ABSTRACT

Title: ECOLOGICAL EFFECTS OF THE
BIOCONTROL INSECTS, *LARINUS PLANUS*
AND *RHINOCYLLUS CONICUS*, ON NATIVE
THISTLES.

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Doctor of Philosophy, 2005

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Biological control of invasive weeds is, by nature, a delicate balance between introducing effective biological control agents and not introducing another invasive species. A disconcertingly similar suite of traits is used to describe invasive insect species and to identify appropriate biological control agents (or candidates): good control agents and invasive exotic species are good dispersers, they are good colonizers, they have high reproduction rates, and they are suited for broad distribution. It shouldn't come as a surprise that two previously released weed biological control agents can now be characterized as invasive species. The purpose of this dissertation is twofold: first, it is to explore the ecological relationship between predispersal seed predation and plant population dynamics, and second, it is to elucidate the risks to native plants involved with introduction and redistribution of exotic species.

Rhinocyllus conicus and *Larinus planus* are Eurasian seed-head weevils, introduced and redistributed broadly across the western U.S. to control exotic thistles.

Exclusion experiments on native thistles, including one that is rare and imperiled, at sites in Colorado present strong evidence that a decrease in seed production due to herbivory by both *R. conicus* and *L. planus* has lead to a reduction in recruitment of the thistles. The density of seedlings in both cases, even in the excluded units, was far below where density dependent effects may play a role in the dynamics of the thistle. Further, a survey of eight western states demonstrates established populations of *L. planus* and effects on seed production in multiple native species in four states. It also documents the near ubiquity and broad diet breadth of *R. conicus*.

The process for approval of phytophagous biocontrol agents has become more cautious and more efforts are made to prevent nontarget herbivory. Nevertheless, land managers still routinely redistribute previously approved, non-regulated agent insects that appear to pose a higher risk to the native flora. The results of this research will benefit resource managers who wish to consider use of phytophagous insects as biological control agents as well as help ecologists and environmental managers understand the risk probabilities of biological control applications.

ECOLOGICAL EFFECTS OF THE BIOCONTROL INSECTS, *LARINUS PLANUS* AND *RHINOCYLLUS CONICUS*, ON NATIVE THISTLES

By

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Dissertation submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
2005

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Dedication

For my wife, Janette.

Acknowledgements

I first and foremost greatly appreciate generous guidance and insight from my advisor and mentor David Inouye and my committee members Doug Gill, Michele Dudash, Irv Forseth, and Bob Denno. Thank you for your support, humor, intellect, and passion for teaching and research. I am also absolutely indebted to Svata Louda whose inspiring research, insight, collaboration, and most generous support were invaluable to this project. I would also like to extend heartfelt thanks to Aaron Inouye, Rebecca Aicher, Cameron Pedersen, Noam Perlmann, Amy Goodstine, Kathy Darrow, Heidi Wenger, Georgia Dendrinos, and Judy Che for their help in the field. David Obenshain, Christopher Cowles, Ben Schapiro, Katryana Hanley-Knutson, Jon Krochmal, Matt Johnson, Emily Hawkins, Kyle Vallecillo, Rubina Rahman, and Silvia Lara were of great assistance in the lab.

Eric Coombs from the Oregon Department of Agriculture was of tremendous assistance in conducting and preparing the manuscript for the survey. Site access and permitting was facilitated by Dan Roddy, Ed Delaney, Marie Curtin, Myron Chase, and Ken Stahlnecker of the National Park Service; Gina Ramos and Bob Troiano of the Bureau of Land Management; Liz Hebertson, Sandy Kominsky, and Gay Austin of the Forest Service; Carey Goss of the Fish and Wildlife Service; and Al Cofrancesco from the U.S. Army Corps of Engineers.

Finally, I owe multitudes of thanks to my many friends at the Rocky Mountain Biological Laboratory and University of Maryland.

Funding was provided by the National Science Foundation, the University of Maryland Center for Biodiversity, and the National Park Service. Gracious logistical support was provided by the Rocky Mountain Biological Laboratory and the University of Maryland Department of Biology.

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Chapter 1: Colonization of and herbivory on native thistles by introduced biocontrol agents *Larinus planus* and *Rhinocyllus conicus*

ABSTRACT

Understanding the risks of introducing phytophagous species as biocontrol agents should be of paramount concern to land managers prior to introducing or redistributing exotic insects. The Eurasian weevil *Larinus planus* has been redistributed extensively throughout the western United States for biological control of an exotic weed, *Cirsium arvense*. From 2001 - 2004, we surveyed 72 *L. planus* release sites in nine states to investigate establishment and nontarget herbivory of exotic weevils on native thistle (*Cirsium*) species. *Larinus planus* was found on four native taxa, occurring at 67% of the sites where it had also established on the target weed species. Unexpectedly, *Rhinocyllus conicus*, another introduced Eurasian weevil, had colonized all but one of the native *Cirsium* taxa encountered and was present at 92% of the *Larinus* release sites. Seed production was reduced more than 50% in plants that hosted the weevils. We found no indication of refugia for native taxa in small or isolated populations. We conclude that *L. planus* establishment and widespread dispersal of *R. conicus* pose a considerable threat to native thistles. The results provide further evidence for risks involved with releasing insects as biocontrol agents against weedy species that have related species in the indigenous flora.

INTRODUCTION

Introduction of non-native phytophagous species as classical biocontrol agents of weeds entails potential risk of herbivory on nontarget species (Simberloff and Stiling 1996, Louda et al. 1997, Strong 1997, Louda 2000, Strong and Pemberton 2000, Louda et al. 2003). The process for approval of phytophagous biocontrol agents has become more cautious over the past decades and efforts are now made to restrict approved agents to narrow specialists (Harris and McEvoy 1995, McEvoy 1996). Nevertheless, land managers still routinely redistribute previously approved, non-regulated agent insects that appear to pose a higher risk to the native flora. A growing number of empirical cases showing both direct and indirect effects of phytophagous biocontrol agents on native organisms illustrates this concern (Simberloff and Stiling 1996, Willis and Ash 1996, Louda et al. 1997, Louda 2000, Gassmann and Louda 2001, Louda and O'Brien 2002, Pearson and Callaway 2003).

Negative effects of nontarget herbivory on important components of plant fitness have been found for native plants in at least three studies to date. *Rhinocyllus conicus*, a Eurasian weevil introduced to North America to control exotic thistle species, has been found to affect adversely the seed production of numerous native thistle species and the abundance and distribution of Platte thistle, *Cirsium canescens* Nutt. (Louda et al. 1997, Louda and Arnett 1999, Louda 2000). The cactus moth (*Cactoblastis cactorum*), released in the Lesser Antilles to control prickly pear cacti (*Opuntia spp.*), has adopted the rare semaphore cactus (*Opuntia spinosissima*) as a host and is likely having demographic effects (Simberloff and Stiling 1996, Johnson and Stiling 1998). Furthermore, recent evidence suggests *Larinus planus,* an adventive weevil being distributed to control

Canada thistle (*Cirsium arvense* L.), is reducing seed production in native thistles in Colorado (Louda and O'Brien 2002, Dodge unpublished data) and is feeding on native plants in Oregon as well (Villegas et al. 2001, E. Coombs pers. com.).

The current extent of nontarget use of native thistles by *L. planus* reported in Colorado (Louda and O'Brien 2002) was not expected. When *L. planus* was considered for redistribution and tested extensively in Alberta, Canada, for feeding and oviposition preference of North American species for its potential as a biocontrol agent, it did not appear to complete its life cycle on large-flowered *Cirsium* species (McClay 1990). Recent reviews of nontarget studies conclude, however, that host specificity testing in lab conditions may not adequately predict field realities and that close relatives of the target species likely face a risk of attack (Pemberton 2000, Louda et al. 2003). Yet, Louda and O'Brien (2002) reported that *L. planus* attacks and successfully develops on Tracy's thistle (*Cirsium undulatum* (Nutt.) Spreng. var. *tracyi* (Rydb.) Welsh), a large-headed species in Colorado. The risk implied to native *Cirsium* thistles of nontarget attack warrants further examination because of the wide array of phytophagous insects released to control exotic taxa in the same genus (*Cirsium*), subtribe (*Carduinae*), and tribe (*Carduidae*). To date, at least 11 insects and two fungi have been introduced or redistributed to control plants in the subtribe *Carduinae* (Gassmann and Kok 2002, Kok and Gassmann 2002, McClay 2002, Appendix A).

In general, information on the magnitude of nontarget impacts is scarce (Louda et al. 2003). *Larinus planus* has been redistributed from the northeast and released throughout the western U.S., and little is known regarding the frequency of colonization, natural dispersion, utilization of native taxa, and the factors that may influence nontarget use. The observations of nontarget feeding by *L. planus* in Colorado and Oregon have not addressed or quantified the geographic extent of these interactions and their effects. Furthermore, *R. conicus* has also been released throughout the western U.S. Reports of *R. conicus* colonizing and using native *Cirsium* are regular (Turner et al. 1987, Louda et al. 1997, Gassmann and Louda 2001), but we still lack a consistent, regional-scale quantitative assessment. We therefore designed a survey to determine the regularity, taxonomic breadth, and magnitude of nontarget host use by *L. planus,* with the secondary goal of providing more extensive information on variation in nontarget use by *R. conicus* across species on a large, regional spatial scale.

The aims of this survey were to address four main questions. First, is the occurrence of *L. planus* feeding on native taxa limited to isolated sites and taxa in local sites in Colorado and Oregon? Second, is the diet breadth and geographical range of nontarget use of native plants by *R. conicus* fully described? Third, what is the numerical impact of *L. planus* and *R. conicus* on seed production of native plants in the survey area? Fourth, what are plant population characteristics that influence probability of colonization and extent of herbivory by *L. planus* and *R. conicus*? Additionally, this survey, by documenting the current use of native taxa by *R. conicus* and *L. planus,* provides both information for immediate management consideration and baseline data for future evaluation of dispersal and host range expansion by *L. planus*.

METHODS

Larinus planus and Rhinocyllus conicus

Larinus planus (Fabricius) (Coleoptera: Curculionidae) is a European seed head weevil that has been widely introduced to the western U.S. as a biocontrol agent against Canada thistle (*Cirsium arvense* (L.) Scop.) (Fig. 1). Canada thistle is an invasive perennial weed species exotic to North America. *Larinus planus* was accidentally introduced in the eastern U.S. and has been established at least since 1968 (White 1972, Wheeler and Whitehead 1985, McClay 1990). It has since been intentionally released in at least 11 states and provinces in western North America (Rees et al., 1996; Louda and O'Brien 2002; personal communications with S. Kominsky, L. Heberston, and G. Austin, USFS; R. Troiano, G. Ramos, BLM; M. Chase, M. Curtin, NPS, C. Goss, USFWS). The bulk of reported releases took place in the 1990s (see Louda and O'Brien 2002). Release sites included public lands, including at least six national parks where conservation of native flora is a stated priority.

Rhinocyllus conicus (Froelich) (*Coleoptera: Curculionidae*) is a Eurasian seed head weevil, released throughout the western states for biocontrol of plumeless thistles (*Carduus spp.* (L.)) (Rees et al. 1996). *R. conicus* was originally released in Canada in 1968 and the U.S. in 1969, and it has since been found in at least 26 states (Zwolfer and Harris 1984, Turner et al. 1987, Louda et al. 1997, Gassmann and Louda 2001).

Larinus planus and *R. conicus* are univoltine, and both complete their larval development within developing capitula of thistles. Both species leave conspicuous, distinctive evidence of oviposition. *Larinus planus* oviposits by chewing a small hole in an immature capitulum, placing an egg in the hole and packing the hole with vegetative matter. This results in a circular scar at the base of a phyllary, followed by necrosis of the phyllary, and sometimes deformation of the capitulum. *R. conicus* lays its eggs on the exterior of developing capitula, covering them with an easily seen cap layer of masticated plant tissue. Larvae hatch and burrow into the flower head. Damage to the flower head by both weevils is primarily through larval feeding on ovules, developing seeds and receptacle tissues, thereby reducing the number of viable seeds matured. Both species undergo metamorphosis in distinctive pupal chambers inside the flower heads, and then emerge as adults.

Prior to a deliberate introduction into the U.S., a potential biocontrol agent must be permitted through the US Department of Agriculture, Animal and Plant Health Inspection Service (APHIS) However, because *L. planus* was introduced accidentally into the eastern U.S., it is not subject to the regulations that accompany deliberately introduced agents for classical biological control, and it does not require a permit for transfer or redistribution. Since the report of significant nontarget effects (Louda et al. 1997), APHIS has denied new permits for interstate transfer of *R. conicus* (Coombs et al. 2004b). Yet, as of 2000, and despite findings of significant levels of nontarget herbivory by both species as well as regulatory changes by APHIS, both *L. planus* and *R. conicus* were still being redistributed and released as biocontrol agents in both public and private lands. For a description of the current protocol for testing and permit process for APHIS approval see Coombs et al. (2004a).

Native Cirsium thistles

The genus *Cirsium* (L.) (Asteraceae: Tribe Cynareae) includes over 96 taxa indigenous to North America (Jordon-Thaden and Louda 2003, USDA and NRCS 2004). Most of the species diversity in the genus occurs west of the continental divide, and it includes several taxa that are recognized as rare, vulnerable, threatened, or endangered (NatureServe 2005).

