reliable predictor of its long-term impact.

Age of invasion at time of agent release

In general, the age of the invasion (5, 25, or 50 years) at the time the biocontrol agent was released had a larger influence on the agent's short-term impact in than it did on its long-term impact.

When introduced to the youngest invasions (five years) at the coast, the agent reduced all measures of invader performance (by very large proportions in the case of c^*) in the 5–15 years immediately following its release (Fig. 1A and D). But this impact largely disappeared over the remaining years of the scenario. When introduced to the older coastal invasions (25 and 50 years), the agent's initial impact was considerably smaller (Fig. 1B, C, E and F), and in all cases (5, 25, and 50 years), c^* and λ fully recovered, as did density when the parasitoid was included. Density in the coastal invasions remained ~20% below that of an uncontrolled invasion in the parasitoid-free model regardless of the age of the invasion when it was first subjected to biocontrol (Fig. 1A).

As with the coast invasions, the younger the Central Valley invasions were when the agent was released, the larger the agent's impact, but again this was only true in the short term. The age of the invasion at the time of agent release made little difference in either the nature (density, c^*, or λ) or magnitude of the agent's impact at the end of five decades of attack. There was one important exception to this. When the agent was introduced to the youngest Central Valley invasions (five years), it was capable of occasionally driving them to extinction. This was especially true when the agent was not parasitized (Table 3): unparasitized weevils drove 12% of the *C. solstitialis* invasions to extinction, while parasitized weevils drove 4% of the invasions to extinction. Local extinction is, of course, the ultimate biocontrol success.

Weevil population performance

The inclusion of the parasitoid had opposing effects on weevil populations at the two sites. At the coast, weevil populations were larger in the absence of its parasitoid. Fifty years after its introduction, median weevil population size was 3.5–4 times greater when it was free of its parasitoid than when it was parasitized. Not only were coastal weevils populations smaller when they were parasitized, but when parasitism was combined with small plant population size (five-year

scenario), fully half of the weevil populations went extinct after managing to persist for years. Only 2% of coastal weevil populations introduced to the five-year-old invasions went extinct when they were not parasitized (Table 3).

We found the opposite pattern among the weevil populations at the Central Valley site. Here, median weevil population size was 1.5–2.5 times larger when the weevil was parasitized than when it suffered no top-down control. No weevil populations went extinct when they were parasitized, while 2% of the nonparasitized weevil populations went extinct after successful establishment (Table 3).

Net impact of the agent on the plant

The net impact of the agent on these different aspects of the invasion process (density, $c*$, and λ) can be measured as final plant abundance after 50 years of biocontrol. Coastal plant populations that were attacked by the biocontrol agent without its parasitoid were 19–25% smaller than an uncontrolled invasion of the same age (Table 3). When they were attacked by parasitized seed predators, these coastal invasions performed as well as invasions that were entirely free of top-down regulation despite the fact that they hosted stable, albeit small, weevil populations (Table 3). At the Central Valley site, the unparasitized biocontrol agent reduced final plant abundance by an impressive 70–88%, while the parasitized weevils reduced final plant abundance by 25–40%, in spite of the fact that these invasions also supported much larger weevil populations (Table 3).

DISCUSSION

In this study we tested whether the seed predator biocontrol agent *E. villosus* affects the density, $c*$, and/or λ of the invasive annual plant *C. solstitialis* and how the generalist parasitoid *P. tritici* changes the outcome of the weed–biocontrol agent interaction at two sites. We demonstrated that an agent can reduce one aspect of invader success (density, and in some cases, $c*$) without reducing others (λ, and in other cases, $c*$). We also demonstrated that the magnitude of the agent's impact changes over the course of decades of attack such that the impact that we observe in the years immediately following agent release may either over- or underestimate its long-term impacts. Surprisingly, we found that the length of time the invader was allowed to spread across the landscape prior to releasing the agent had little impact on either the nature of control (density, $c*$, or λ) or its magnitude over the long term. Finally, we found that a generalist parasitoid fundamentally altered the outcome of the weed–biocontrol agent interaction by inducing a trophic cascade, the strength of which varied between the two sites.

It is interesting to note that Shea et al. (2010), working with a perennial invader (*Carduus nutans*) and three species of biocontrol agent (two seed predators and a root-crown weevil), found a similar outcome. In that system, an agent capable of reducing the weed's $c*$ did not also reduce its λ. Also similar to Shea et al. (2010), we found that the nature and the magnitude of the agent's impact were contingent upon local plant population dynamics. The similarity of our findings suggests that these outcomes may be common in weed–biocontrol systems.

