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Biocontrol insect impacts population growth of its target plant species but not an incidentally used nontarget

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Abstract. Understanding the impact of herbivory on plant populations is a fundamental goal of ecology. Damage to individual plants can be visually striking and affect the fates of individuals, but these impacts do not necessarily translate into population-level differences in vital rates (survival, growth, or fecundity) or population growth rates. In biological control of weeds, quantitative assessments of population-level impacts of released agents on both target invasive plants and native, nontarget plants are needed to inform evaluations of the benefits and risks of releasing agents into new regions. Here we present a 3-yr experimental demographic field study using the European root-feeding biocontrol weevil, *Mogulones crucifer*, first released in Canada in 1997 to control the invasive weed *Cynoglossum officinale* (Boraginaceae). *Mogulones crucifer* is an effective "search and destroy" agent in Canada, but sporadically feeds, oviposits, and develops on native nontarget Boraginaceae. We investigated the population-level impacts of this biocontrol insect on its target weed and a native nontarget plant, *Hackelia micrantha* (Boraginaceae), by releasing large numbers of weevils into naturally occurring patches of *H. micrantha* growing isolated from or interspersed with *C. officinale*. We followed the fates of individual plants on release and nonrelease (control) sites for two transition years, developed matrix models to project population growth rates (λ) for each plant species, and examined the contributions from differences in vital rates to changes in λ using life table response experiments (LTRE). In contrast to studies of the insect–plant interaction in its native range, as a biocontrol agent, *M. crucifer* increased mortality of *C. officinale* rosettes in the year immediately following release, depressing the weed's λ to below the population replacement level. However, λ for *H. micrantha* was never depressed below the replacement level, and any differences between release and nonrelease sites in the nontarget could not be explained by significant contributions from vital rates in the LTRE. This study is the first to simultaneously and experimentally examine target and nontarget population-level impacts of a weed biocontrol insect in the field, and supports the theoretical prediction that plant life history characteristics and uneven herbivore host preferences can interact to produce differences in population-level impacts between target and nontarget plant species.

Key words: biocontrol; *Cynoglossum officinale*; *Hackelia micrantha*; herbivory; houndstongue; life table response experiment; matrix population models; *Mogulones crucifer*; *Mogulones cruciger*; nontarget impacts; target impacts; weed biocontrol.

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Introduction

Insect herbivores can have enormous influence on plant populations (Huntly 1991, Agrawal et al. 2012, Kim et al. 2013), however, the link between herbivory damage and plant population dynamics is complex, poorly understood, and difficult to predict (Crawley 1989, Halpern and Underwood 2006, Maron and Crone 2006). The occurrence of herbivory does not necessarily affect plant performance (Trumble et al. 1993), but when it does, impacts can be considered at the individual and population levels. First, impact on an individual plant's performance occurs when insect damage causes it to die or depresses its growth or reproduction. Second, individual impacts can collectively translate into herbivore-driven changes in plant abundance (i.e., population-level impacts) provided that (1) individual impacts are severe and widespread enough to affect demographic vital rates (survival, growth, reproduction) at the population scale, and (2) population growth rate (λ) is sensitive to changes in affected vital rates (Ehrlén et al. 2005). Depending on a plant's life history characteristics, λ will be differentially sensitive to changes in vital rates of various plant life stages (Franco and Silvertown 2004). For example, λ for short-lived plants tends to be vulnerable to changes in growth and reproduction, while λ of long-lived, iteroparous plants is generally responsive to mortality of established individuals (Silvertown et al. 1993, Lesica 1995). Characterizing and understanding changes in vital rates can be accomplished through collecting simple plant performance data, but predicting changes in λ requires knowledge and integration of a plant's complete life history into population growth models (Ehrlén 2003, Ehrlén et al. 2005).

Understanding and predicting the connection between herbivory impacts to individual plants and consequent reductions in plant abundance is directly relevant to classical biological control of invasive plants (henceforth referred to as 'weed biocontrol'). The goal of this applied ecological discipline is to use host-specific insect herbivores to sustainably suppress invasive plant populations to below ecologically and/or economically relevant threshold levels (McFadyen 1998, Hoddle 2004, Suckling 2013). However, quantitative evidence for the magnitudes and mechanisms of impact by released agents on their target weeds is scarce (Carson et al. 2008, Morin et al. 2009). Many postrelease impact studies have been observational and uncontrolled, where plants near releases were monitored without equivalent attention to control areas without agents (e.g., Hoffmann and Moran 1998, Grevstad 2006, Seastedt et al. 2007). Even for those impact studies that have involved controlled and replicated manipulation of insect density, posttreatment data collection has often been restricted to individual plant performance (e.g., Sheppard et al. 2001, Corn et al. 2006), or to phenomenological differences in plant density or cover (e.g., Butler et al. 2006, De Clerck-Floate and Wikeem 2009) without linking the changes in plant abundance to underlying vital rate driver. Experimental studies that measure aspects of both target weed performance and density come closer to uncovering mechanisms behind population-level impacts of agent exposure (e.g., Dhileepan 2001, Seastedt et al. 2003, Tipping et al. 2009) or lack of impacts (Garren and Strauss 2009, Ortega et al. 2012), but rarely empirically determine how observed changes in vital rates explain responses in plant population growth rate through population modeling. Experimental demography and population modeling reveal how vital rates affect population growth rate and combined with biological knowledge of a system, can uncover the mechanisms by which an agent affects (or fails to affect) plant populations (Kriticos 2003). To our knowledge, the only postrelease field-based experimental demographic study of an introduced specialist biocontrol insect on its invasive target weed is that by Dauer et al. (2012) on the *Jacobaea vulgaris* L. [=*Senecio jacobaea* L.] system.

Understanding the impact of released biocontrol agents at the plant population level is useful for assessing risks to nontarget species. Insects given regulatory approval for release in countries practicing classical weed biocontrol can often use nontarget plants phylogenetically related to their target weed host to some degree (Sheppard et al. 2005), particularly when the insects are at high densities (e.g., Baker et al. 2004, Dhileepan et al. 2006, Pratt et al. 2009). While minor feeding on individual plants of nontarget species classified as threatened or endangered (also known as 'at risk') may be legally relevant in some countries (Sheppard et al. 2003), ecological relevance occurs at the plant population level (Delfosse

2005, Suckling and Sforza 2014). In that regard, the suite of observational demographic studies showing population-level impact on native nontarget thistles from the seed-feeding biocontrol weevils *Rhinocyllus conicus* Frölich and *Larinus planus* Fabricius (Louda et al. 1997, 2005, Louda and O'Brien 2002, Rose et al. 2005, Havens et al. 2012) have been highly influential and set a new standard for future nontarget studies. However, we know of no experimental demographic studies involving biocontrol agents and nontarget plants.