Cirsium taxonomy is, in some aspects, unresolved (Barlow-Irick 2002, Kelch and Baldwin 2003). Taxonomic categories in this paper include the sources used for determining taxa as well as original authors (see Peck 1961, Hitchcock and Cronquist 1973, Great Plains Flora Association 1986, Hickman 1993, Welsh et al. 1993, Weber and Wittmann 2001). In most cases, and in all unresolved cases, we collected representative samples from the populations as vouchers. Furthermore, latitude and longitude coordinates of all populations sampled are available (online Appendix B at https://drum.umd.edu/dspace/bitstream/1903/2199/1/Appendix+B.doc). For this analysis, we combined recognized subspecies, except in the case of Tracy's thistle (*Cirsium undulatum* (Nutt.) Spreng. var. *tracyi* (Rydb.) Welsh) and Wavyleaf thistle (*Cirsium undulatum* var. *undulatum* (Nutt.) Spreng.) where sample sizes were large enough to permit independent investigation. All of the *C. undulatum* found on the western slope of the Continental Divide in Colorado and in Utah were classified as *C. undulatum* var. *tracyi* and all others were included as *C. undulatum* var. *undulatum* (Weber and Wittmann 2001, USDA and NRCS 2004).

Study sites

To determine the geographic and taxonomic ranges of nontarget feeding by *L. planus* as well as feeding by *R. conicus*, we employed a general sampling plan to quantify both the occurrence and magnitude of herbivory by *L. planus* in relation to reported release sites in the upper Great Plains. If we also found *R. conicus*, we quantified its occurrence and damage as well. Because almost nothing is known about *L. planus* and its colonization of native thistle taxa, we located as many *L. planus* release sites as possible using latitude and longitude coordinates provided by weed control agencies, public land managers, and private land owners.

Multiple releases are frequently made in close proximity in order to increase probability of establishment of the agents. In order to reduce effects of such non-independent events and avoid pseudoreplication in our analyses, we defined groups of release sites with less than 10 km separating any two of the releases as a release cluster. A population of native plants was considered part of that cluster if it was within 10 km of any of the related known release sites.

In July of each year (2001-2004), we surveyed at total of 72 *L. planus* release sites in 33 release clusters in Colorado, Idaho, Nebraska, Nevada, Oregon, South Dakota, Utah, Washington, and Wyoming. The survey resulted in collection of data from 186 populations of 14 native *Cirsium* taxa. We chose the dates of the survey to coincide with the average flowering phenology of the native thistles. We attempted to visit populations when the bulk of the main flower heads were maturing seed but prior to seed release.

Each site visit began by examining the nearest Canada thistle populations for evidence of *L. planus* establishment. We looked at flower buds at different phenological stages for evidence of weevil oviposition and larval development, and inspected plants for adult weevils. When oviposition scars were encountered, capitula were opened to verify presence of larvae, pupae, or teneral adults. Following this assessment, we searched the surrounding areas for up to five discrete populations per species of each native *Cirsium* thistle species present in the area. These populations were easily definable due to clumped distributions at the sampling sites. At each locale we determined population sizes of the plants by counting the number of flowering plants in the contiguous population (as defined by within 100m of another plant).

In addition to visiting *L. planus* release sites, we also sampled thistle populations encountered by scanning roadsides and associated disturbed areas. In particular, we surveyed roadside populations in counties where *L. planus* purportedly has been released but for which we found no specific release site locations reported.

Detection of L. planus and R. conicus colonization and phenological assessment

We quantified stage of plant phenological development and level of weevil oviposition by randomly selecting five flowering plants from each population and examining all of the capitula on them. In some cases (n = 16), populations at a site consisted of fewer than five flowering plants – in which case all of the plants were sampled; also, in most cases fewer than five populations of native thistles could be found within 10 km of a *Larinus* release site. For each plant, we sampled the main terminal capitulum and all of the flower heads on alternating branches along the main stem. Each capitulum was assigned a distinct identification number, based on its position in the plant architecture. If fewer than 10 capitula were available using this method, we examined all of the heads on the other branches. In cases of multiple flowering stalks stemming from a single basal rosette, we randomly chose two stems to quantify intensively.

Diameter of each capitulum was measured, enclosing the phyllaries surrounding the receptacle, but not including the spreading spines. Flower head developmental stage and evidence of oviposition by either *L. planus* or *R. conicus* were recorded. Capitula that had not extended beyond the subtending leaves (and therefore were not yet susceptible to weevil oviposition) were not included. We did not measure capitulum diameter if the phyllaries had already reflexed and released seed.

Statistical analysis

Analysis of the association between *L. planus* colonizing the target host, *C. arvense,* and each native *Cirsium* species was performed using a chi-square contingency test. Analysis of factors associated with colonization (yes/no) by *L. planus* on *C. arvense* and on native thistle plants per release cluster was performed using logistic regression (logit binary model SAS® ver. 8.02 : SAS Inst., 1999). For prediction of *L. planus* colonization of *C. arvense*, independent variables for the model included: number of *L. planus* releases in the cluster, number of years since first release, average latitude, average longitude, average elevation, average size of the plant populations, and average distance of native populations to nearest release point. For prediction of *L. planus* colonization of native *Cirsium* species, the independent variables included the above variables, plus: colonization of target *C. arvense*, average distance of native populations to nearest release, and number of native populations sampled. The factor "colonization of target *C. arvense*" had to be removed from the logistic regression analysis predicting colonization of native *Cirsium* due to the issue of multicollinearity; and, it was tested independently using a chi-square contingency test. All other variables were assessed using a stepwise inclusion (p = 0.20) and removal (p = 0.10) from the final regression model. These p-values were chosen to increase sensitivity and decrease probability of type II errors.

We also performed analyses of factors that influence colonization of native *Cirsium* populations by *R. conicus*. Independent variables included: latitude, longitude, elevation, number of native populations sampled in a cluster, and average size of native populations. Variables were assessed using a stepwise inclusion (p = 0.20) and removal (p = 0.10) from the final regression model.

Measurement of oviposition rates

Plant-level oviposition intensity was defined as the percent of susceptible capitula with evidence of oviposition. Susceptible capitula were those that were as large or larger than the stage of development during which *L. planus* or *R. conicus* is reported to oviposit (large buds). Population-level oviposition intensity was calculated by averaging the plant-level oviposition intensity per population. We compared plant phenology and weevil oviposition (to determine temporal refugia for plants) per population by calculating oviposition intensity per inflorescence class (sequentially produced primary, secondary, tertiary positions on branches). Primary capitula were defined as main terminal capitula and all terminal heads on branches from the main stem, which are produced early in the growing season. Secondary capitula were defined as terminal heads from sub-branches off main branches, and tertiary capitula were those that subtend secondary capitula and are produced latest during the season. All of the *Cirsium* species included in this study flower indeterminately, with primary capitula maturing before secondary and secondary before tertiary. Not all species or individuals in a species produce all classes of capitula described above.

We assessed the relationship between population-level oviposition intensity and population size in *C. undulatum* by examining the correlation between the natural log of population-level intensity and the natural log of population size. We investigated this relationship with *C. undulatum* at the subspecific level (vars. *tracyi* and *undulatum*) to increase the information provided for the subtaxon reported as vulnerable (var. *tracyi*). We were only able to investigate this relationship for subspecific variants only within *C. undulatum* due to smaller sample sizes for other native thistle species.

Measurement of effects of herbivory on seed production

We also collected flower heads from the five randomly selected plants, taking the main terminal flower head plus the terminal flower heads on the alternate subtending branches. We collected those capitula that had completed flowering and had not yet dispersed seed. We also collected capitula that had aborted after initiating development (>10mm diameter). These would have been expected to develop based on examination of development of other capitula in the same position on the plant, taking into account the sequential flowering nature of these species. The collected capitula were dissected. We recorded number of filled, undamaged seeds, evidence of insect feeding (including the number of weevil pupal chambers), and any developing insects in the capitulum. Insects were classified by species and stage of development. We were unable to collect and dissect mature capitula from a few populations (n = 2) that we visited due to underdeveloped or overdeveloped flowering by plants in the population. In some of these cases we could still determine weevil presence and colonization by the presence or absence of oviposition evidence on underdeveloped heads and by the presence of oviposition evidence plus pupal chambers in overdeveloped capitula. However, these data were not used in the analysis of weevil effects on seed production.

We used the data from the dissected capitula to evaluate relationships between successful seed predator development and viable seed production in each thistle species. Average seed produced per capitulum per plant was used as a component estimate of effects on plant fitness (as in Rand and Louda 2004). Each plant was assigned to one of four categories: 1) hosting *R. conicus*, 2) hosting *L. planus*, 3) hosting both, or 4) hosting neither. We corrected for the preponderance of zero values in the seed production per capitulum data, and the resulting non-normal departures from the means, by using mean number of seeds per capitulum averaged per plant and assigning each plant to one of the hosting categories. The plant mean values were square root transformed and satisfied ANOVA assumptions. A mixed model ANOVA was used to compare seed production between categories across all species, with species as a random factor and hosting category as fixed. Category means were compared using Tukey-Kramer adjustment. Within-species analysis was conducted similarly, with population as a random factor and hosting category as fixed. In all cases where we found fewer than three populations of a species to sample and in the cases of *C. canovirens* and *C. flodmanii* we did not block by population, as there were no measurable population effects. All four categories were not available for most of the within-species analyses and in all cases the design is unbalanced. Thus, the ANOVAs were performed using Type III sums of squares.

Additional notes on statistical comparisons

The issue of independence of observations on presence or absence of the insect herbivores is a concern. Obviously capitula on the same plant are not independent, however we combined heads on a plant to evaluate population effects. Also, plants within a population are arguably not independent; however, we contrast populations. Further, neighboring populations may not be independent; however, our clusters reduce the chance that our main findings present pseudoreplication. In sum, we make comparisons on multiple levels, working to present conservative interpretations since we acknowledge the potential for non-independent observations.

RESULTS

Colonization of plant populations by L. planus and R. conicus

Larinus planus had established on the target species, *C. arvense*, at 12 (36%) of the 33 *L. planus* release clusters we visited. Among the 12 clusters where *L. planus* had colonized *C. arvense,* no native thistles were observed at three; and, *L. planus* had colonized native thistle populations at six (67%) of the remaining nine clusters. Further, 21 distinct populations of *C. undulatum* var. *undulatum*, five populations of *C. undulatum* var. *tracyi*, three populations of *C. perplexans*, and one population of *C. flodmanii* were colonized by *L. planus* across the survey range (Table 1-1, Fig. 1-1a). We found no evidence of colonization by *L. planus* on *C. ochrocentrum* and *C. neomexicanum*, the only other native *Cirsium* species found in the surveyed areas where *L. planus* had established. A full list of survey sites, sorted by species, with locations and selected attributes is available (online Appendix B at https://drum.umd.edu/dspace/bitstream/1903/2199/1/Appendix+B.doc). Selected attributes sorted by survey cluster and species is also available (online Appendix C at https://drum.umd.edu/dspace/bitstream/1903/2199/2/Appendix+C.doc).

Rhinocyllus conicus was detected on *C. arvense* at 29 (89%) of the 33 release clusters visited. Additionally, *R. conicus* had colonized native populations at 23 (92%) of the 25 visited release clusters in which we encountered native *Cirsium* (Table 1-1, Fig. 1-1b). We found no native thistles in seven of the areas searched. Overall, *R. conicus* had colonized 15 species of *Cirsium* thistles. Although we found some populations of native thistles that had not been colonized by *R. conicus*, we encountered *R. conicus* on every species of native thistle investigated in the survey, except for *C. remotifolium*.

Table 1-1. Native *Cirsium* species included in survey and colonization by *Larinus planus* (LAPL) and *Rhinocyllus conicus* (RHCO). Proportion of populations hosting LAPL and/or RHCO is given as a percent (e.g. % Pop’s hosting LAPL). ID sources: A: (Great Plains Flora Association, 1986); B: (Hickman, 1993); C: (Hitchcock and Cronquist, 1973); D: (Peck, 1961); E: (Weber and Wittmann, 2001); F: (Welsh et al., 1993).