In an ideal biocontrol scenario, the agent drives the weed to a new, lower equilibrium abundance that is below some threshold deemed to be ecologically or economically acceptable and the two species will coexist in a way that is stable over the long term, with the agent providing continuous control (Smith and van den Bosch 1967, van Driesche et al. 2008). When it works as described here, biocontrol provides an elegant solution to the otherwise intractable problem of widespread, high-density populations of invasive plants. The *Hypericum perforatum*–*Chrysolina quadrigemina* system is an example of just such a successful biocontrol effort. At the peak of its invasion, *H. perforatum* infested nearly one million hectares in northern California. The introduction of the biocontrol agent *C. quadrigemina* reduced the plant's abundance by several orders of magnitude in just over a decade, and the two species now coexist at low densities (Huffaker and Kennett 1959) with the agent presumably providing continuous control.

Similarly, the results from our simulation model indicate that at the Central Valley site, where *C. solstitialis* has been so problematic, *E. villosus* has been successful in both reducing plant density and establishing its own populations that we predict will maintain this lower plant density over the long term. This is in spite of the fact that in the field more than one-third of the larvae are killed by a generalist parasitoid. But our results do not suggest that the agent is causing the plant populations to decline over time (λ is unaffected). In other words, the degree of control achieved by *E. villosus* thus far likely represents its maximum impact. The general consensus among land managers is that *E. villosus*, alone or in combination with the other agents that have been released for *C. solstitialis* in California, has not reduced *C. solstitialis* densities to acceptably low levels (DiTomaso and Healy 2007).

In contrast, at our coastal site, the parasitoid is predicted to eliminate the impact of the agent on the plant by suppressing weevil population size. Why did coastal weevil populations respond so differently to parasitism in our simulations? One possibility is the difference in plant performance, i.e., oviposition opportunities, at the two sites (lower in the coastal populations and higher in the Central Valley). We initially expected that lower plant density and fewer inflorescences per plant would limit weevil population growth at the coast, but that does not appear to be the case because, on average, 60% of the coast plants remained unattacked at the end of each scenario run and the weevils have the

ability to detect and move to unattacked plants. A second possibility is the large differences in larval mortality due to causes other than parasitism (high in the coastal populations and very low in the Central Valley). Forty-one percent of the larvae died in the coastal populations due to causes other than parasitoid attack and, while this was high, the weevil populations were nevertheless persistent and offered some degree of control of the weed. The inclusion of the parasitoid seems to push the weevil population over a threshold and suggests that parasitism combined with what appears to a poor fit between the weevil and the coastal climate is limiting the weevil's own success and therefore its impact on the plant.

At the Central Valley, the parasitoid substantially reduced the impact of the weevil on the plant, just as it had at the coast, but here it also bolstered weevil populations. This nonintuitive outcome arises because the reproductive success of the plant and the weevil are tightly coupled to one another. In the absence of the parasitoid, *E. villosus* drove the *C. solstitialis* populations to low enough numbers that oviposition opportunities became scarce and the weevil's own populations were reduced. When the parasitoid was included in the model, the plant population was significantly larger and supported a larger weevil population because oviposition opportunities were no longer limiting.

We were surprised by how little influence the age of the invasion at the time of agent release had on the agent's ability to control the weed. We expected that the agent would have its largest impact on the weed when it was introduced to the smallest invasions (five years). Very small populations that are in the earliest stages of establishment are expected to experience pronounced seed-limited recruitment and thus be quite sensitive to seed predation. We therefore expected to see a larger impact on both density and λ in these smallest invasions. Further, because these youngest invasions occupied such a small area when the agent was released and the agent was dispersed over the entirety of that area, we expected the agent might have its largest impact on c^*. In the first 15 years of biocontrol, the agent did have a larger impact in the youngest invasions than it did in the older invasions at both sites. But after 50 years of attack, the magnitude of impact was similar regardless of whether the agent was introduced to 5-, 25-, or 50-year-old invasions. There was one important exception to this. At the Central Valley where the agent's impact was greater, the parasitoid-free agent drove 12% of the youngest invasions to extinction and the parasitized agent drove 4% of the invasions to extinction. (The agent drove none of the 25- and 50-year-old invasions to extinction regardless of the whether the agent was parasitized.) The invasions that went extinct were very small when the agent was released (2–21 plants), which was below the median (Table 2). This suggests that there is a threshold invasion size below which the agent is capable of the ultimate success (local extinction), but this threshold

population size appears to be very small. We did not see a similarly elevated extinction rate at the coast, where the agent had a much smaller impact on the plant even when it was not parasitized. Taken together, we conclude that a biocontrol agent's impact can be larger in younger invasions, but primarily when small invasion size is combined with large agent impacts and no parasitism.