We conducted a 3-yr manipulative factorial field experiment to assess herbivory impacts of a root-feeding biocontrol weevil *Mogulones crucifer* Pallas (=*Ceutorhynchus cruciger* Herbst, =*Mogulones cruciger* Herbst, Coleoptera:Curculionidae), on its target invasive host plant *Cynoglossum officinale* L. (Boraginaceae), and a native, nontarget host plant, *Hackelia micrantha* (Eastw.) J.L. Gentry (Boraginaceae). We released *M. crucifer* into naturally occurring patches of the nontarget plant in Canada growing interspersed or isolated from the target weed and compared the demographic response of target and nontarget plants on release sites to those on nonrelease (control) sites for two transition years after release. Herbivory patterns documented in this experiment indicated that *M. crucifer* fed on both plant species, but relative to the target weed, *H. micrantha* herbivory was localized (Catton et al. 2014), temporary, rare, mild, and driven by a spillover mechanism (Catton et al. 2015). Catton et al. (2015) therefore predicted that population-level impacts were unlikely for the nontarget plant, but noted that quantitative demographic investigation was needed. Theory predicts that the difference in relative preference of a biocontrol agent for its target and nontarget hosts may not translate into proportional impacts if the nontarget species is especially vulnerable (or robust) to the herbivory experienced (Holt and Hochberg 2001). Here, we use demographic data and population modeling to address these follow-up questions regarding target and nontarget impacts in this experiment. Specifically, we address: (1) Did the biocontrol insect cause population-level impacts to the target or nontarget plant, and if so, how? and (2) Did population-level impacts persist after the year of release? To our knowledge, this is the first experimental demographic study to simultaneously investigate the magnitudes and mechanisms of impact of a biocontrol agent on both its target weed (*C. officinale*) and a co-occurring native, nontarget plant (*H. micrantha*).

Methods

Study system

The system for the current study has previously been described (Catton et al. 2014, 2015), but relevant information is briefly summarized here for our focal species. *Cynoglossum officinale* (houndstongue) is a biennial or short-lived perennial Eurasian tap-rooted forb that has invaded disturbed grassland and forested habitats in North America. This weed is problematic to the cattle industry, primarily due to its toxicity and fur-clinging, burred nutlets (Upadhyaya et al. 1988). *Cynoglossum officinale* seeds germinate in the spring and overwinter as vegetative rosettes with taproots (De Jong and Klinkhamer 1988*a*). When a threshold size is reached (Wesselingh et al. 1997), in the second or later spring, plants flower (i.e., 'bolt') and produce adhesive burred nutlets for dispersal by epizoochory (De Clerck-Floate 1997). Flowering is usually fatal for *C. officinale*, but 2–45% of flowering *C. officinale* in the introduced range may survive to flower a second time (Williams 2009). *Cynoglossum officinale* reproduces by seed only and generally requires small-scale soil disturbances to establish in both its native and introduced ranges (Klinkhamer and De Jong 1988, Williams et al. 2010). The plant therefore has patchy distributions and exhibits metapopulation dynamics in both its native and introduced ranges (van der Meijden et al. 1992, DeClerck-Floate 1996).

The European root-feeding weevil *M. crucifer* damages *C. officinale* predominately through larval root-feeding (Prins et al. 1992), though adults feed on leaf blades, petioles, and bolting stems. Individual *C. officinale* plants can commonly contain >20 and occasionally >100 *M. crucifer* eggs or larvae (De Clerck-Floate and Schwarzländer 2002, Van Hezewijk et al. 2008, Catton et al. 2015). The weevil is functionally univoltine but can reach outbreak population densities within several generations when not limited by *C. officinale* availability (Schwarzlaender 1997, De Clerck-Floate et al. 2005). *Mogulones crucifer* is an

excellent example of a "search and destroy" biocontrol agent (Murdoch et al. 1985). Approved for release in Canada in 1997, the weevil has quickly suppressed populations of its target weed and dispersed to new infestations (De Clerck-Floate et al. 2005, De Clerck-Floate and Wikeem 2009). In some cases on rangeland in the interior of British Columbia, Canada, *M. crucifer* has eliminated local *C. officinale* within 2 yr after release when interacting with drought conditions (De Clerck-Floate et al. 2005, De Clerck-Floate and Wikeem 2009). In its native range, *M. crucifer* is known to decrease fecundity of *C. officinale* by 30–35%, but not affect mortality of vegetative rosettes (Prins et al. 1992, Williams et al. 2010). However, the fecundity impact observed in Europe is not sufficient itself to cause decreases in *C. officinale* populations in North America (Maron et al. 2010, Williams et al. 2010). In addition to controlling *C. officinale* in the introduced range, *M. crucifer* also sporadically feeds, oviposits, and develops in native nontarget Boraginaceae species in Canada (De Clerck-Floate and Schwarzländer 2002, Andreas et al. 2008). Nontarget use was expected for this oligophagous insect, as pre- and post-release host specificity tests revealed that the weevil's host range included several confamilial species, albeit both its preference and performance were much stronger for *C. officinale* (De Clerck-Floate and Schwarzländer 2002). Notably, the demographic consequences of *M. crucifer* herbivory have not been studied at the individual or population level for any nontarget species.

Hackelia micrantha (Boraginaceae), commonly known as 'blue stickseed', is a polycarpic taprooted perennial forb native to North America. *Hackelia micrantha* grows sympatrically with the confamilial, invasive *C. officinale* in semiforested rangeland in British Columbia, Alberta, and the western United States (Douglas et al. 1998), and is similar to *C. officinale* in shoot morphology and phenology, although is more long-lived. *Mogulones crucifer* accepts *H. micrantha* as a field host for feeding, oviposition, and larval development, but prefers the nontarget species to a lesser degree than *C. officinale*. Field surveys by Catton et al. (2015) found 17% of sampled *H. micrantha* were colonized with *M. crucifer* eggs or larvae compared with 83% of *C. officinale*, with 39 *M. crucifer* eggs or larvae in *C. officinale* for every one in *H. micrantha*. The maximum number of juvenile *M. crucifer* found in a *H. micrantha* plant was 10, compared to 116 for *C. officinale*. *Hackelia micrantha* supports complete development of *M. crucifer* (R. De Clerck-Floate and H. Catton, *unpublished manuscript*), but the weevil did not establish on release sites in this experiment where *C. officinale* was absent ("Target Rare" sites, see Methods: Rangeland release experiment; Catton et al. 2015). The global conservation status of *H. micrantha* is abundant and secure (NatureServe 2012).