Species	**ID source**	**States found**	**No. populations**	**LAPL detected**	**RHCO detected**	**% Pop’s hosting LAPL**	**% Pop’s hosting RHCO**	**% Pop's hosting LAPL or RHCO**
C. brevistylum	C	OR	4	No	Yes	0	25	25
C. calcareum	F	UT	4	No	Yes	0	50	50
C. callilepis	C	OR	2	No	Yes	0	100	100
C. canovirens	C	OR	9	No	Yes	0	89	89
C. eatonii	E, F	CO, UT	2	No	Yes	0	50	50
C. flodmanii	A	NE, SD, WY	6	Yes	Yes	17	100	100
C. neomexicanum	F	NV, UT	5	No	Yes	0	80	80
C. occidentale	B	OR	2	No	Yes	0	50	50
C. ochrocentrum	A	CO, NE	4	No	Yes	0	100	100
C. peckii	D	OR	3	No	Yes	0	100	100
C. perplexans	E, F	CO	14	Yes	Yes	21	64	64
C. remotifolium	C	OR	1	No	No	0	0	0
C. scariosum	E, F	CO, ID, NV, OR, UT	14	No	Yes	0	71	71
C. scopulorum	E, F	UT	1	No	Yes	0	100	100
C. subniveum	C	OR	2	No	Yes	0	50	50

Species	**ID source**	**States found**	**No. populations**	**LAPL detected**	**RHCO detected**	**% Pop’s hosting LAPL**	**% Pop’s hosting RHCO**	**% Pop's hosting LAPL or RHCO**
C. undulatum var. tracyi	E, F	CO, UT	21	Yes	Yes	24	57	76
C. undulatum var undulatum	A, E, F	CO, ID, NE, NV, OR, SD, UT, WY	88	Yes	Yes	24	81	82

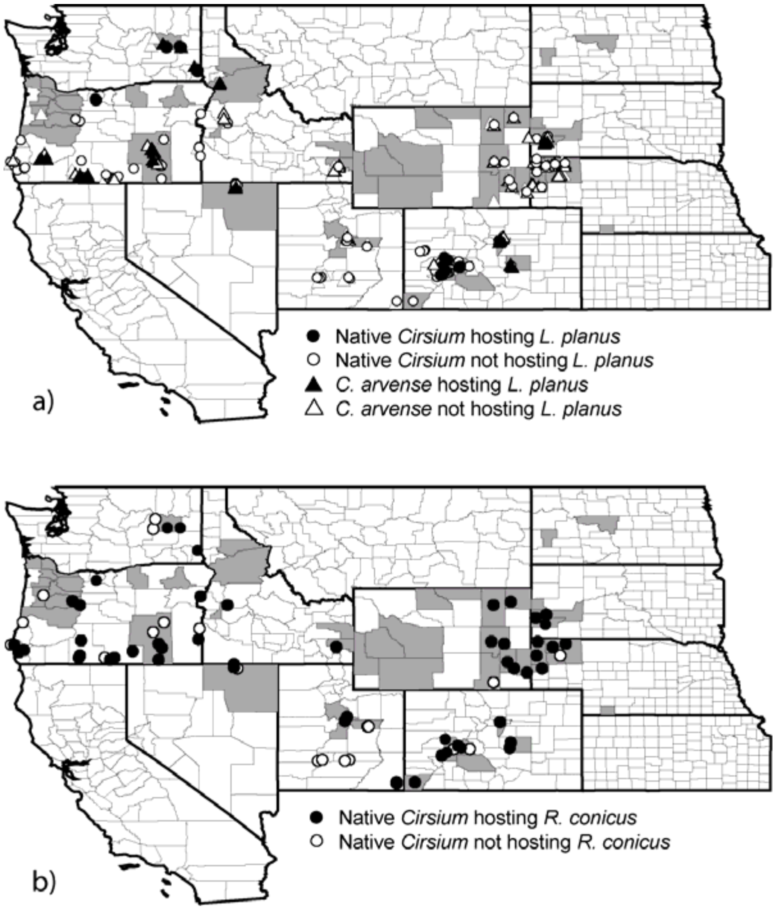

Figure 1-1. Maps of counties in the western US with known intentional releases of *Larinus planus* (shaded areas). Filled circles on a) represent areas (clusters or individual populations) where *L. planus* established on native populations (see CO, OR, SD, and WA). Colonized sites comprise four taxa of native *Cirsium*. Filled triangles indicate clusters or populations where *L. planus* has established on *C. arvense* (and not native *Cirsium*). Open circles and open triangles indicate sampled populations of native *Cirsium* and *C. arvense*, respectively, where we found no *L. planus*. Filled circles on b) represent sites where *Rhinocyllus conicus* established on native populations, and open circles represent areas where native *Cirsium* thistles were found not hosting *R. conicus*. The colonized sites comprise 16 taxa of native *Cirsium*.

Colonization of native *Cirsium* species by *L. planus* was highly related to its establishment on *C. arvense* (chi-square = 14.36, p<0.01). Logistic regression on potential factors influencing *L. planus* colonization of *C. arvense* populations per release cluster showed no relationship (p=0.10 to remain in model) between colonization of *L. planus* of its adapted host plant and any of the measured parameters: average host plant population size, number of releases in the cluster, number of years since first release, elevation, latitude, or longitude. Similarly, logistic regression on potential factors influencing *L. planus* colonization of the native *Cirsium* populations per release cluster also showed no relationship (p=0.10 to remain in model) between colonization of *L. planus* and any of the measured parameters: average plant population size, number of native populations surveyed in the cluster, number of releases in the cluster, number of years since first release, average distance of native populations to the nearest release site, elevation, latitude, or longitude.

In addition, *R. conicus* occurrence and establishment on native *Cirsium* populations at *Larinus* release sites also showed no relationship to any of the tested independent variables: average plant population size, number of native populations surveyed in the cluster, elevation, latitude, and longitude.

We surveyed 71 native *Cirsium* populations that were not proximate (as far as we know) to *L. planus* release sites. Of these, six (8%) hosted *L. planus* and 47 (66%) hosted *R. conicus*. The six added populations hosting *L. planus* were in Oregon and Washington.

Oviposition levels and plant phenology

Larinus planus oviposition intensity (average ovipositions per plant per population) was variable across 23 distinct populations of four native taxa (Table 1-2). *R. conicus* oviposition intensity also varied across 102 distinct populations of the 15 taxa of native *Cirsium* species observed (Table 1-2). In both cases, oviposition levels per species were found in excess of 50% of capitula per population.

Table 1-2. Average *Larinus planus* and *Rhinocyllus conicus* oviposition intensity on native *Cirsium* species in colonized plant populations. Averages are based on average percent capitula attacked per plant per population, averaged over populations.

***Larinus planus* oviposition intensity on native *Cirsium* species**

Plant species	**No. populations**	**Total No. plants**	**Average intensity per plant per pop.**	**Std dev of averages**	**Maximum average per pop.**	**Minimum average per pop.**
C. flodmanii	1	5	11%	N/a	11%	11%
C. perplexans	3	9	10%	0.06	17%	6%
C. undulatum var. *tracyi*	3	15	60%	0.29	80%	27%
C. undulatum var. *undulatum*	16	57	28%	0.25	100%	5%

***Rhinocyllus conicus* oviposition intensity on native *Cirsium* species**

Plant species	**No. populations**	**Total no. plants**	**Average intensity per plant per pop.**	**Std dev of averages**	**Max. average per pop.**	**Min. average per pop.**
C. brevistylum	1	5	7%	N/a	7%	7%
C. calcareum	2	3	85%	0.01	85%	86%
C. callilepis	2	6	9%	0.13	18%	0%
C. canovirens	3	10	68%	0.26	91%	40%
C. eatonii	1	8	72%	N/a	72%	72%
C. flodmanii	5	25	33%	0.26	7%	66%
C. neomexicanum	2	10	97%	0.04	100%	95%
C. occidentale	1	5	80%	N/a	80%	80%
C. ochrocentrum	3	15	5%	0.03	7%	2%
C. peckii	2	2	69%	0.44	100%	38%
C. perplexans	9	38	45%	0.25	76%	4%
C. subniveum	1	2	100%	N/a	100%	100%
C. scariosum	6	20	77%	0.23	100%	43%
C. undulatum var. *tracyi*	7	32	58%	0.27	78%	3%
C. undulatum var. *undulatum*	56	243	38%	0.22	93%	5%

None of the usual parameters found to influence plant-insect interactions, which we measured, were significant in predicting oviposition frequency by either *L. planus* or *R. conicus*. For example, no common trends across populations emerged in the relationship between plant phenology and *L. planus* oviposition or *R. conicus* oviposition. Some populations had a higher proportion of the earlier inflorescences attacked; others had a higher proportion of the later inflorescences attacked; and, yet others had consistent levels across early and later inflorescences (see Fig. 1-2 for *L. planus* oviposition patterns; *R. conicus* oviposition summaries per population are available online at Appendix D at https://drum.umd.edu/dspace/bitstream/1903/2199/3/Appendix+D.doc). Also, no significant relationship emerged for either *L. planus* or *R. conicus* between population size and oviposition intensity for any of the native thistle species (for *L. planus*: r = 0.25, p = 0.31; for *R. conicus*: r = -0.056, p = 0.67).

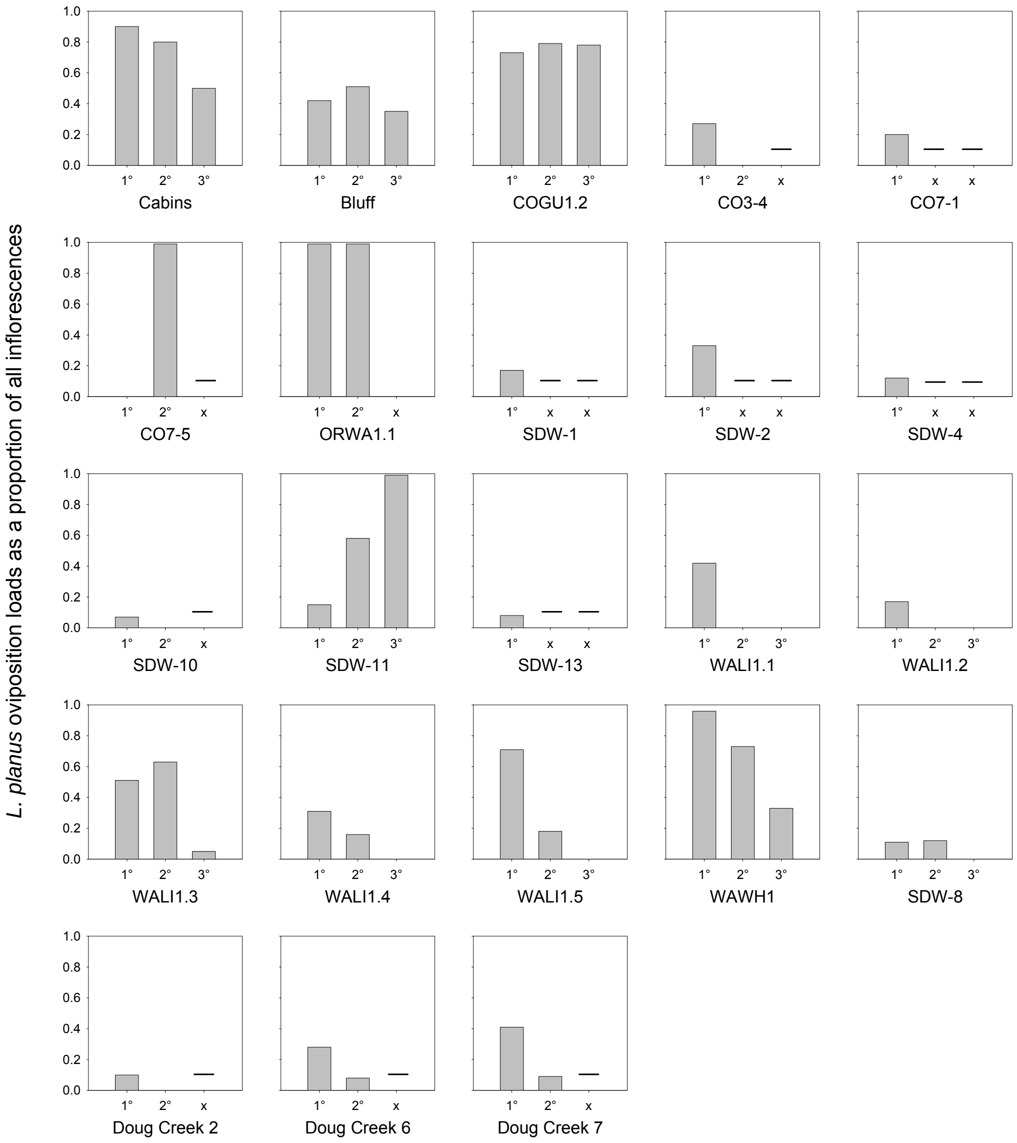

Figure 1-2. Average proportion of inflorescences per plant oviposited on by *Larinus planus* in the three populations of *Cirsium undulatum* var. *tracyi* (Cabins, Bluff, COGU1.2), 16 populations of *C. undulatum* var. *undulatum*, one population of *C. flodmanii* (SDW-8), and three populations of *C. perplexans* (Doug Creek 2, 6, 7) colonized by *L. planus*. Location data for study sites are in Appendix B. The columns in each graph represent primary, secondary and tertiary inflorescences, respectively. Columns depicting no inflorescences existed in a class are distinguished from zero percent oviposition by columns marked with “x” and a dash in the column space.

Effects of floral herbivory on seed production

Mixed model ANOVA results across species indicated that plants that hosted either *L. planus* and/or *R. conicus* produced at least 50% fewer seeds per capitulum than did those that did not host either weevil ($F_{3,\,507} = 31.67$, $p < 0.01$) (Table 1-3, Fig. 1-3). Values of mean seed production per head with weevils present are all significantly lower than those without weevils present. There was no measurable difference in seed production between plants hosting *L. planus* vs. those hosting *R. conicus* using planned contrasts and p values adjusted for multiple comparisons ($\alpha = 0.05$, Tukey-Kramer). Within-species analyses showed that in five of the 10 native taxa, plants that hosted weevils, either *L. planus* and/or *R. conicus,* produced fewer seeds than those that did not host either weevil (Table 1-3, Fig. 1-4). In the other 5 taxa, the trend of reduced seed production per plan with weevil presence was similar, but the differences were not significant at $\alpha = 0.05$.

Table 1-3. Average seed production per capitulum per plant for each of 12 native *Cirsium* taxa (square root transformed). Five of the nine taxa we could test showed significantly reduced seed production when hosting *Larinus planus* (LAPL) and/or *Rhinocyllus conicus* (RHCO). Three of the remaining four showed a non-significant but consistent trend of reduced seed production. Five other taxa were not testable because all plants encountered on the survey hosted *R. conicus*, and two species were not mature enough at the time of sampling to collect seed production data. In cases with more than one comparison, similar letters in superscript accompanying the mean values indicate non-significant differences at $\alpha = 0.05$.