Limitations of the findings

Fully 54% of all biocontrol agents are seed predators or florivores that reduce seed production (Coombs et al. 2004), but not all invading species or even all populations of the same species will be sensitive to seed predation. Those species that have an annual life cycle and depend on the current year's seed rain for recruitment will be most sensitive to seed loss (Crawley 1989, Louda and Potvin 1995), and *C. solstitialis* possesses these traits. Elsewhere, we have shown that *C. solstitialis* density can be suppressed by reducing seed input whether via seed-feeding biocontrol agents or by other means, but that even when density was reduced, the populations persisted (Swope and Parker 2010). These results from field experiments are consistent with our model predictions presented here in that seed predation reduced plant density but had little effect on λ. Even some populations of annual invaders that would otherwise be sensitive to seed predation may be less so at sites (or in years) in which germination microsites are scarce (Louda 1983, Crawley 1989, Sheppard et al. 2002). For those invasive species that possess life history traits that ought to buffer their populations against seed losses, e.g., high fecundity, perennial, and long-lived seed banks, seed predators are predicted to be ineffective biocontrol agents (e.g., Parker 2000).

Biocontrol agents and native parasitoids

It has long been recognized that generalist parasitoids and predators readily attack novel insect species as hosts (Settle and Wilson 1990, Cornell and Hawkins 1993) including biocontrol agents (Goeden and Louda 1976, Paynter et al. 2010). Cornell and Hawkins (1993) evaluated parasitoid attack for 87 exotic insects and found that all but nine (over 89%) were attacked by one or more native parasitoid in the introduced range. Paynter et al. (2010) surveyed the literature for 28 of the 30 species of weed biocontrol agent established in New Zealand and found no relationship between parasitoid richness and the agent's residence time, suggesting that agents are rapidly colonized by parasitoids present in the introduced range (see also Hill and Hulley 1995).

Predation and parasitism may reduce both the likelihood that agents establish and their impact on the target weed. Paynter et al. (2010) found a significant correlation between the degree of parasitism and the failure of the agent to control the target weed. Indeed, in our simulations, parasitized agents at the coast had higher extinction rates than nonparasitized agents

(Table 3) and the parasitoid substantially reduced the agent's effectiveness at both sites. At the coast, the parasitoid eliminated the modest degree of control the agent provided and at the Central Valley, the parasitoid turned a potentially very effective agent into a modestly effective one.

It is often argued that it is precisely the release from top-down control that transforms an innocuous herbivore in the native range into an effective biocontrol agent in the introduced range (Strong et al. 1984, Lawton 1985, Hunt-Joshi et al. 2005, van Driesche et al. 2008). This is simply the "enemy release" hypothesis used to explain agent impact rather than invader success. But it is possible that agents will frequently be attacked by native enemies that may reduce and even eliminate the impact of an otherwise effective biocontrol agent on its weed by inducing a trophic cascade. Weed–biocontrol systems may be especially prone to trophic cascades because the agent is typically highly host specific (i.e., has narrow diet; Leibold 1989, Abrams 1993), and was presumably selected precisely because it was expected to have a strong impact on the plant's populations. In other words, there is a strong link between the first and second trophic levels, a prerequisite for a trophic cascade (Paine 1980, Strong 1992). Predation therefore has the potential to fundamentally alter the outcome of the interaction between the herbivore (biocontrol agent) and the plant (weed). Interestingly, this means that, for a given level of parasitism, an effective agent will be more adversely affected than a less effective one. The contrasting results from our coast and Central Valley populations support this.