Rangeland release experiment

A factorial field experiment using *M. crucifer* releases was conducted from 2009 to 2011 to characterize the demography of *C. officinale* and *H. micrantha* in the presence and absence of the biocontrol weevil. In 2008, 12 sites with naturally occurring patches of *H. micrantha* were located within a radius of 7.5 km under aspen *Populus tremuloides* Michx. canopy on grazed rangeland in the Foothills Fescue Natural Region in southern Alberta, Canada (Downing and Pettapiece 2006). Sites were ≥360 m apart and were separated by unshaded grassland (i.e., habitat not suitable for either study plant species), and showed no signs of *M. crucifer* presence prior to our releases. Each site contained 29–85 *H. micrantha* rosettes (vegetative) or bolting (reproductive) plants generally within a radius of 4–22 m (mean ± SD = 0.44 ± 0.49 *H. micrantha*/m^2, n = 12 sites). On six sites, herein referred to as "Target Common" sites, the *H. micrantha* plants were growing interspersed with 35–98 naturally occurring rosette or bolting *C. officinale* plants (0.26 ± 0.35 *C. officinale*/m^2, n = 6 sites). The other six sites were classified as "Target Rare" sites, and their low numbers of *C. officinale* (0–7 plants) within their evaluation radii were manually removed upon discovery, beginning in July 2009 until August 2011. "Target Common" and "Target Rare" sites were included in the experiment to test a separate question regarding the ability of *M. crucifer* to persist on *H. micrantha* in the absence of *C. officinale* (described in Catton et al. 2015), and are not directly relevant to the questions addressed in this manuscript. However, we deemed it necessary to analyze the impacts of *M. crucifer* releases on *H. micrantha* on "Target Common" and "Target Rare" sites separately because after 2 yr, *M. crucifer* populations had established on "Target Common" release sites,

but not on "Target Rare" release sites, indicating different weevil population histories (Catton et al. 2015).

On 4 June 2009, 300 *M. crucifer* were released at each of three "Target Common" and three "Target Rare" sites. These releases were each three times the size of those typically used in the *M. crucifer* biocontrol release program (De Clerck-Floate et al. 2005), and were meant to represent a local *M. crucifer* outbreak. Weevils used for the releases were overwintered, ovipositing adults collected from an outdoor rearing plot in southern Alberta and had an estimated female:male ratio of 2.2:1, based on a pooled subsample of 300 weevils. Weevils were released on the ground at a single marked point per site within 1 m of nontarget and target plants (when present), to force movement to host plants.

Fates of individual plants on all 12 sites were tracked by marking 1 × 1 m permanent quadrats around release points or, for nonrelease sites, central reference points. Where possible, we sampled 30 rosettes, 20 bolting plants, and 50 seedlings of each species as near to the release or reference point as possible. The radius sampled and number of quadrats laid per site varied with plant density (8–38 quadrats per site, total = 228 quadrats). *Hackelia micrantha* and *C. officinale* plants within quadrats were mapped to the nearest ~10 cm once per year during 3–26 July 2009 (4–7 weeks after release), 29 June–23 July 2010, and 5 July–3 August 2011. Plants of both species in each quadrat were assigned in each year to one of the following developmental stages: (1) seedling: a plant with cotyledons or ≤3 true leaves, (2) small rosette: a vegetative plant with 4–9 true leaves, with all leaves <30 cm long, (3) large rosette: a vegetative plant with ≥10 true leaves, or at least one leaf ≥30 cm long, or (4) bolter: a plant with ≥1 bolt (i.e., flowering shoot). Flowering *H. micrantha* were later classified as small bolters (1–2 bolts) and large bolters (≥3 bolts) due to high variability in seed production. By recording the exact plant locations within the permanent quadrats, survival and growth of individual small rosettes, large rosettes, and bolters could be followed up for the two transitions following *M. crucifer* release; 2009–2010 ("Year 0–1") and 2010–2011 ("Year 1–2"). Quadrat plants also were examined each year for visible indications of distinctive *M. crucifer* adult herbivory. Spatial and temporal patterns of *M. crucifer* herbivory on both plant species in this experiment are described in detail by Catton et al. (2014, 2015), but generally were widespread and persistent for *C. officinale* and limited to within 4.25 m of release points and to Year 0 for *H. micrantha* regardless of target plant density. This pattern indicates that the nontarget herbivory was driven by a spillover mechanism from the temporary outbreak densities of weevils onsite following releases (Catton et al. 2014, 2015).

Fecundity of all reproductive plants was estimated in each year. Both plant species produce multiple bolts per plant, each with many flowers. Each flower produces a maximum of four nutlets (i.e., a tetrad). The number of tetrads per bolt was counted once each year when seed set of both species was complete (July/August 2009–2011). Between 43 and 50% of *H. micrantha* bolts and 4–13% of *C. officinale* bolts were <75% intact because of trampling damage or seed removal by grazing cattle. Based on our experimental questions regarding the impacts of the biocontrol weevil on plant population dynamics, we estimated the biological potential for plants to produce seeds, disregarding trampling damage. When bolts were ≥75% intact, we obtained an exact count of the number of tetrads that appeared to contain ≥1 viable nutlet. When bolts were not intact, the number of tetrads per bolt was estimated for each year with the best available information, in the following order; site-specific or all-sites regressions between bolt height and tetrad count, or site-specific or all-sites average tetrad count per bolt (Appendix S1). Total viable seed counts per bolt per year were calculated by multiplying the number of tetrads by the average number of viable nutlets per tetrad for each species (*C. officinale* mean = 2.60, n = 484 tetrads; *H. micrantha* mean = 2.16, n = 2209 tetrads). These values were calculated from a subsample of inflorescence branches destructively sampled from quadrat plants between 28 July and 6 August 2010 (25 *C. officinale* from five study sites and 93 *H. micrantha* from 11 study sites).