Species	**Mean seed LAPL**	**SE LAPL**	**Mean seed RHCO**	**SE RHCO**	**Mean seed both**	**SE both**	**Mean seed no weevils**	**SE no weevils**	**ANOVA p value**
Across all species	0.23^{a}	0.65	1.49^{a}	0.19	1.28^{a}	0.56	3.24^{b}	0.22	<0.01
C. brevistylum	n/a		2.44	0.76	n/a		n/a		n/a
C. callilepis	n/a		2.77	1.57	n/a		2.65	0.71	0.95
C. calcareum	Too early in year for seed production data								
C. canovirens	n/a		0.57	0.49	n/a		3.73	0.55	<0.01
C. eatonii	n/a		1.01	0.29	n/a				n/a
C. flodmanii	n/a		1.37	0.49	n/a		2.23	0.59	0.35
C. neomexicanum	n/a		0.67	0.18	n/a				n/a
C. occidentale	n/a		1.45	0.45	n/a				n/a
C. ochrocentrum	n/a		1.05	1.24	n/a		1.94	0.52	0.52
C. peckii	n/a		0.00	2.00	n/a		4.68	1.59	0.04

Species	**Mean seed LAPL**	**SE LAPL**	**Mean seed RHCO**	**SE RHCO**	**Mean seed both**	**SE both**	**Mean seed no weevils**	**SE no weevils**	**ANOVA p value**
C. perplexans	n/a		1.22	0.30	n/a				n/a
C. remotifolium	Too early in year for seed production data								
C. scariosum	n/a		0.84	0.43	n/a		4.70	0.70	<0.01
C. subniveum	n/a		0.61	0.61	n/a		5.89	0.87	0.13
C. undulatum var. tracyi	0.97^{a}	0.80	1.59^{a}	0.50	n/a		5.25^{b}	0.51	<0.01
C. undulatum var. undulatum	1.38^{a}	1.09	2.05^{a}	0.19	1.43^{a}	0.53	3.03^{b}	0.17	<0.01

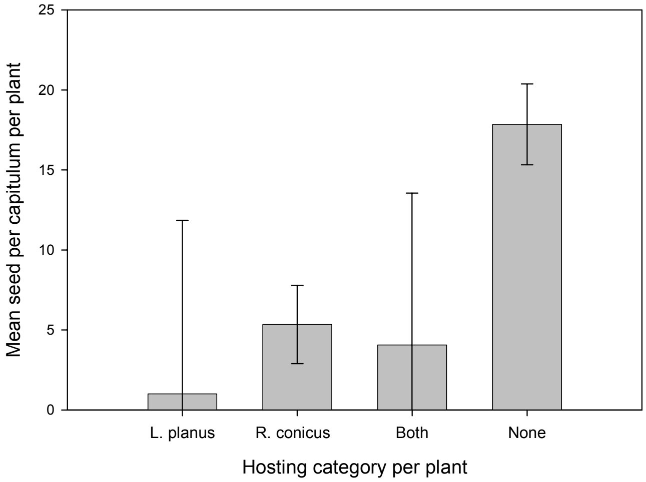

Figure 1-3. Average seed production per capitulum per plant (not square root transformed) across all 12 species of native *Cirsium*, by four categories of insect presence: 1) *Larinus planus* only, 2) *Rhinocyllus conicus* only, 3) Both *R. conicus* and *L. planus*, and 4) No weevils. Seed production is reduced significantly in presence of *R. conicus*, *L. planus*, or both across the 12 species (square root transformed, $F_{3,507} = 31.67$, $p < 0.01$). Values representing weevil presence are all significantly different from weevil absence (None) using Tukey-Kramer adjusted post-hoc comparisons ($\alpha = 0.05$, Tukey-Kramer). Error bars represent two SE.

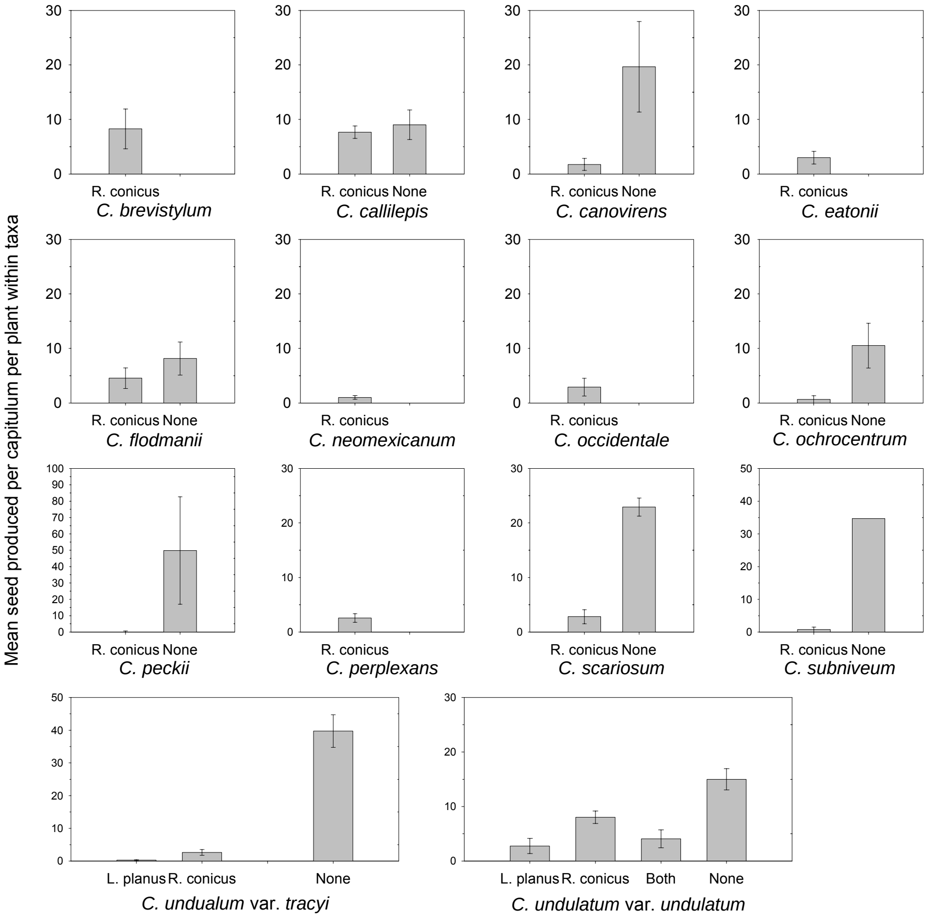

Figure 1-4. Average seed production per capitulum per plant (no transformation) within all 14 taxa of native *Cirsium* for which we gathered seed production data. Data are arranged by categories of insect presence: *Larinus planus*, *Rhinocyllus conicus*, “Both” *R. conicus* and *L. planus*, and no weevils (“None”). See Table 3 for statistical comparisons using square root transformed data.

DISCUSSION

Colonization

We found that *L. planus* had colonized native *Cirsium* taxa in six out of eight of the clusters (75%) where it had become established on the exotic *C. arvense* and where we found native thistle taxa in the vicinity. *Larinus planus* successfully colonized four species: Flodman's thistle (*C. flodmanii*), Rocky Mountain thistle (*C. perplexans*), Wavyleaf thistle (*C. undulatum* var. *undulatum*) and Tracy's thistle (*C. undulatum* var. *tracyi*). The colonization of native *Cirsium* taxa by *R. conicus* was pandemic throughout the upper Great Plains and Intermountain regions sampled; 16 of the 17 taxa of *Cirsium* observed hosted *R. conicus*, and the vast majority of all the populations sampled was infested.

These data suggest that the native *Cirsium* species of this region are highly vulnerable to negative effects of these exotic weevils. Refugia from the weevils could dampen the effects of these floral herbivores. However, we have no evidence that such population refugia exist to help local persistence of native *Cirsium* species. *Larinus planus* was found at both high (highest = 2621m) and low (lowest = 82m) elevation sites. Further, *R. conicus* was also found at both high (2823m) and low (76m) elevation sites. However, as Louda et al. (1997) found for a Rocky Mountain *Cirsium* species, we found that neither *L. planus* nor *R. conicus* occurred in our highest population (3749m); and, in the Pacific Northwest, neither weevil was prevalent within five miles of the coast (E. Coombs, OR Dept. of Ag., personal communication), similar to the situation for *Cirsium occidentale* along the central California coast (Maron et al. 2002). So, elevation or coastal exposure may provide some refuge for high altitude and coastal species. Regardless, both weevils have the ability to survive and reproduce across the elevation gradient that includes most thistle species in North America. The similarity in years since first release may have influenced our ability to detect any impact this factor may have; releases at most of the sites we visited took place within a ten-year period.

It is interesting, and somewhat surprising, that we found no significant relation between native plant population size and colonization of either of the exotic weevils. Both apparency theory and metapopulation / island biogeography spatial models predict that larger populations of flowering thistles should attract dispersing weevils more easily; they should also better sustain annual populations of non-dispersing weevils. However, it may be the case that the missing variable in our study is historic population size.

The strong relationship between the occurrence of *L. planus* on native species when it occurred on its targeted host, *C. arvense,* could reflect a variety of factors. It is likely that *L. planus* dispersed from *C. arvense* at the release site to proximate native populations, representing a “spillover” effect similar to that found for *R. conicus* onto native thistles in mid and tallgrass prairie (Rand and Louda 2004). It is not likely that a source pool of *L. planus* on *C. arvense* continuously feeds sink pools of *L. planus* on native *Cirsium*. Further, in this case, the presence of native *Cirsium* may positively influence the probability of use of *C. arvense* at least at some sites. For example, *L. planus* has colonized and maintained its populations on *C. undulatum* var. *tracyi* in Almont, CO at least since 1999, yet this is an area where *C. arvense* populations are small, phenologically delayed, and less used by *L. planus* (Louda and O’Brien 2002).

Further, we regularly found populations of *L. planus* on native *Cirsium* species even where *C. arvense* did not occur as a host source plant in sympatry.

Oviposition levels and plant phenology

The oviposition levels of both *L. planus* and *R. conicus* on the plants were variable, but tremendously high in some cases. Average levels are regularly above 50% in a population; and, for each weevil there were native thistle populations that had oviposition levels close to 100%. However, we found no evidence relating oviposition levels to plant population size.

Unexpectedly, based on previous work on weevil-thistle interactions, we found no obvious consistent phenological refugia for native plants across the taxa studied. Although in some populations early flower heads appear to escape oviposition, since the primary terminals had lower oviposition rates, other populations did not show this pattern, and in fact had higher oviposition rates on flower heads emerging later in the growing season. These findings may reflect the fact that both weevils have a prolonged oviposition period that covers a large proportion of most populations' flowering periods. However, consistently later flowering taxa, such as *C. calcareum*, *C. flodmanii* and *C. remotifolium* and, interestingly, *C. arvense*, may have some temporal refuge from complete seed destruction due to their later flowering phenologies. Similarly, late season rainfall in areas with long growing seasons can lead to a second flush of flower production; these flower heads may then escape heavy oviposition intensity.

Effects of floral herbivory on seed production

Seed production per plant is the best estimate of plant fitness for which we have data. The results show in all cases either trends or significant reductions of seed production by those plants that hosted *L. planus* or *R. conicus* or both. Across all taxa, seed production was reduced approximately 75% in plants that hosted one or both weevils (Fig. 1-3). In five out of the 10 individual taxa tested, seed production was significantly reduced by about 30% to 95% (Fig. 1-4).

Whether or not the observed considerable decline in seed production will result in population-level effects depends on the degree to which a population is seed limited (Harper 1977, Fenner 1992), which is common for native *Cirsium* species (Louda et al. 1990, Louda and Potvin 1995, Maron et al. 2002, Rose et al. in press). Populations of *C. canescens*, *C. occidentale*, *C. perplexans*, and *C. undulatum* var. *tracyi* respond positively to predispersal seed predator exclusion experiments or seed addition experiments (Louda et al. 1990, Louda and Potvin 1995, Maron et al. 2002, Dodge unpublished data, Rose et al. in press). Arguably, characteristics of plant species most often limited by seed production include those that: inhabit early successional or disturbed habitats (see Turnbull et al. 2000), are monocarpic, and do not reproduce vegetatively (Louda 1989b, Fenner 1992). Native *Cirsium* species generally fall into some or all of the above categories.

Conservation concerns

Reduced seed production will have demographic effects in all populations that are seed limited, such as Platte thistle in sand prairies (Louda and Potvin 1995, Rose et al. in press). Extinction due to the weevils may be less likely since most thistles spend some part of their life as a vegetative rosette that, in theory, could purge a population of the weevils in the absence of flowering adults, i.e., the weevils cannot persist in the absence of flower heads. However, when exotic thistles are sympatric with one or more native thistle species that can act as a source pool for weevil persistence, the scenario becomes much more perilous.

Nontarget floral herbivory by *L. planus* is the second case where the tests for host specificity of phytophagous biocontrol agents distributed to control thistles did not anticipate the magnitude of the nontarget effects on the less preferred native species. The same underestimate was made for *R. conicus* before its release in the late 1960s (Zwolfer and Harris 1984, Gassmann and Louda 2001). However, these cases are quite different legally, in that *L. planus* is adventive and was, thus, never cleared by APHIS for introduction into the United States. Regulation of movement of organisms for biological control once they are established is controlled by the recipient states and supervised by the USDA APHIS Plant Protection and Quarantine unit. However, in practice, the two weevils have been used quite similarly in the western United States.