Agents that harbor generalist parasitoids may have other indirect effects on native insects. Carvalheiro et al. (2008) found a negative correlation between native insect richness and abundance and biocontrol agent abundance. The native insects and biocontrol agents share predators and parasitoids and the authors used a food web approach to identify apparent competition as the likely cause of native insect declines. The parasitoid in our study, *P. tritici*, is known to attack a wide range of taxa from Lepidoptera and Coleoptera to Hymenoptera (Childers 1982, Alvarado-Rodriguez 1987, Hoschele and Tanigoshi 1993, Menezes et al. 2009). In fact, *P. tritici* itself was even once considered as a potential biocontrol agent for the red imported fire ant (*Solenopsis invicta*; Thorvilson et al. 1987). We do not know whether populations of *E. villosus* indirectly impact other insect species (including pollinators and other biocontrol agents) by bolstering *P. tritici* populations, but the breadth of the parasitoid's diet suggests that this is possible. This argues for not releasing ineffective biocontrol agents even when they are demonstrably highly host specific so as to reduce any potential indirect impacts such as apparent competition on other insects including any prospective agents that have yet to be identified or released.

Management implications

Whether the agent was successful in controlling the weed varied by site and depended on how and when impact was measured and whether the agent was subjected to attack by the parasitoid. Interestingly, agent impact was not strongly influenced by how long the invasion had been allowed to spread before the agent was introduced. This is encouraging news for land managers who often face the challenge of reining in invaders that are already well established. Even when introduced to high-density populations spread across the largest area (50-year invasions), the agent was able to suppress weed density to the same degree as when it was introduced to the smallest invasions. The exception, of course, being those occasions in which the agent drove the weed to extinction. This occurred only when the agent was able to impose strong top-down control (Central Valley, without and, to a lesser degree, with the parasitoid) and only in the youngest invasions. In a field setting, this may occur when small satellite invasions establish some distance from the source population. These satellite invasions may advance the invasion front in a saltatory fashion and so the agent may play an especially important role in controlling invasions at the landscape scale. Unfortunately, our best evidence for this comes from the parasitoid-free scenarios, which may be uncommon in a field setting.

A cautionary lesson from our data is that we should not assume that the agent's impact observed in the years immediately following its release is a good predictor of its long-term impact. In the field, the impact of an agent is likely to be most evident, or simply most frequently measured, in the years immediately following its release. Assessing the agent's impact on the plant in the first several years after its release would have underestimated its long-term impact in some cases and substantially overestimated it others. A land manager carefully measuring *C. solstitialis* response to biocontrol in the five years following release of the agent would rightly conclude that the plant's populations were in rapid decline (in one case, λ was reduced by half; Fig. 2A) but would be wrong to assume that that trend would continue and that the invasion would go extinct. In fact, after half a century of biocontrol, the invasions were growing as quickly as those with no biocontrol agents, despite the fact that all were hosting large and generally stable populations of seed predators. This is important because land managers may well make decisions about how to spend scarce funds for control efforts based on the data collected immediately following agent release. It is conceivable that, after assessing the agent's impact in the first five years of biocontrol, they would assume that the invader's population(s) were in rapid decline and bound to go locally extinct and turn their attention elsewhere.

Of all the variables that we explored, the presence of the generalist parasitoid had the largest impact on the agent's ability to control all aspects of invader success.

This is unfortunate because most agents are probably susceptible to colonization by such enemies (Cornell and Hawkins 1993, Paynter et al. 2010), and biocontrol practitioners have limited means of coping with this challenge. Paynter et al. (2010) found that biocontrol agents with ecological analogues in the introduced range tended to be more heavily parasitized than those agents with few close relatives and those with a different feeding niche. They also found that those agents with more parasitoids in the native range tended to accumulate more in the introduced range, too. Thus, one way to minimize the complications of generalist enemies is to avoid using insects as potential biocontrol agents that have closely related taxa in the introduced range, who feed on the plant in a similar manner, and are heavily parasitized at home. Additionally, biocontrol agents that do not serve as reservoirs for native parasitoids may also reduce the risk of indirect impacts on native insects and other biocontrol agents via apparent competition.

Even in the absence of the parasitoid, the agent's ability to control the weed differed markedly by site. When information about local, generalist parasitoids is combined with estimates of agent population performance in their absence, practitioners may be better able to identify sites where the agent is most likely to be successful and focus release efforts there. In other words, practitioners need to explicitly consider the biotic as well as the abiotic environment, and the interaction between the two, when evaluating how well matched a prospective agent is to a site because high agent population performance may be required for it to have a significant impact on the plant when subjected to parasitism as we saw in our Central Valley site.

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Supplemental Material

Appendix

Quantifying *Centaurea solstitialis* recruitment from the seed bank (*Ecological Archives* A022-001-A1).