Construction and analysis of matrix population models

Stage-based transition matrices were developed by pooling plants in each treatment (release or nonrelease), transition (Year 0–1 or Year 1–2), and species (i.e., *C. officinale* on "Target Common" sites, *H. micrantha* on "Target Common" sites,

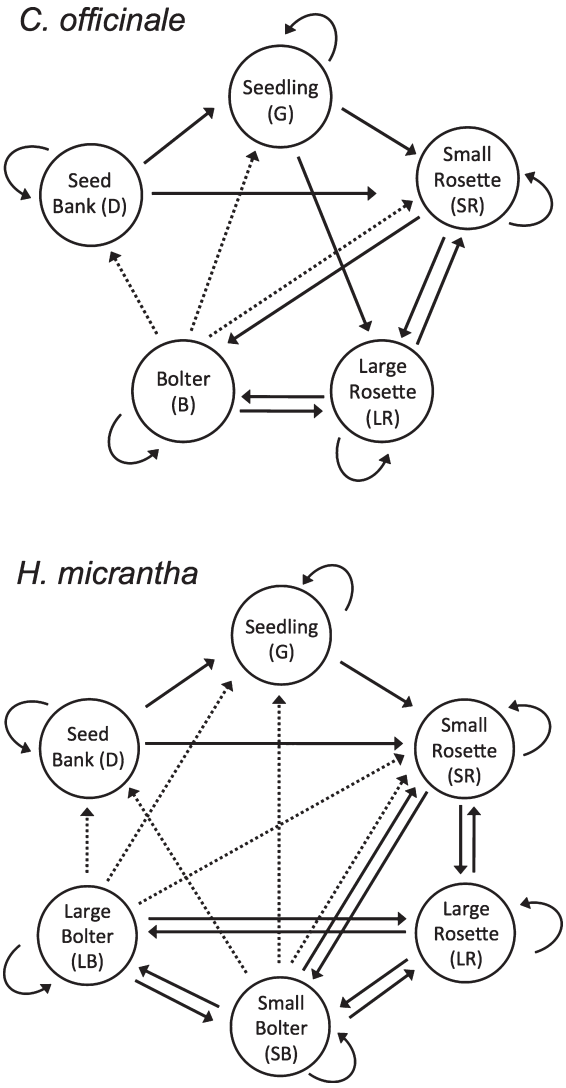

Fig. 1. Life cycle diagrams for *C. officinale* and *H. micrantha.* Arrows represent the possible fates of surviving plants in each life stage from 1 yr to the next. Death can occur at any stage. Dotted lines represent transitions for newly produced seed.

or *H. micrantha* on "Target Rare" sites) combination, according to the life history models formulated from our observations in the field (Figs. 1 and 2, Table 1). Plants were pooled among the three sites within each treatment category to mitigate the effects of small sample sizes for some stages (occasionally ≤5 plants across all three sites, Appendix S2), following Horvitz and Schemske (1995) and Rooney and Gross (2003). Rates of rosette and bolter transitions between stages were separated into binomial probabilities (e.g., survival, growth or transitioning given survival, bolting given transitioning, etc.) and parameterized using overall pooled proportions in the first year of each transition (Morris and Doak 2002). Fecundity values were calculated using a generalized linear model (GLM) with Poisson errors and a log link function. Sample sizes for each stage in each matrix are detailed in Appendix S2. Seed survival, germination and dormancy, and seedling survival vital rates were parameterized from a separate seedling emergence field experiment in our study area (detailed in Appendix S3), and the same set of these values was used in all matrices for each species. The only seedling vital rate that could be calculated from the rangeland release experiment was seedling to rosette growth.

Density-dependence was not included in the matrix models to conserve the ability to analyze models analytically, and because excluding intraspecific density-dependence from models may be appropriate in situations where interspecific competition exists as it did on our natural rangeland study sites (Crone et al. 2011). However, the possibility of intraspecific density-dependence in the early life stage vital rates of seedling and rosette survival and growth were evaluated and judged to be likely inconsequential to the comparisons between release and nonrelease sites (Appendix S4).

The dominant eigenvalues of the transition matrices represent the projected stable population growth rate of a population under consistent conditions (λ, Caswell 2001), and were calculated using the package 'popbio' in R v2.15.2 (Stubben and Milligan 2007, R Development Core Team 2014). For each vital rate and for λ, 95% bootstrap confidence intervals (CI) were determined as the 2.5% and 97.5% quantiles from a distribution of re-calculated values derived from re-sampling plant fates with replacement within plant life stages, holding sample sizes constant, for 10,000 iterations (Caswell 2001).

The likelihood that the observed differences in matrix vital rates and λ between treatments (release vs. nonrelease) or transition years occurred by chance was assessed using randomization tests (Caswell 2001). Observed differences in λ and vital rates between categories were compared with null distributions generated from 10,000 random permutations of individuals between the matrices

C. officinale

	Seed Bank (D)	Seedling (G)	Small Rosette (SR)	Large Rosette (LR)	Bolter (B)
Seed Bank (D)	sD (1−gD)	0	0	0	F sF hF (1−gF)
Seedling (G)	sD gD (1−jD)	sG (1−gG)	0	0	F sF hF gF (1−jF)
Small Rosette (SR)	sD gD jD	sG gG (1−lG)	sSR (1−gSR)	sLR gLR (1−tLR)	F sF hF gF jF
Large Rosette (LR)	0	sG gG lG	sSR gSR (1−bSR)	sLR (1−tLR)	sB tB
Bolter (B)	0	0	sSR gSR bSR	sLR tLR bLR	sB (1−tB)

H. micrantha

	Seed Bank (D)	Seedling (G)	Small Rosette (SR)	Large Rosette (LR)	Small Bolter (SB)	Large Bolter (LB)
Seed Bank (D)	sD (1−gD)	0	0	0	FSB sF hF (1−gF)	FLB sF hF (1−gF)
Seedling (G)	sD gD (1−j)	sG (1−gG)	0	0	FSB sF hF gF (1−j)	FLB sF hF gF (1−j)
Small Rosette (SR)	sD gD j	sG gG(1−lG)	sSR (1−gSR)	sLR tLR (1−bLR)	sSB tSB (1−bSB) (1−lSB) + FSB sF hF gF j	FLB sF hF gF j
Large Rosette (LR)	0	sG gG lG	sSR gSR (1−bSR)	sLR (1−tLR)	sSB tSB (1−bSB) lSB	sLB tLB (1−bLB)
Small Bolter (SB)	0	0	sSR gSR bSR	sLR tLR bLR (1−lLR)	sSB (1−tSB)	sLB tLB bLB
Large Bolter (LB)	0	0	0	sLR tLR bLR lLR	sSB tSB bSB	sLB (1−tLB)

Fig. 2. Transition matrix frameworks for *C. officinale* and *H. micrantha.* Abbreviated parameters are defined in Table 1.

being tested, keeping sample sizes for each life stage constant with the observed data. *P*-values were calculated as the proportion of the random permutations that were greater (one-tailed) or more extreme (two-tailed) than the observed difference, and were compared to the predetermined level for statistical significance of $\alpha = 0.05$. We used one-tailed tests when testing for a treatment effect within transition years for λ under the alternate hypothesis that *M. crucifer* releases would reduce λ, and two-tailed tests for the transition year effect within treatments for λ and for all vital rate comparisons because expected effects for these comparisons were not intuitive. The null distribution for the effects of treatment within transition year was generated by randomly permuting individuals across release and nonrelease sites within transition years. Similarly, the null distribution for the effect of transition year within treatment was generated by randomly permuting individuals across transition years within treatments. Interactions were tested by comparing the observed standard deviation of the differences in parameters between transition years (i.e., slopes) within treatments to the null distribution of standard deviations generated from the random permutations (Caswell 2001).