To date, there have been at least six other agents in addition to *L. planus* and *R. conicus* that have been released or redistributed for the control of exotic *Cirsium* species thistles: two chrysomelid leaf beetles, *Altica carduorum* Guerin-Meneville, and *Cassida rubiginosa* Muller; a third weevil, *Hadroplontus (Ceutorhynchus) litura* Fabricius; two tephritid flies, *Urophora cardui* L. and *U. stylata* Fabricius; and, a rust fungus, *Puccinia punctiformis* (Str.) Rohl. (Appendix A). Of these, five are known to also feed on North American native species and the sixth (*Urophora cardui* L.) apparently was not tested on North American natives (Gassmann and Kok 2002, McClay 2002). There have been at least seven species of agents released for the control of *Carduus* thistles (Appendix A). Of these, five readily accepted *Cirsium* thistles as hosts, one fungus (*Puccinia carduorum* Jacky) could colonize but did not persist on *Cirsium* species, and one (*Psylliodes chalcomera* Illiger) fed on European *Cirsium* but not the North American *Cirsium undulatum* in a small field trial in Italy (Dunn and Campobasso 1993).

Two of these other agents are of major concern now. The first is *Trichosirocallus horridus* Panzer, a rosette weevil released to control musk thistle (*Carduus nutans*) and bull thistle (*Cirsium vulgare* (Savi) Tenore) (Kok and Gassmann 2002). We recorded the presence of both adults of *T. horridus* on rosettes, and larvae that fit the description of *T. horridus* in flowering stalks, of *C. perplexans*. The second is *Cheilosia corydon*, a root crown fly released to control plumeless thistles (*Carduus spp.*). This fly has established, and has been recovered from, a *Cirsium* species in Oregon (E. Coombs, Oregon Department of Agriculture, personal communication 2004). When insects are released in the field in a new environment, they are thrust into novel relationships for survival relative to those they experienced in greenhouse tests or in their native ranges. The fact that there is evidence that the majority of species introduced to control exotic *Cirsium* and *Carduus* thistles can, and in fact are likely, to feed to some extent on native taxa is a serious challenge for the native flora. We expect more instances of nontarget herbivory on native relatives of targeted weeds, as currently documented here and in the literature (Pemberton 2000), will be found as additional investigations proceed and expand.

A primary management policy issue at hand is the continued redistribution of biocontrol agents. Redistribution of adventive insects and previously approved insects is far less regulated than initial importation and release. The burden of thorough investigation into risk in the case of proposed redistribution lies in the hands of biocontrol practitioners and land managers. Research on criteria for decisions to redistribute both previously approved and adventive agents lag behind other research since most land managers may not follow or have access to the most current research on risks to native plants. As noted earlier, both *L. planus* and *R. conicus* were still being released in some states as of 2000, despite the existing literature demonstrating *R. conicus*' negative effects for native plants. The concern over likely nontarget effects is especially important when we consider that on public lands protecting the native flora is a primary objective of management. In many native thistle populations it is apparent that the introduced biological control agent can have more negative ecological effects than the exotic thistles do via competition. If biocontrol practitioners adhere to the 12 guidelines outlined in the International Code of Best Practices for Classical Biological Control of Weeds (Balciunas and Coombs 2004), then the incidence of this type of nontarget impacts should be reduced.

Monitoring and evaluation

To date, monitoring and evaluation of releases by the releasing organization is the exception rather than the norm. Few of the people we contacted who provided information on releases of insects had information on the establishment of *L. planus* on its target *C. arvense* at most of the release sites, either on public lands or private land. Presumably the lack of quantitative monitoring information is due, at least in part, to limited funding and personnel available for it in most biocontrol programs. The lack of time and funding for other control options is one of the reasons biocontrol solutions were sought in the first place.

The only informal or published reports suggesting nontarget feeding by *L. planus* we found were Villegas (2001) and in southern Oregon and Louda and O'Brien (2002) in western Colorado. During the time we were conducting the survey, *L. planus* was discovered on a *C. arvense* flower head in California - it is suspected that the insects dispersed from Oregon (B. Villegas, California Department of Food and Agriculture, personal communication 2004). Also, during this time period *L. planus* was recovered from *C. callilepis* (Greene) Jeps. in Oregon (E. Coombs, Oregon Department of Agriculture, personal communication). Given the length of time necessary to detect establishment and efficacy of biocontrol agents, the data in this survey do more than quantify the current spatial spread and diet breadth of these two weevils in this region; they also form a valuable baseline dataset for evaluating the scope of any further spread, or diet breadth expansion, or subsequent impacts of these two introduced weevils across this region.

Larinus planus may be still in the initial stages of its population increase and spread, and it remains to be seen whether it will follow a similar path to that of *R. conicus*. Establishment of a biocontrol agent is not immediate – it may take up to 10 years before levels of herbivory reach a point where control can be measured, and analysis of efficacy should occur only after 10-20 years have elapsed since introduction (McFadyen 1998). *R. conicus* was first introduced in the US in 1969 and actively redistributed throughout the early 1970s (Rees 1977, Louda 2000). Nontarget feeding on native *Cirsium* was not reported until 1977 (Rees 1977). Subsequently, Turner et al. (1987) reported the broad range of nontarget feeding of *R. conicus* from surveys done in the mid-1980s (Turner et al. 1987); and, not until 28 years after introduction were ecological effects reported (Louda et al. 1997). Thus, the long-term studies of insect use of native *Cirsium* taxa in Nebraska showed that colonization by *R. conicus* of native species in the sand prairie without the targeted weed took over 20 years from the time it was released in Nebraska (Louda 2000). To date, however, approximately a third (30 species) of the 102+ native *Cirsium* taxa now known have been documented hosts of *R. conicus* (see Pemberton 2000).

Conclusions

Our survey documents the wide current distribution of *L. planus* associated with release sites against Canada thistle over a large portion of the west-central United States. It clearly reveals that the occurrence of *L. planus* feeding on native plants is not isolated to a single area or a single native species. Our findings also increase the range of species on which *R. conicus* has been found completing its life cycle, and the results document a wider geographic range of occurrence of nontarget effects than previously reported. Additionally, the decreased seed production by plants attacked by the weevils quantified here provides evidence that both *L. planus* and *R. conicus* likely are affecting population densities of numerous thistle species. Lastly, we found no evidence of spatial refugia for native taxa from these exotic weevils.

Further studies of the effects of introduced species on native populations, and the potential for insect herbivores to regulate plant populations, will help ecologists and environmental managers understand the risk probabilities of biocontrol applications, as well as theory of population dynamics and regulation, species invasions, and trophic level interactions. Concern for risk to native floral guilds is increasing with the growing evidence of phytophagous insects’ ability to limit plant populations. The results of this research will be beneficial to resource managers who wish to consider use of phytophagous insects as biological control agents.

APPENDICES

Appendix A. Classical and adventive biocontrol agents released or redistributed to control plants in the tribe *Cardueae*, subtribe *Carduinae*. The subtribe comprises 36 genera, 2 of which (*Cirsium* and *Saussurea*) have native representations in North America. Feeding habits are from lab and field studies. Adventive species are indicated in the first column (*ADVENTIVE), all others were introduced through a USDA, Animal and Plant Health Inspection Service (APHIS) permit. "Released widely" and "established widely" refers to cases where the insect was released or has established in more than ten states, including states both east and west of the Mississippi River. Release and establishment information, unless otherwise stated comes from Coombs et al. (2004ab). Note that the last column "APHIS permit status" reflects the position of APHIS in regulation. State agriculture departments may still restrict redistribution.

Agent	**Description**	**Target(s)**	**Release Actions**	**Feeding Habit (and source)**	**Status of Agent in US**	**APHIS permit status**
Altica carduorum Guerin-Meneville (Coleoptera: Chrysomelidae)	Leaf beetle	*C. arvense*	Released DE, IN, MD, MN, NJ, SD, WI in 1966 and in MD, NJ,SD in 1970	"Readily feed on all *Cirsium* species tested" (McClay 2002)	No recorded establishment	No restrictions – however a race from China not approved
Cassida rubiginosa Muller (Coleoptera: Chrysomelidae) *ADVENTIVE	Leaf beetle	*C. vulgare, C. arvense, Carduus spp.*	Redistributed widely	Adults accept species from *Arcticum*, *Carduus*, *Cirsium*, *Silybum*, *Onopordum*, and *Centaurea* (McClay 2002).	Unknown	Adventive, no restrictions
Psylliodes chalcomera Illiger (Coleoptera: Chrysomelidae)	Leaf beetle	*Carduus nutans*	Released KS, MD, TX First release in 1997	Fed on European *Carduus* and *Cirsium spp.* did not use 3 NA *Cirsium* species (Gassmann and Kok 2002).	No recorded establishment	No restrictions
Hadroplontus (Ceutorhynchus) litura Fabricius (Coleoptera: Curculionidae)	Stem weevil	*C. arvense*	Released widely First release in 1972	"Normal larval development occurs on all *Cirsium* species tested, including three NA *spp.*" (McClay 2002).	Established in ID, MT, NE, ND, OR, SD, UT, VA, WA, WY	No restrictions
Larinus planus Fabricius (Coleoptera: Curculionidae) *ADVENTIVE	Seed head weevil	*C. arvense*	Redistributed widely	Feeds on many species in *Cirsium* ; was not expected to develop on large headed spp. (McClay 1989).	Established widely	Adventive, no restrictions

Agent	**Description**	**Target(s)**	**Release Actions**	**Feeding Habit (and source)**	**Status of Agent in US**	**APHIS permit status**
Rhinocyllus conicus Frolich (Coleoptera: Curculionidae)	Seed head weevil	*Carduus spp.* *C. arvense*	Released widely First release in 1969	Larvae feed and develop *Carduus*, *Cirsium*, *Sylibum*, *Onopordum* (Gassmann and Kok 2002).	Established widely	All permits for interstate shipment revoked in 2000
Trichsirocallus horridus Panzer (Coleoptera: Curculionidae)	Rosette weevil	*Carduus spp.*	Released widely First release in 1974	Preferred hosts were *Cirsium*, *Carduus*, and *Onorpordum* (Gassmann and Kok 2002).	Established in CO, ID, KA, MD, MI, MT, OR, VA, WA, WY	Release in CA is prohibited
Cheilosia corydon Harris (Diptera: Syrphidae)	Root crown fly	*Carduus spp.*	Released MD, NJ, MT, NV, OR, TX First release in 1990	Larvae survived on all *Carduus spp.* as well as NA native *Cirsium crassicaule* (Green) Jeps. In field found on *C. palustre* L. (Gassmann and Kok 2002).	Established in OR	No restrictions
Urophora cardui L. (Diptera: Tephritidae)	Gall fly	*C. arvense*	Released widely First release in 1980	Apparently not tested on NA species, but limited feeding on *C. vulgare* and *Carduus acanthoides* L. (McClay 2002).	Established in CA, MD, MT, NV, OR, VA, WA, WY No nontarget effects reported	No restrictions
Urophora solstitalis L. (Diptera: Tephritidae)	Seed fly	*Carduus spp.*	Released MD, MT, OR. First release in 1993	(Gassmann and Kok 2002)	No recorded establishment	No restrictions
Urophora stylata Fabricius (Diptera: Tephritidae)	Seed-feeding fly	*C. vulgare*	Released CA, CO, MD, MT, WA First release in 1983	Field records in Europe include feeding on *Carduus* and *Cirsium spp.* (Kok and Gassmann 2002)	Established in CO, MD, OR, WA	No restrictions
Puccinia carduorum Jacky (Uredinales: Pucciniaceae)	Rust Fungus	*Carduus nutans*	Released widely. First release in 1987 (accidentally prior to 1987).	Limited infestation of *Cirsium*, *Cynara*, *Saussurea*, and *Sylibum* in greenhouse. Didn't persist on tested 22 NA *Cirsium spp.* (Gassmann and Kok 2002).	Established widely	No restrictions
Puccinia punctiformis (Str.) Rohl. (Uredinales: Pucciniaceae) *ADVENTIVE	Rust Fungus	*C. arvense*	Redistributed widely.	No record of persistence on tested NA *Cirsium spp.* (P. Backman, Penn St. Univ.).	Established widely	No restrictions

Appendix B. Native *Cirsium* sites included in the survey, sorted by species, state, and release cluster. The word "None" in the "Release" and "Cluster" columns indicates cases where the native population was not within 10 kilometers of a known release site. Latitudes and longitudes are in decimal degrees. Presence of *L. planus* is indicated in the column "LAPL" and *R. conicus* in the column "RHCO", and Oviposition itensity of *R. conicus* on *Cirsium* species.

Species	St	County	Lat.	Lon.	Elev	Release	Cluster	Population ID	Year Visited	Pop Size	LAPL	RHCO
C. brevistylum	OR	Curry	42.8304	-124.5241	30	None	None	OR3	2003		No	No
C. brevistylum	OR	Curry	42.6842	-124.4241	85	None	None	OR5	2003	2	No	No
C. brevistylum	OR	Curry	42.5006	-124.2889	76	None	None	OR6	2003	75	No	Yes
C. brevistylum	OR	Douglas	43.6837	-124.1487	60	None	None	OR1	2003		No	No
C. calcareum	UT	Utah	39.9304	-111.2166	2438	UTUT4	Manti	UTUT1	2002	2	No	Yes
C. calcareum	UT	Utah	39.9262	-111.2247	2438	UTUT4	Manti	UTUT2.2	2002	1	No	Yes
C. calcareum	UT	Utah	39.8197	-111.23	2743	UTUT6	Manti	UTUT8	2002	8	No	No
C. calcareum	UT	Wayne	38.211	-111.1852	761	None	None	UTCR2.1	2002	5	No	No
C. callilepsis	OR	Jefferson	44.4361	-121.8222	1280	None	None	ORJE1.1	2003		No	Yes
C. callilepsis	OR	Linn	44.5256	-121.9945	1036	None	None	ORLI1.1	2003		No	Yes
C. canovirens	OR	Harney	42.782	-118.7571	1554	None	None	ORHA1.3	2003		No	Yes
C. canovirens	OR	Harney	42.6782	-118.6792	2225	None	None	ORHA1.4	2003		No	Yes
C. canovirens	OR	Klamath	42.224	-121.7144	1371	None	None	ORKL1.2	2003		No	Yes
C. canovirens	OR	Klamath	42.2467	-121.7833	1325	None	None	ORKL1.3	2003		No	Yes
C. canovirens	OR	Lake	42.2242	-120.6895	1584	None	None	OR10	2003		No	Yes
C. canovirens	OR	Lake	42.1831	-120.5294	1523	None	None	OR11	2003		No	Yes
C. canovirens	OR	Lake	42.2023	-120.6737	1554	None	None	ORLA1.2	2003		No	Yes
C. canovirens	OR	Lake	42.565	-119.697	1706	None	None	ORLA1.3	2003		No	Yes
C. canovirens (x subniveum)	OR	Harney	42.7835	-118.7718	1889	ORHA3	Malheur P-ranch	OR12	2003		No	No
C. eatonii	CO	Gunnison	38.8957	-106.8901	2823	None	None	Brush	2001	50	No	Yes
C. eatonii	UT	Piute	38.2548	-112.331		None	None	UTPI2	2002	5	No	No
C. flodmanii	NE	Dawes	42.6774	-103.4748	1205	NEDA1	Ft. Robinson	NEDA.6	2001	180	No	Yes

Species	**St**	**County**	**Lat.**	**Lon.**	**Elev**	**Release**	**Cluster**	**Population ID**	**Year Visited**	**Pop Size**	**LAPL**	**RHCO**
C. flodmanii	NE	Sheridan	42.7838	-102.7146	1162	NESH1	Hay Springs	NESH.1	2001	52	No	Yes
C. flodmanii	NE	Sheridan	42.8035	-102.732	1089	NESH1	Hay Springs	NESH.4	2001	20	No	Yes
C. flodmanii	SD	Custer	43.6252	-103.4903	1412	SDW	Wind Cave	SDW.8	2001	100	Yes	Yes
C. flodmanii	WY	Crook	44.5887	-104.7167	1325	WYCR4	Devils Tower	WYCR4.2	2001	30	No	Yes
C. flodmanii	WY	Crook	44.5922	-104.725	1239	WYCR4	Devils Tower	WYCR4.4	2001	50	No	Yes
C. neomexicanum	NV	Elko	41.8432	-115.7053		None	None	NVEL2.1	2002	10	No	Yes
C. neomexicanum	NV	Elko	41.9831	-115.7046		None	None	NVEL4	2002	30	No	Yes
C. neomexicanum	NV	Elko	41.9794	-115.7017		None	None	NVEL5	2002	40	No	Yes
C. neomexicanum	NV	Elko	41.9296	-115.675		NVEL6	Rattlesnake Cyn	NVEL6.1	2002	30	No	Yes
C. neomexicanum	UT	San Juan	37.3255	-109.334		None	None	UTSJ1	2002	500	No	No
C. occidentale	OR	Curry	42.5833	-124.0215	274	None	None	OR7	2003	3	No	No
C. occidentale	OR	Curry	42.6012	-123.9888	548	None	None	OR8	2003	73	No	Yes
C. ochrocentrum	CO	El Paso	38.6749	-104.7785	1828	COC	Ft. Carson	COC.4	2001	35	No	Yes
C. ochrocentrum	NE	Scotts Bluff	41.9262	-103.9297	1225	NESC1	Scotts Bluff East	NESC.1	2001	60	No	Yes
C. ochrocentrum	NE	Scotts Bluff	41.9399	-104.0261	1225	NESC1	Scotts Bluff East	NESC.2	2001	32	No	Yes
C. ochrocentrum	NE	Scotts Bluff	41.9292	-104.0038	1225	NESC1	Scotts Bluff East	NESC.3	2001	20	No	Yes
C. peckii	OR	Harney	42.7496	-118.6811	2103	None	None	ORHA1.1	2003		No	Yes
C. peckii	OR	Harney	42.6696	-118.5914	2804	None	None	ORHA1.2	2003		No	Yes
C. peckii	OR	Harney	42.1648	-118.6046	1310	None	None	ORHA1.5	2003		No	Yes
C. perplexans	CO	Montrose	38.3633	-107.5043	2377	None	None	Sign	2001	70	No	Yes
C. perplexans	CO	Montrose	38.3304	-107.5373	2468	None	None	View	2001	40	No	Yes
C. perplexans	CO	Montrose	38.4429	-107.547	2346	None	None	Wires	2001	10	No	Yes
C. remotifolium	OR	Curry	42.8356	-124.556	15	None	None	OR4	2003	40	No	No
C. scariosum	CO	Gunnison	38.6936	-106.4758	3749	None	None	CPASS	2001	53	No	No
C. scariosum	ID	Valley	44.3519	-116	1463	None	None	IDVA2	2003	4	No	Yes
C. scariosum	ID	Caribou	42.6478	-111.6282	1798	IDCA1	Soda Springs	IDCA1.1	2003	1	No	Yes
C. scariosum	ID	Caribou	42.6925	-111.5942	1859	IDCA2	Soda Springs	IDCA2.1	2003	6	No	Yes

Species	**St**	**County**	**Lat.**	**Lon.**	**Elev**	**Release**	**Cluster**	**Population ID**	**Year Visited**	**Pop Size**	**LAPL**	**RHCO**
C. scariosum	NV	Elko	41.8432	-115.7053		None	None	NVEL2.2	2002	25	No	Yes
C. scariosum	NV	Elko	41.9296	-115.675		NVEL6	Rattlesnake Cyn	NVEL6.2	2002	15	No	Yes
C. scariosum	OR	Klamath	42.8791	-121.762	1402	None	None	ORKL1.1	2003		No	Yes
C. scariosum	OR	Lake	42.3269	-120.8016	1645	None	None	OR9	2003		No	No
C. scariosum	UT	Sanpete	39.7072	-111.2955	2743	UTSP1	Gooseberry	UTSP1.2	2002			
C. scariosum	UT	Sanpete	39.7034	-111.2973	2743	UTSP2	Gooseberry	UTSP2	2002	100	No	Yes
C. scariosum	UT	Carbon	39.8007	-111.214	2377	UTCA1	Manti	UTCA1.3	2002	100	No	Yes
C. scariosum	UT	Carbon	39.7726	-111.1978	2346	UTCA2	Manti	UTCA2.3	2002	20	No	Yes
C. scariosum	UT	Carbon	39.7751	-111.1973	2346	UTCA3	Manti	UTCA3.1	2002		No	Yes
C. scariosum	UT	Utah	39.8387	-111.2785	2743	UTUT6	Manti	UTUT6	2002	150	No	No
C. scopulorum	UT	Carbon	39.8007	-111.214	2377	UTCA1	Manti	UTCA1.4	2002	100	No	Yes
C. subniveum	OR	Baker	44.7376	-117.0565	1158	None	None	ORBA1.1	2003		No	Yes
C. subniveum	OR	Malheur	43.4315	-117.1173	1402	None	None	ORMA1.3	2003		No	Yes
C. subniveum	OR	Malheur	43.4064	-117.1242	1036	None	None	ORMA1.4	2003		No	No
C. undulatum var. undulatum	CO	El Paso	38.7289	-104.8106	1828	COC	Ft. Carson	COC.1	2001	50	Yes	Yes
C. undulatum var. undulatum	CO	El Paso	38.6749	-104.7748	1828	COC	Ft. Carson	COC.2	2001	170	No	Yes
C. undulatum var. undulatum	CO	El Paso	38.6636	-104.7726	1828	COC	Ft. Carson	COC.3	2001	115	No	Yes
C. undulatum var. undulatum	CO	El Paso	38.6752	-104.78	1828	COC	Ft. Carson	COC.5	2001	135	No	Yes
C. undulatum var. undulatum	CO	El Paso	38.708	-104.7822	1828	COC	Ft. Carson	COC.6	2001	180	No	Yes
C. undulatum var. undulatum	CO	Jefferson	39.8328	-105.2275	1767	COJE2	Jefferson Cty	COJE2.1	2001	16	No	No
C. undulatum var. undulatum	CO	Jefferson	39.8752	-105.1176	1767	COJE2	Jefferson Cty	COJE2.2	2001	10	No	Yes
C. undulatum var. undulatum	CO	Jefferson	39.8748	-105.1162	1767	COJE2	Jefferson Cty	COJE2.3	2001	13	No	Yes
C. undulatum var. undulatum	CO	Jefferson	39.7325	-105.237	1767	COJE2	Jefferson Cty	COJE3.1	2001	5	No	Yes
C. undulatum var. undulatum	CO	Jefferson	39.7325	-105.2374	1767	COJE2	Jefferson Cty	COJE3.2	2001	3	No	Yes
C. undulatum var. undulatum	CO	Jefferson	39.7319	-105.2389	2194	COJE3	Jefferson Cty	COJE3.3	2001	6	No	No
C. undulatum var. undulatum	CO	Jefferson	39.7419	-105.2396	2194	COJE3	Jefferson Cty	COJE3.4	2001	6	Yes	Yes
C. undulatum var. undulatum	CO	Jefferson	39.7463	-105.2397	2194	COJE3	Jefferson Cty	COJE3.5	2001	5	No	No

Species	**St**	**County**	**Lat.**	**Lon.**	**Elev**	**Release**	**Cluster**	**Population ID**	**Year Visited**	**Pop Size**	**LAPL**	**RHCO**
C. undulatum var. undulatum	CO	Jefferson	39.75	-105.2403	2194	COJE3	Jefferson Cty	COJE3.5.1	2001	2	No	Yes
C. undulatum var. undulatum	CO	Jefferson	39.7429	-105.235	2194	COJE3	Jefferson Cty	COJE3.6	2001	12	No	Yes
C. undulatum var. undulatum	CO	Jefferson	39.8002	-105.2486	1920	COJE5	Jefferson Cty	COJE5.1	2001		No	No
C. undulatum var. undulatum	CO	Jefferson	39.6778	-105.2432	2011	COJE7	Jefferson Cty	COJE7.1	2001	3	Yes	No
C. undulatum var. undulatum	CO	Jefferson	39.6677	-105.2547	2011	COJE7	Jefferson Cty	COJE7.2	2001	4	No	No
C. undulatum var. undulatum	CO	Jefferson	39.667	-105.2517	2011	COJE7	Jefferson Cty	COJE7.3	2001	5	No	Yes
C. undulatum var. undulatum	CO	Jefferson	39.6673	-105.2521	2011	COJE7	Jefferson Cty	COJE7.4	2001	1	No	Yes
C. undulatum var. undulatum	CO	Jefferson	39.6667	-105.2519	2011	COJE7	Jefferson Cty	COJE7.5	2001	4	Yes	Yes
C. undulatum var. undulatum	ID	Caribou	42.5511	-111.8296	1950	IDCA3	Beaver Basin	IDCA3.1	2003	9	No	Yes
C. undulatum var. undulatum	ID	Caribou	42.5381	-111.8545	1950	IDCA3	Beaver Basin	IDCA3.2	2003	8	No	Yes
C. undulatum var. undulatum	ID	Valley	44.3519	-116	1463	None	None	IDVA1	2003	1	No	Yes
C. undulatum var. undulatum	NE	Sioux	42.437	-103.7881	1406	NESI2	Agate	NESI.6	2001	14	No	Yes
C. undulatum var. undulatum	NE	Sioux	42.4108	-103.7908	1369	NESI2	Agate	NESI.7	2001	7	No	Yes
C. undulatum var. undulatum	NE	Sioux	42.3988	-103.8104	1366	NESI2	Agate	NESI.8	2001	50	No	No
C. undulatum var. undulatum	NE	Sioux	42.4222	-103.7693	1348	NESI2	Agate	NESI.9	2001	15	No	Yes
C. undulatum var. undulatum	NE	Dawes	42.7709	-103.0864	1091	NEDA4	Chadron	NEDA.1	2001	5	No	No
C. undulatum var. undulatum	NE	Dawes	42.727	-103.1114	1092	NEDA4	Chadron	NEDA.2	2001	11	No	Yes
C. undulatum var. undulatum	NE	Dawes	42.6775	-103.1859	1164	NEDA4	Chadron	NEDA.3	2001	14	No	Yes
C. undulatum var. undulatum	NE	Dawes	42.6686	-103.1873	1167	NEDA4	Chadron	NEDA.4	2001	12	No	Yes
C. undulatum var. undulatum	NE	Dawes	42.6727	-103.4543	1194	NEDA1	Ft. Robinson	NEDA.5	2001	7	No	Yes
C. undulatum var. undulatum	NE	Dawes	42.6859	-103.4734	1238	NEDA1	Ft. Robinson	NEDA.7	2001	20	No	Yes
C. undulatum var. undulatum	NE	Sioux	42.7214	-103.7469	1286	NESI3	Harrison	NESI.1	2001	31	No	No
C. undulatum var. undulatum	NE	Sioux	42.7848	-103.8014	1227	NESI3	Harrison	NESI.2	2001	30	No	Yes
C. undulatum var. undulatum	NE	Sioux	42.8254	-103.7981	1203	NESI3	Harrison	NESI.3	2001	19	No	Yes
C. undulatum var. undulatum	NE	Sheridan	42.7842	-102.7149	1115	NESH1	Hay Springs	NESH.2	2001	1	No	No
C. undulatum var. undulatum	NE	Sheridan	42.7915	-102.718	1106	NESH1	Hay Springs	NESH.3	2001	9	No	Yes
C. undulatum var. undulatum	NE	Sheridan	42.8003	-102.7259	1096	NESH1	Hay Springs	NESH.5	2001	17	No	No