Table 1. Symbols and definitions of vital rates used to construct *C. officinale* and *H. micrantha* transition matrices (displayed in Fig. 2).

Symbol	Definition
Both plant species	
sF†	Survival of viable new seed
hF‡	Viable new seed lands on suitable habitat, given survival
gF†	Viable new seed germinates, given survival and suitable habitat
sD†	Survival of seed in seed bank
gD†	Seed in seed bank germinates, given survival
j‡	Seed grows to SR (i.e., skipping G), given survival and germination
sG†	Survival of G
gG†	G grows, given survival
lG	G becomes LR, given survival and growth
sSR	Survival of SR
gSR	SR grows, given survival
bSR	SR bolts, given survival and growth
sLR	Survival of LR
tLR	LR transitions, given survival
bLR	LR bolts, given survival and transitioning
C. officinale	
sB	Survival of B
tB	B becomes LR, given survival
F	Number of viable seeds produced
H. micrantha	
lLR	LR becomes LB, given survival, transitioning, and bolting
sSB	Survival of SB
tSB	SB transitions, given survival
bSB	SB bolts, given survival and transitioning (i.e., becomes LB)
lSB	SB becomes LR, given survival, transitioning, and nonbolting
FSB	Number of viable seeds produced by SB
sLB	Survival of LB
tLB	LB transitions, given survival
bLB	LB bolts, given survival and transitioning (i.e., becomes SB)
FLB	Number of viable seeds produced by LB

Notes: All definitions refer to proportions (= probabilities), except fecundities, which are counts. Life stage abbreviations (in capital letters) are as in Figs. 1 and 2. F = viable new seed.

†Parameter value determined from a separate seedling emergence experiment (see Appendix S3).

‡Parameter value assigned arbitrarily.

We conducted a retrospective analysis of how each vital rate contributed to differences in λ in each comparison using a modified life table response experiment (LTRE) as described by Elderd and Doak (2006):

$$\lambda^t - \lambda^r = \sum_{m,n} \sum_{p} \left(v_p^t - v_p^r \right) \frac{\partial \lambda}{\partial a_{m,n}} \frac{\partial a_{m,n}}{\partial v_p} \Big|_{\frac{1}{2}(\mathbf{A}^t + \mathbf{A}^r)}$$

where λ^t and λ^r are the treatment (release) and reference (nonrelease) population growth rates, respectively, a is the matrix element, m and n are the row and column, v_p is an individual vital rate, p is the number of vital rates, $\mathbf{A}^t$ and $\mathbf{A}^r$ are the treatment and reference transition matrices, respectively.

Confidence intervals and the probability of a value occurring under the null hypothesis of equal populations (P-values) for LTRE contributions were calculated using identical methods to those for vital rates and λ, using two-tailed tests. Interactions between treatment and transition year effects for each vital rate contribution were tested as the probability of the observed difference between transition years occurring given the generated null distribution. We did not conduct prospective analyses such as elasticity calculations because these analyses are only valid for very small hypothetical perturbations in vital rates, while our interest lay in retrospectively identifying and understanding the observed demographic changes in our experiment (e.g., Bruna and Oli 2005).

Finally, because nontarget herbivory was highly aggregated (95% of *M. crucifer* nontarget herbivory was limited to Year 0 and to <4.25 m from release points, Catton et al. 2014, 2015), we performed an additional, more conservative analysis testing for potential population-level impacts to *H. micrantha* near release points. Additional *H. micrantha* matrices for each transition year were calculated using plants pooled from all release sites either within or outside the 4.25 m radius from release points. Due to the fine-scale nature of this within-site spatial analysis, five *H. micrantha* plants >15 m from release points were excluded from analysis as distance outliers, and three very large *H. micrantha* with extremely high seed counts that all by chance occurred >4.25 m from release points were excluded from analysis as fecundity outliers. These outlier plants were removed only from the within-patch spatial analysis.

Results

Cynoglossum officinale *dynamics*

Population growth rate for the target plant, *C. officinale*, ranged from 0.807 to 1.138, with the only λ value below the replacement rate of 1.0 occurring on release sites in Year 0–1

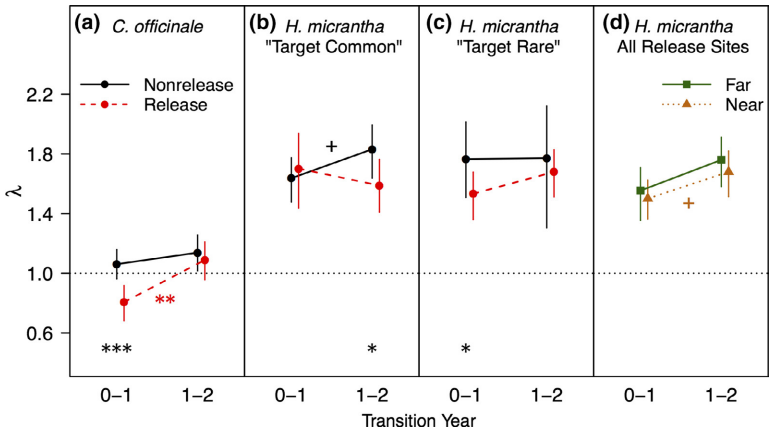

Fig. 3. Population growth rate (λ) for (a) *C. officinale* and (b, c) *H. micrantha* on *M. crucifer* release and nonrelease sites in transitions Year 0–1 and Year 1–2. λ values in (a–c) were calculated using matrices constructed from *C. officinale* or *H. micrantha* plants pooled from three sites per category. Values in (d) were calculated from pooled *H. micrantha* plants near (≤4.25 m) or far (>4.25 m) from release points on all six release sites. Values above the dotted reference line of λ=1.0 represent projected population growth, while values below the line represent projected population shrinkage. Bars represent 95% bootstrap CIs. Statistically significant differences calculated by randomization tests are indicated by the symbols: *** = $P < 0.001$, ** = $0.001 < P \leq 0.010$, * $0.010 < P \leq 0.050$, + = $0.050 < P \leq 0.100$. Symbols for the effect of treatment (release or nonrelease) within transition year are immediately above transition year numbers, and symbols for the effect of transition year within treatment are near the lines connecting the points. There were no significant interactions of transition year × treatment.