Species	**St**	**County**	**Lat.**	**Lon.**	**Elev**	**Release**	**Cluster**	**Population ID**	**Year Visited**	**Pop Size**	**LAPL**	**RHCO**
C. undulatum var. undulatum	NE	Sheridan	42.7583	-102.6955	1170	NESH1	Hay Springs	NESH.6	2001	10	No	Yes
C. undulatum var. undulatum	NE	Sioux	42.9126	-103.8201	1171	NESI1	Ogalala	NESI.4	2001	58	No	Yes
C. undulatum var. undulatum	NE	Sioux	42.9165	-103.8694	1193	NESI1	Ogalala	NESI.5	2001	73	No	Yes
C. undulatum var. undulatum	OR	Harney	43.7119	-118.5126	1280	None	None	ORHA1.6	2003		No	No
C. undulatum var. undulatum	OR	Lake	42.2504	-120.3165	1645	None	None	ORLA1.1	2003		No	Yes
C. undulatum var. undulatum	OR	Malheur	43.0574	-117.0704	1371	None	None	ORMA1.1	2003		No	Yes
C. undulatum var. undulatum	OR	Malheur				None	None	ORMA1.2	2003			
C. undulatum var. undulatum	OR	Wasco	45.2691	-121.1897	365	None	None	ORWA1.1	2003		Yes	Yes
C. undulatum var. undulatum	SD	Custer	43.7373	-103.8169	1713	None	None	SDC.1	2001	20	No	Yes
C. undulatum var. undulatum	SD	Custer	43.8408	-103.3914	1416	None	None	SDC.2	2001	25	No	Yes
C. undulatum var. undulatum	SD	Custer	43.5806	-103.4849	1306	SDW	Wind Cave	SDW.1	2001	10	Yes	Yes
C. undulatum var. undulatum	SD	Custer	43.6293	-103.4366	1430	SDW	Wind Cave	SDW.10	2001	11	Yes	Yes
C. undulatum var. undulatum	SD	Custer	43.6274	-103.3767	1206	SDW	Wind Cave	SDW.11	2001	12	Yes	Yes
C. undulatum var. undulatum	SD	Custer	43.6282	-103.3755	1270	SDW	Wind Cave	SDW.12	2001	14	Yes	Yes
C. undulatum var. undulatum	SD	Custer	43.6237	-103.3766	1270	SDW	Wind Cave	SDW.13	2001	15	Yes	Yes
C. undulatum var. undulatum	SD	Custer	43.5813	-103.4833	1296	SDW	Wind Cave	SDW.2	2001	10	Yes	Yes
C. undulatum var. undulatum	SD	Custer	43.5599	-103.4909	1313	SDW	Wind Cave	SDW.3	2001	20	Yes	Yes
C. undulatum var. undulatum	SD	Custer	43.5608	-103.4926	1314	SDW	Wind Cave	SDW.4	2001	25	Yes	Yes
C. undulatum var. undulatum	SD	Custer	43.5589	-103.4957	1315	SDW	Wind Cave	SDW.5	2001	8	No	No
C. undulatum var. undulatum	SD	Custer	43.5579	-103.4941	1315	SDW	Wind Cave	SDW.6	2001	20	No	No
C. undulatum var. undulatum	SD	Custer	43.6156	-103.4931	1416	SDW	Wind Cave	SDW.7	2001	9	Yes	Yes
C. undulatum var. undulatum	SD	Custer	43.5934	-103.4895	1291	SDW	Wind Cave	SDW.9	2001	20	No	Yes
C. undulatum var. undulatum	WA	Lincoln	47.3639	-118.4075	188	WALI6	Coal Creek	WALI1.5	2003	35	Yes	Yes
C. undulatum var. undulatum	WA	Lincoln	47.3828	-117.8273	198	WALI1	Fish Trap	WALI1.1	2003	9	Yes	Yes
C. undulatum var. undulatum	WA	Lincoln	47.3794	-117.8126	198	WALI1	Fish Trap	WALI1.2	2003	2	Yes	Yes
C. undulatum var. undulatum	WA	Lincoln	47.3735	-117.8126	198	WALI1	Fish Trap	WALI1.3	2003	4	Yes	Yes
C. undulatum var. undulatum	WA	Lincoln	47.3272	-117.8748	198	WALI3	Fish Trap	WALI1.4	2003	7	Yes	Yes

Species	St	County	Lat.	Lon.	Elev	Release	Cluster	Population ID	Year Visited	Pop Size	LAPL	RHCO
C. undulatum var. undulatum	WA	Whitman	46.4253	-117.1775	82	None	None	WAWH1	2003	10	Yes	Yes
C. undulatum var. undulatum	WA	Whitman	46.4559	-117.2108	91	None	None	WAWH2	2003	9	Yes	Yes
C. undulatum var. undulatum	WA	Lincoln	47.5349	-118.5187	198	WALI7	Twin Lakes	WALI7.1	2003	17	No	Yes
C. undulatum var. undulatum	WY	Crook	44.5835	-104.7236	1196	WYCR4	Devils Tower	WYCR4.1	2001	15	No	Yes
C. undulatum var. undulatum	WY	Crook	44.5988	-104.7239	1280	WYCR4	Devils Tower	WYCR4.3	2001	15	No	Yes
C. undulatum var. undulatum	WY	Converse	42.8411	-105.486	1488	WYCO15	Ft. Fetterman	WYCO15.2	2001	4	No	No
C. undulatum var. undulatum	WY	Converse	42.8347	-105.4935	1496	WYCO15	Ft. Fetterman	WYCO15.3	2001	10	No	Yes
C. undulatum var. undulatum	WY	Converse	42.8545	-105.5002	1500	WYCO15	Ft. Fetterman	WYCO15.4	2001	6	No	Yes
C. undulatum var. undulatum	WY	Campbell	44.3014	-105.4989	1383	WYCA1	Gillette	WYCA1.1	2001	15	No	Yes
C. undulatum var. undulatum	WY	Goshen	41.6376	-104.2115	1458	WYGO2	La Grange	WYGO2.1	2001	60	No	Yes
C. undulatum var. undulatum	WY	Goshen	41.6552	-104.2339	1430	WYGO2	La Grange	WYGO2.2	2001	31	No	No
C. undulatum var. undulatum	WY	Converse	42.8253	-105.1715	1556	WYCO5	Shawnee	WYCO5.1	2001	14	No	Yes
C. undulatum var. undulatum	WY	Converse	42.8251	-105.2101	1584	WYCO5	Shawnee	WYCO5.2	2001	7	No	Yes
C. undulatum var. undulatum	WY	Platte	41.8738	-104.7886	1543	WYPL1	Slater	WYPL1.2	2001	30	No	Yes
C. undulatum var. undulatum	WY	Platte	41.9025	-104.7887	1585	WYPL1	Slater	WYPL1.3	2001	20	No	Yes
C. undulatum var. undulatum	WY	Platte	41.887	-104.7886	1563	WYPL1	Slater	WYPL1.4	2001	10	No	Yes
C. undulatum var. undulatum	WY	Platte	41.8714	-104.7684	1610	WYPL1	Slater	WYPL1.5	2001	15	No	No
C. undulatum var. undulatum	WY	Platte	42.1046	-104.9075	1402	WYPL5	Wheatland	WYPL5.1	2001	50	No	Yes
C. undulatum var. undulatum	WY	Platte	42.1046	-104.9068	1402	WYPL5	Wheatland	WYPL5.2	2001	2	No	Yes
C. undulatum var. undulatum	WY	Platte	42.121	-104.8589	1359	WYPL5	Wheatland	WYPL5.3	2001	3	No	Yes
C. undulatum var. undulatum	WY	Platte	42.1211	-104.8563	1359	WYPL5	Wheatland	WYPL5.4	2001	3	No	Yes
C. undulatum var tracyi	CO	Gunnison	38.6509	-106.8632	2438	COGU2	Almont	Bluff	2001	25	Yes	No
C. undulatum var tracyi	CO	Gunnison	38.6648	-106.8424	2444	COGU2	Almont	Cabins	2001	25	Yes	No
C. undulatum var tracyi	CO	Gunnison	38.731	-106.7537	2621	COGU2	Almont	Onemile	2001	40	Yes	No
C. undulatum var tracyi	CO	Gunnison	38.653	-106.8609	2438	COGU2	Almont	Tree	2001	10	Yes	No
C. undulatum var tracyi	CO	Montezuma	37.2974	-108.4124	2164	COMESA	Comesa	COMESA1	2002	5	No	Yes
C. undulatum var tracyi	CO	Montezuma	37.2723	-108.4609	2164	COMESA	Comesa	COMESA2	2002	5	No	Yes

Species	**St**	**County**	**Lat.**	**Lon.**	**Elev**	**Release**	**Cluster**	**Population ID**	**Year Visited**	**Pop Size**	**LAPL**	**RHCO**
C. undulatum var tracyi	CO	Montezuma	37.2537	-108.5528	2164	COMESA	Comesa	COMESA3	2002	6	No	Yes
C. undulatum var tracyi	CO	Gunnison	38.5477	-107.3217	2377	None	None	Soap	2001	6	No	Yes
C. undulatum var tracyi	CO	Gunnison	38.4461	-107.308	2285	None	None	Cove	2001	32	No	Yes
C. undulatum var tracyi	CO	Gunnison	38.4595	-107.2573	2438	None	None	Cut	2001	15	No	Yes
C. undulatum var tracyi	CO	Gunnison	38.453	-107.3299	2285	None	None	Lake	2001	21	No	Yes
C. undulatum var tracyi	CO	Gunnison	38.4462	-107.3409	2362	None	None	Pine	2001	27	No	Yes
C. undulatum var tracyi	CO	Montezuma	37.3236	-108.6911	2164	None	None	COMO1	2002	6	No	Yes
C. undulatum var tracyi	CO	Gunnison	38.9292	-107.3467	1981	COGU1	Paonia Res.	COGU1.1	2002	25	No	Yes
C. undulatum var tracyi	CO	Gunnison	38.9428	-107.3529	1981	COGU1	Paonia Res.	COGU1.2	2002	12	Yes	Yes
C. undulatum var tracyi	UT	Utah	39.8828	-111.2642	2590	UTUT4	Manti	UTUT3	2002	5	No	Yes
C. undulatum var tracyi	UT	Emery	39.4832	-110.5123		None	None	UTEM1	2002	20	No	No
C. undulatum var tracyi	UT	Piute	38.2623	-112.2968		None	None	UTPI1	2002	1	No	No
C. undulatum var tracyi	UT	Piute	38.2229	-112.3862		None	None	UTPI3	2002	30	No	No
C. undulatum var tracyi	UT	Piute	38.2492	-112.4823		None	None	UTPI4	2002	15	No	No
C. undulatum var tracyi	UT	Wayne	38.259	-111.2369	761	None	None	UTCR1	2002	25	No	No

Appendix C. Release clusters and presence/absence of *Larinus planus* and *Rhinocyllus conicus* on both *Cirsium arvense* and native *Cirsium* taxa.

Release Cluster and Native *Cirsium* Encountered	**State**	**First Known Release**	**Number of Known Releases**	**Elevation (m)**	**No. Native *Cirsium* Populations**	***L. planus* on *C. arvense***	***L. planus* on Native *Cirsium***	***R. conicus* on *C. arvense***	***R. conicus* on Native *Cirsium***
ALMONT	CO	1992	2	2438		Yes		No	
C. undulatum var. tracyi					4		Yes		No
FORT CARSON	CO	1997	2	1828		Yes		Yes	
C. undulatum var. undulatum					5		No		Yes
C. ochrocentrum					1		No		Yes
JEFFERSON CNTY	CO	1993	6	1932		Yes		Yes	
C. undulatum var. undulatum					16		Yes		Yes
PAONIA RESERVOIR	CO	1997	1	1981		Yes		Yes	
C. undulatum var. tracyi					2		Yes		No
RAINBOW LAKE	CO	1992	2	3314		No		No	
No native taxa found							--		--
SODA SPRING	ID	2000	2	1798		No		Yes	
C. scariosum					2		No		Yes
AGATE	NE	1997	1	1349		No		Yes	
C. undulatum var. undulatum					4		No		Yes
BOX BUTTE	NE	1997	2	1212		No		Yes	
No native taxa found							--		--
CHADRON	NE	1996	6	1098		No		Yes	
C. undulatum var. undulatum					4		No		Yes
FORT ROBINSON	NE	1996	1	1205		No		Yes	
C. undulatum var. undulatum					2		No		Yes
C. flodmanii					1		No		Yes
HARRISON	NE	1997	1	1244		No		Yes	
C. undulatum var. undulatum					3		No		Yes
HAY SPRINGS	NE	1997	2	1184		No		Yes	
C. flodmanii					2		No		Yes
C. undulatum var. undulatum					4		No		Yes
OGALALA GRASSLAND	NE	1996	1	1143		No		Yes	
C. undulatum var. undulatum					2		No		Yes
SCOTTS BLUFF EAST	NE	1998	4	1184		No		Yes	
No native taxa found							--		--
SCOTTS BLUFF WEST	NE	1998	7	1224		No		Yes	
C. ochrocentrum					3		No		Yes