(Fig. 3a). In that transition year, λ on release sites was significantly lower than on nonrelease sites, but just one transition year later, λ from both treatments were above 1.0 and indistinguishable. The impact on λ of transition year within treatment was significant for release sites and not nonrelease sites (i.e., slopes in Fig. 3a), but the interaction between treatment and transition year (i.e., the difference in slopes) was not statistically significant ($P = 0.104$).

LTRE analysis identified several *C. officinale* vital rates that made contributions to differences in λ on release relative to nonrelease sites that were unlikely to be due to chance, in particular, survival of small and large rosettes (Fig. 4). A significant contribution means that there was a sufficiently nonrandom distribution of plant fates and their impacts between release and nonrelease sites. It should be noted that significance of each vital rate contribution is measured in response to its own null distribution rather than the magnitudes of other vital rates. Therefore, significant observed contributions needed not be overly large (e.g., sSB in Fig. 5a), while large observed contributions could be indistinguishable from randomly generated values (e.g., F in Fig. 4). For *C. officinale* on release relative to nonrelease sites in Year 0–1, the sum of significant contributions was sufficient to cause a significant decrease in λ and a shift from positive population growth to negative growth (Fig. 4, Table 2). Therefore, we can be confident that the difference in λ observed in this comparison is a nonrandom treatment effect resulting from decreased rosette survival. This same effect was not observed in Year 1–2. Significant interactions between how contributions to differences in λ between release relative to nonrelease sites differed between the transition years (i.e., differences in the heights of gray and black bars for each vital rate in Fig. 4) demonstrate that the contributions of small and large rosette survival were significantly more negative in Year 0–1 than Year 1–2 ($P \leq 0.035$), and nearly significant for transitioning of surviving large rosettes ($P = 0.053$). Interactions were calculated based on their own null distributions generated from subtracting Year 0–1 permutation contributions from Year 1–2 permutation contributions and therefore did not always correspond

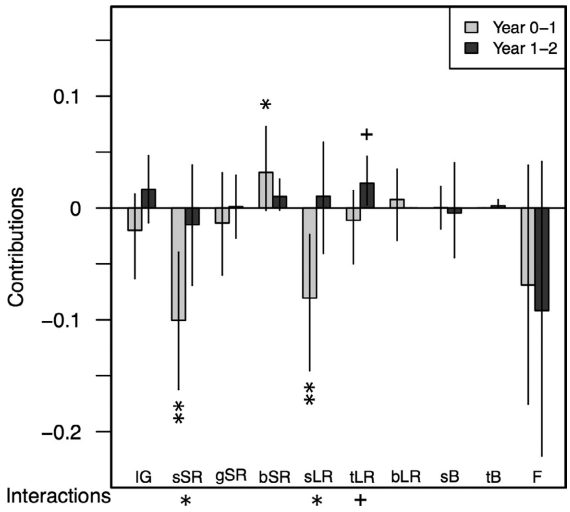

Fig. 4. Contributions of differences in *C. officinale* vital rates to differences in λ on *M. crucifer* release sites relative to nonrelease sites within transitions Year 0–1 and Year 1–2. Bar heights and error bars represent life table response experiment values for the observed data and their 95% bootstrap CIs. Vital rates with contributions significantly different than zero according to randomization tests are indicated by the symbols: *** = $P < 0.001$, ** = $0.001 < P \leq 0.010$, * = $0.010 < P \leq 0.050$, + = $0.050 < P \leq 0.100$. Symbols for significant interactions are immediately below the panel.

with significance or nonsignificance in annual vital rate contributions.

Raw vital rate values and comparisons are presented in Appendix S5. LTREs for the transition year effect within treatments yielded very similar results to the treatment effect within transition year, and are presented in Appendix S6. Number of plants for each species and site category per year are presented in Appendix S7.

Hackelia micrantha *dynamics*

Nontarget λ values ranged from 1.533 to 1.831, with all predicting positive long-term population growth ($\lambda > 1.0$, Fig. 3b,c). Upon returning to several release sites 8 d after release, we observed some individual impacts of reduced fecundity on *H. micrantha* as some bolting stems within 3 m of release points were girdled by adult *M. crucifer* feeding damage, preventing seed development on those stems. However, these individual impacts did not translate to the population level as the comparison of *H. micrantha* plants from all *M. crucifer* release sites within or outside 4.25 m of release points indicated no significant differences in λ in treatment or transition year (Fig. 3d).

The only significant effects of treatment (i.e., release vs. nonrelease) within transition year on λ occurred on "Target Common" sites in Year 1–2, and on "Target Rare" sites in Year 0–1 (Fig. 3b, c, Table 2), although all *H. micrantha* λ (and 95% CIs) were still well above 1.0. LTRE analysis for the two significant comparisons demonstrated that differences in several vital rates made significant contributions to differences in λ observed (Fig. 5). These contributions mostly involved small bolters, but were insufficient to account for significant decreases in λ (Table 2). In other words, we cannot be confident that the observed difference in *H. micrantha* λ between release and nonrelease sites in the two comparisons in question did not occur simply by chance.

Discussion

The analyses and results presented here demonstrate the value of performing demography experiments to examine both the magnitude of and mechanisms behind target and nontarget population-level impacts in weed biocontrol. As a "search and destroy" agent (Murdoch et al. 1985) that also happens to be oligophagous, *M. crucifer* presents a rich opportunity to study context-specific impacts of herbivory on populations of both its preferred and target host *C. officinale* and the novel nontarget host, *H. micrantha*. Furthermore, as opposed to the seed-feeding agents used in earlier nontarget studies (e.g., *R. conicus* in Louda et al. 1997), *M. crucifer* feeds on both root and shoot tissue of its target and nontarget hosts (Catton et al. 2015, R. De Clerck-Floate and H. Catton, *unpublished manuscript*). Damage to conductive and storage tissues can reduce the vital rates of all plant life stages (Hunter 2001), therefore the experimental demographic approach used here was necessary to uncover the magnitude and mechanism of any *M. crucifer* impact on population growth rates. The data demonstrate that if the most extreme (i.e., Year 0) *M. crucifer* densities in this study were to persist for many years, vital rate impacts on release sites would cause the *C. officinale* populations to eventually

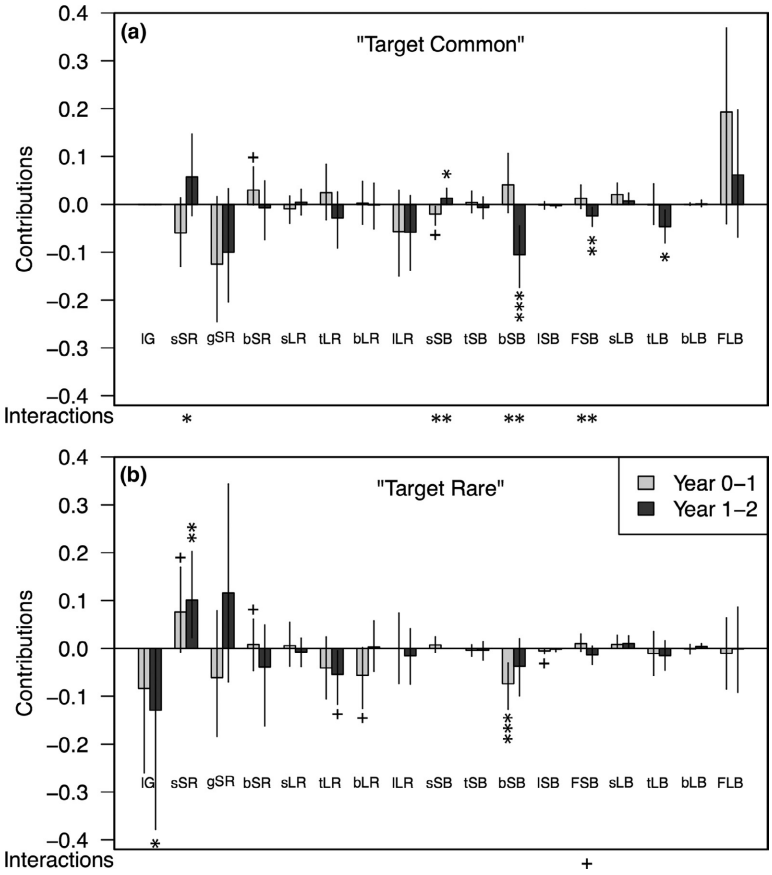

Fig. 5. Contributions of differences in *H. micrantha* vital rates to differences in λ on *M. crucifer* release sites relative to nonrelease sites within transitions Year 0–1 and Year 1–2 on (a) "Target Common" and (b) "Target Rare" sites. Bar heights and error bars represent life table response experiment values for the observed data and their 95% bootstrap CIs. Vital rates with contributions significantly different than zero according to randomization tests are indicated by the symbols: *** = $P < 0.001$, ** = $0.001 < P \leq 0.010$, * = $0.010 < P \leq 0.050$, + = $0.050 < P \leq 0.100$. Symbols for significant interactions are immediately below each panel.

decline to extirpation ($\lambda < 1.0$), while the *H. micrantha* populations would continue to grow ($\lambda > 1.4$). By determining which vital rates contributed most to differences in both target and nontarget λ between *M. crucifer* release and nonrelease sites, we can infer from our biological knowledge of the herbivore and its host plants which vital rate impacts can reasonably be attributed to damage caused by the insect. In the following sections, we interpret the quantitative demographic impacts observed in context with the known biology of this system. Such an interpretation is a crucial step for connecting mechanism with effect, for gaining insight into differences in the agent–target weed interaction between the native and introduced ranges, and for understanding the nature of the herbivore–plant interactions in this biocontrol system.

Impact of M. crucifer *on* C. officinale

The matrix models indicated that *C. officinale* λ values always projected positive population growth ($\lambda > 1.0$), except on *M. crucifer* release sites in Year 0–1. In that "outbreak" transition year, λ on release sites was significantly lower than on nonrelease sites, a difference demonstrated by the LTRE to be driven by decreases in survival of both small and large rosettes. Because >90% of flowering *C. officinale* plants die after seed production, and the seed bank for this species lasts only 1–2 years (Van Breemen

Table 2. Observed and hypothetical λ for *C. officinale* and *H. micrantha* on *M. crucifer* release and nonrelease sites within transitions Year 0–1 and Year 1–2.

Year	Observed λ_{nrel}	Observed λ_{rel}	Observed λ_{rel}–λ_{nrel}	P_{obs}	λ_{diff} at $P = 0.05$	$\sum$ significant contributions	P_{sig}	λ_{hrel}
C. officinale								
Year 0–1	1.061	0.807	−0.255	<0.001	−0.135	−0.149	0.031	0.912
Year 1–2	1.138	1.089	−0.049	0.336	−0.168	0	0.516	1.138
H. micrantha "Target Common"								
Year 0–1	1.637	1.701	0.064	0.618	−0.300	0	0.490	1.637
Year 1–2	1.831	1.588	−0.243	0.037	−0.227	−0.163	0.121	1.668
H. micrantha "Target Rare"								
Year 0–1	1.765	1.533	−0.232	0.044	−0.223	−0.074	0.282	1.691
Year 1–2	1.772	1.679	−0.092	0.290	−0.267	−0.028	0.420	1.744

Notes: Reported for each transition year are observed λ for release (λ_{rel}) and nonrelease (λ_{nrel}) sites, the probability of the observed difference in λ between release and nonrelease sites occurring if sites were identical according to randomization tests (P_{obs}), the difference in λ necessary for statistical significance (λ_{diff} at $P = 0.05$), the sum of the significant vital rate contributions from the life table response experiment (see Figs. 4 and 5), the probability of the difference in λ derived from only significant vital rate contributions occurring if sites were identical (P_{sig}), and the hypothetical new λ_{rel} (=λ_{hrel}) calculated by discounting λ_{nrel} by significant vital rate contributions.

1984, Williams et al. 2010), the target weed population is highly dependent on the survival and growth of rosettes for seed supply from year to year. *Cynoglossum officinale* rosette mortality can be linked to *M. crucifer* as target plants sampled from 10 sites in our study area 0–4 years after weevil release (including the "Target Common" sites 2 yr after release) had a high probability of containing eggs or larvae (Catton et al. 2015). Even very small *C. officinale* rosettes with root crown diameters of <5 mm contained between 1 and 9 *M. crucifer* eggs or larvae more than half the time (Catton et al. 2015), which would severely reduce the availability of connective tissue between root and shoot in these small plants. Similarly, the congeneric root crown weevil *Mogulones larvatus* Goeze, released as a biocontrol agent in Australia against *Echium plantagineum* L. (Boraginaceae) also decreased survival rates of its target weed rosettes (Sheppard et al. 2001).

Interestingly, our experiment revealed a key difference in the *M. crucifer*–*C. officinale* interaction between its native and introduced ranges. Observational and experimental impact studies of the *M. crucifer*–*C. officinale* interaction in Europe demonstrated a significant decrease of 30–35% in plant fecundity, but no effect of the weevil on rosette mortality (Prins et al. 1992, Williams et al. 2010). The strong impact on rosette survival observed in our study may explain why *M. crucifer* has been an effective biocontrol insect for *C. officinale* in Canada. The quick 2-yr collapse of *C. officinale* populations after release observed by De Clerck-Floate and Wikeem (2009) occurred in too short of a time frame for differences in fecundity alone to impact plant population abundance, although in that study, weevil effects were interacting with drought conditions. Fecundity values for *C. officinale* on our release sites were not significantly lower than on nonrelease sites; however, we suspect our experiment may have underestimated the impact of *M. crucifer* on this target weed vital rate. A single, within-season insect release may not approximate the cumulative impact on *C. officinale* of mild *M. crucifer* root-feeding over multiple years that is common in the native range (Schwarzlaender 1997), and is likely to occur in the introduced range when and where *M. crucifer* are at low densities prior to population explosions. Further study for a longer time period with sub-outbreak *M. crucifer* densities would better determine any fecundity impact on *C. officinale* population growth rates with and without *M. crucifer* in the introduced range. Ironically, the main limitation to conducting such an experiment is the difficulty in finding robust *C. officinale* populations multiple years after *M. crucifer* release in Canada (R. De Clerck-Floate, *personal observation*), as rapid local weed extirpation is the usual outcome of weevil introduction.

It should be noted that the impacts discussed above were only observed in the transition immediately following release (Year 0–1). Just

one transition year later, *C. officinale* λ values were nearly identical on release and nonrelease sites, driven mainly by recovery in rosette survival and growth on release sites, and 2 yr after release, absolute numbers of small rosettes rebounded to prerelease numbers (Appendix S7). This recovery may be the result of two factors: (1) site-level changes in weevil density between the years, and (2) moisture availability. Our releases of 300 weevils per site were three times higher than the standard release density for *M. crucifer* biological control (De Clerck-Floate et al. 2005, De Clerck-Floate and Wikeem 2009), and likely represented a temporary "outbreak" of biocontrol agents that could not be sustained by the *C. officinale* available onsite. Weevil density was not measured over the 3 yr in this study, however, a drop in site-level agent density after release can be inferred because the proportion of *C. officinale* with visible *M. crucifer* adult feeding damage dropped significantly 1 yr after release (Catton et al. 2015). *Cynoglossum officinale* has a short-term seed bank (1–2 yr, Van Breemen 1984, Williams et al. 2010), therefore in this experiment new rosettes could still appear from older seed in Years 1 and 2 after the temporary weevil outbreak. However, in situations where *C. officinale* populations are sufficient to support local *M. crucifer* outbreaks for multiple years, sustained reduction of rosette survival would deplete the seed bank, thus leading to a reduction in compensatory target weed recruitment over time. In terms of climate, Year 1–2 was a better transition year for multiple *C. officinale* vital rates on nonrelease sites (Appendix S5), including small rosette growth and bolting probability of large rosettes. While Year 0 (2009) had an average amount of spring and summer precipitation, Year 1 was more than twice as wet in the study region (Appendix S8). *Cynoglossum officinale* is water-limited, especially early in its life cycle (De Jong and Klinkhamer 1988*b*). If moisture availability in the first year of a transition is more influential on a vital rate than in the second year, then Year 1–2 *C. officinale* plants could have benefited from the extra precipitation in Year 1. The stress imposed on plants by root-feeding may be increased with decreasing moisture availability (Blossey and Hunt-Joshi 2003).

Impact of M. crucifer *on* H. micrantha

In contrast with *C. officinale*, all calculated λ's for *H. micrantha* were well above 1.0, projecting positive population growth under all observed circumstances. This result is logical given that this plant is polycarpic and survival of reproductive individuals was very high, between 80 and 100%. Unlike *C. officinale*, reproductive *H. micrantha* create new plants without being lost from the population themselves, resulting in higher λ values in the nontarget plant. Most λ comparisons for *H. micrantha* showed no significant differences, including plants on release sites within and outside of the 4.25 m radius where 95% of nontarget herbivory was observed (Fig. 3d). The data show no evidence that *H. micrantha* populations will decline from *M. crucifer* herbivory, regardless of the weevil outbreak conditions of the release year or the distance from release points. The only significant differences between *H. micrantha* release and nonrelease site λ's were on "Target Common" sites in Year 1–2 and "Target Rare" sites in Year 0–1, but all λ's were still well above 1.0, and magnitudes of significant vital rate contributions were not sufficient to explain the differences in λ observed. Therefore, the differences *H. micrantha* in λ values cannot be distinguished from simple random variation.

We conclude from the combination of the results presented here that there is no conclusive evidence that *H. micrantha* suffered any reduction in λ or threat to population persistence in response to *M. crucifer* releases in this experiment. Theory predicts that if impact of target and nontarget herbivory at the individual plant level is equal and neither species experiences refuges from herbivory, nontarget plant populations with a high ratio of λ to herbivory rate are likely to persist (Holt and Hochberg 2001). *Hackelia micrantha* displayed this robustness with a higher λ and a much lower herbivory rate than *C. officinale* (Catton et al. 2015). The nontarget plant further benefitted from spatial, temporal, and probabilistic refuges from *M. crucifer* herbivory (Catton et al. 2014, 2015), which served to 'dilute' the influence of any individual impacts on population-level *H. micrantha* vital rates and population growth rates. Therefore, even as local *M. crucifer* and *C. officinale* populations likely boom and bust around them (Catton et al. 2015), *H. micrantha* populations are able to withstand the occurrence of incidental nontarget herbivory resulting from weevil outbreaks. However, care

should be taken when extrapolating these results to other regions or other nontarget species. For example, plants may be more vulnerable to the effects of root-feeding under dry conditions (Blossey and Hunt-Joshi 2003). And, while it is tempting to extrapolate our results to other nontarget species that may be used by *M. cruciferer*, it must be kept in mind that even congeners can display very different population dynamics (Buckley et al. 2010).

The results of this study provide quantitative support for the idea that herbivory can, but does not always, lead to population-level impacts to plants (Crawley 1989, Maron and Crone 2006). Therefore, herbivory on individual target and nontarget plants in weed biocontrol should not be assumed to necessarily cause population-level impacts without further examination of herbivory patterns in conjunction with individual impacts, plant life histories, vital rates, and population growth rates. Experimental demographic postrelease field studies such as this one are imperative for simultaneously evaluating population-level impacts of oligophagous weed biocontrol agents on target and nontarget plants. Such knowledge can help decision-makers evaluate future potential agents with a balance of risk and benefit in mind.

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Supporting Information

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.1280/supinfo

Data Availability

Data associated with this paper have been deposited in Dryad: http://dx.doi.org/10.5061/dryad.p3fg9