Release Cluster and Native *Cirsium* Encountered	**State**	**First Known Release**	**Number of Known Releases**	**Elevation (m)**	**No. Native *Cirsium* Populations**	***L. planus* on *C. arvense***	***L. planus* on Native *Cirsium***	***R. conicus* on *C. arvense***	***R. conicus* on Native *Cirsium***
RATTLESNAKE CYN	NV	1995	1	1500		Yes		Yes	
C. neomexicanum					4		No		Yes
C. scariosum					2		No		Yes
EEWILSON	OR	1985	1	76		Yes		Yes	
No native taxa found							--		--
MALHEUR BENSON	OR	1995	1	1219		Yes		Yes	
No native taxa found							--		--
WIND CAVE	SD	1992	13	1298		Yes		No	
C. flodmanii					1		Yes		Yes
C. undulatum var. undulatum					12		Yes		Yes
GOOSEBERRY	UT	1999	2	2743		No		Yes	
C. scariosum					2		No		Yes
MANTI	UT	1996	6	2524		No		Yes	
C. calcareum					3		No		Yes
C. undulatum var. tracyi					1		No		Yes
C. scariosum					4		No		Yes
C. scopulorum					1		No		Yes
COAL CREEK	WA	2000	2	188		Yes		Yes	
C. undulatum					1		Yes		Yes
FISH TRAP	WA	1999	4	198		Yes		Yes	
C. undulatum					4		Yes		Yes
MARLIN	WA	1999	2	146		Yes		Yes	
No native taxa found							--		--
WILSON CREEK	WA	1999	1	182	0	Yes		No	
No native taxa found							--		--
FORT FETTERMAN	WY	1997	6	1496		No		Yes	
C. undulatum var. undulatum					3		No		Yes
DEVILS TOWER	WY	1996	4	1180		No		Yes	
C. flodmanii					2		No		Yes
C. undulatum var. undulatum					2		No		Yes
GILLETE	WY	1996	1	1381		No		Yes	
C. undulatum var. undulatum					1		No		Yes
LA GRANGE	WY	1993	1	1479		No		Yes	
C. undulatum var. undulatum					2		No		Yes
SHAWNEE	WY	1996	1	1575		No		Yes	
C. undulatum var. undulatum					2		No		Yes

Release Cluster and Native *Cirsium* Encountered	**State**	**First Known Release**	**Number of Known Releases**	**Elevation (m)**	**No. Native *Cirsium* Populations**	***L. planus* on *C. arvense***	***L. planus* on Native *Cirsium***	***R. conicus* on *C. arvense***	***R. conicus* on Native *Cirsium***
SLATER	WY	1994	2	1587		No		Yes	
C. undulatum var. undulatum					4		No		Yes
SUMMIT INFO	WY	1996	1	2604		No		Yes	
No native taxa found							--		--
WHEATLAND	WY	1997	2	1391		No		Yes	
C. undulatum var. undulatum					4		No		Yes

Appendix D. Oviposition intensity of *R. conicus* on *Cirsium* species. Oviposition intensity is calculated as proportion of susceptible capitula with external evidence of oviposition and/or feeding larvae. Primary (1˚) capitula are the main terminal capitulum and then all terminal heads on branches from the main stem. Secondary (2˚) capitula are terminal heads from sub-branches off main branches, and tertiary (3˚) capitula are those that subtend secondary capitula. N = sample size, SD = standard deviation. Some elevations have been estimated with the use of maps (noted "e").

Species	**State**	**County**	**Pop.**	**Lat.**	**Long.**	**Elev. (m)**	**Avg. Ovip Freq. 1˚ Capitula**	**N (1˚)**	**SD (1˚)**	**Avg. Ovip Freq. 2˚ Capitula**	**N (2˚)**	**SD (2˚)**	**Avg. Ovip Freq. 3˚ Capitula**	**N (3˚)**	**SD (3˚)**
C. brevistylum	OR	Curry	OR6	42.501	-124.289	76	0.70	20	0.47	n/a	0		n/a	0	
C. calcareum	UT	Utah	UTUT1	39.93	-111.217	2438	1.00	10	0.00	0.33	3	0.58	n/a	0	
C. calcareum	UT	Utah	UTUT2.2	39.926	-111.225	2438	1.00	6	0.00	0.00	1	0.00	n/a	0	
C. callilepsis	OR	Linn	ORLI1.1	44.526	-121.994	1036	0.43	7	0.53	n/a	0		n/a	0	
C. canovirens	OR	Harney	ORHA1.4	42.678	-118.679	2225	0.53	19	0.51	0.40	5	0.55	n/a	0	
C. canovirens	OR	Lake	OR10	42.224	-120.69	1584	0.90	31	0.30	0.52	23	0.51	n/a	0	
C. canovirens	OR	Lake	ORLA1.2	42.202	-120.674	1554	1.00	21	0.00	0.82	17	0.39	n/a	0	
C. eatonii	CO	Gunnison	BRUSH	38.896	-106.89	2823	0.84	56	0.37	0.54	26	0.51	0.33	3	0.58
C. flodmanii	NE	Dawes	NEDA.6	42.677	-103.475	1205	0.19	31	0.40	0.00	39	0.00	0.00	2	0.00
C. flodmanii	NE	Sheridan	NESH.1	42.784	-102.715	1162	0.10	21	0.30	0.00	3	0.00	n/a	0	
C. flodmanii	SD	Custer	SDW.8	43.625	-103.49	1412	0.64	44	0.49	0.29	31	0.46	0.00	1	0.00
C. flodmanii	WY	Crook	WYCR4.2	44.589	-104.717	1325	0.72	29	0.45	0.18	11	0.40	n/a	0	
C. flodmanii	WY	Crook	WYCR4.4	44.592	-104.725	1239	0.59	17	0.51	n/a	0		n/a	0	
C. neomexicanum	NV	Elko	NVEL2.1	41.843	-115.705	1500e	1.00	26	0.00	1.00	16	0.00	n/a	0	
C. neomexicanum	NV	Elko	NVEL4	41.983	-115.705	1500e	0.96	28	0.19	0.89	19	0.32	n/a	0	
C. occidentale	OR	Curry	OR8	42.601	-123.989	548	0.77	26	0.43	n/a	0		n/a	0	
C. ochrocentrum	CO	El Paso	COC.4	38.675	-104.779	1828	0.07	14	0.27	n/a	0		n/a	0	
C. ochrocentrum	NE	Scotts Bluff	NESC.1	41.926	-103.93	1225	0.07	27	0.27	0.00	1	0.00	n/a	0	
C. ochrocentrum	NE	Scotts Bluff	NESC.2	41.94	-104.026	1225	0.05	22	0.21	0.00	12	0.00	n/a	0	
C. peckii	OR	Harney	ORHA1.1	42.75	-118.681	2103	0.38	8	0.52	n/a	0		n/a	0	
C. peckii	OR	Harney	ORHA1.2	42.67	-118.591	2804	1.00	5	0.00	n/a	0		n/a	0	

Species	**State**	**County**	**Pop.**	**Lat.**	**Long.**	**Elev. (m)**	**Avg. Ovip Freq. 1° Capitula**	**N (1°)**	**SD (1°)**	**Avg. Ovip Freq. 2° Capitula**	**N (2°)**	**SD (2°)**	**Avg. Ovip Freq. 3° Capitula**	**N (3°)**	**SD (3°)**
C. perplexans	CO	Montrose	SIGN	38.363	-107.504	2377	0.83	63	0.38	0.37	35	0.49	n/a	0	
C. perplexans	CO	Montrose	VIEW	38.33	-107.537	2468	0.66	74	0.48	0.46	106	0.50	0.18	11	0.40
C. perplexans	CO	Montrose	WIRES	38.443	-107.547	2346	0.68	71	0.47	0.40	25	0.50	0.00	2	0.00
C. perplexans	CO	Mesa	De Beq. N	39.342	-108.213	1560	0.00	42	0.00	0.00	48	0.00	n/a	0	
C. perplexans	CO	Delta	Paonia Hill	38.865	-107.598	1724	0.00	10	0.00	0.00	24	0.00	n/a	0	
C. perplexans	CO	Ouray	Chaf Gulch	38.277	-107.736	2148	0.04	24	0.09	0.00	0	0.00	n/a	0	
C. perplexans	CO	Montrose	Doug Ck 1	38.647	-107.568	2095	0.77	18	0.25	0.41	18	0.31	n/a	0	
C. perplexans	CO	Montrose	Doug Ck 2	38.649	-107.554	2124	0.65	13	0.49	0.86	7	0.00	n/a	0	
C. perplexans	CO	Montrose	Doug Ck 6	38.658	-107.551	2160	0.54	19	0.49	0.23	62	0.33	n/a	0	
C. perplexans	CO	Montrose	Doug Ck 7	38.649	-107.551	2159	0.90	12	0.14	0.58	39	0.19	n/a	0	
C. perplexans	CO	Mesa	Pyr Rock E	39.311	-108.268	2000e	0.00	35	0.00	0.00	28	0.00	n/a	0	
C. perplexans	CO	Montrose	W Landfill	38.525	-107.825	1890	0.12	27	0.14	0.00	48	0.00	n/a	0	
C. perplexans	CO	Mesa	Wint Flats 2	39.291	-108.422	1856	0.48	31	0.43	0.34	14	0.24	n/a	0	
C. scariosum	ID	Caribou	IDCA1.1	42.648	-111.628	1798	0.74	19	0.45	n/a	0		n/a	0	
C. scariosum	ID	Caribou	IDCA2.1	42.692	-111.594	1859	1.00	48	0.00	0.00	1	0.00	n/a	0	
C. scariosum	UT	Carbon	UTCA1.3	39.801	-111.214	2377	1.00	25	0.00	n/a	0		n/a	0	
C. scariosum	UT	Carbon	UTCA2.3	39.773	-111.198	2346	0.68	25	0.48	n/a	0		n/a	0	
C. scariosum	UT	Carbon	UTCA3.1	39.775	-111.197	2346	0.75	20	0.44	n/a	0		n/a	0	
C. undulatum	ID	Caribou	IDCA3.1	42.551	-111.83	1950	0.84	31	0.37	0.22	18	0.43	0.00	2	0.00
C. undulatum	ID	Caribou	IDCA3.2	42.538	-111.854	1950	0.92	36	0.28	0.70	61	0.46	0.18	17	0.39
C. undulatum	OR	Lake	ORLA1.1	42.25	-120.316	1645	0.75	20	0.44	0.31	32	0.47	0.00	13	0.00
C. undulatum	OR	Wasco	ORWA1.1	45.269	-121.19	365	0.75	4	0.50	0.00	2	0.00	n/a	0	
C. undulatum	WA	Lincoln	WALI1.1	47.383	-117.827	198	0.16	31	0.37	0.00	16	0.00	n/a	0	
C. undulatum	WA	Lincoln	WALI1.2	47.379	-117.813	198	0.58	26	0.50	0.06	49	0.24	0.00	13	0.00
C. undulatum	WA	Lincoln	WALI1.3	47.374	-117.813	198	0.91	11	0.30	0.59	22	0.50	0.00	22	0.00
C. undulatum	WA	Lincoln	WALI1.5	47.364	-118.407	188	0.75	40	0.44	0.23	64	0.43	0.00	26	0.00

Species	State	County	Pop.	Lat.	Long.	Elev. (m)	Avg. Ovip Freq. 1° Capitula	N (1°)	SD (1°)	Avg. Ovip Freq. 2° Capitula	N (2°)	SD (2°)	Avg. Ovip Freq. 3° Capitula	N (3°)	SD (3°)
C. undulatum	WA	Lincoln	WALI7.1	47.535	-118.519	198	0.85	60	0.36	0.13	116	0.34	0.00	3	0.00
C. undulatum	WA	Whitman	WAWH1	46.425	-117.177	82	0.39	18	0.50	0.09	47	0.28	0.00	54	0.00
C. undulatum var. tracyi	CO	Gunnison	COGU1.1	38.929	-107.347	1981	0.08	36	0.28	0.00	57	0.00	0.00	23	0.00
C. undulatum var. tracyi	CO	Gunnison	COGU1.2	38.943	-107.353	1981	0.92	13	0.28	0.30	10	0.48	0.00	1	0.00
C. undulatum var. tracyi	CO	Gunnison	PINE	38.446	-107.341	2362	0.73	30	0.45	0.52	108	0.50	0.06	68	0.24
C. undulatum var. tracyi	CO	Montezuma	COMESA2	37.272	-108.461	2164	1.00	20	0.00	0.89	37	0.31	0.33	24	0.48
C. undulatum var. tracyi	CO	Montezuma	COMESA3	37.254	-108.553	2164	0.96	25	0.20	0.67	36	0.48	0.31	16	0.48
C. undulatum var. tracyi	CO	Montezuma	COMO1	37.324	-108.691	2164	0.95	37	0.23	0.64	56	0.48	0.71	7	0.49
C. undulatum var. tracyi	UT	Utah	UTUT3	39.883	-111.264	2590	0.92	24	0.28	0.76	38	0.43	0.00	6	0.00
C. undulatum var. undu	CO	El Paso	COC.1	38.729	-104.811	1828	0.48	23	0.51	0.20	5	0.45	n/a	0	
C. undulatum var. undu	CO	El Paso	COC.2	38.675	-104.775	1828	0.50	14	0.52	n/a	0		n/a	0	
C. undulatum var. undu	CO	El Paso	COC.3	38.664	-104.773	1828	0.67	9	0.50	n/a	0		n/a	0	
C. undulatum var. undu	CO	El Paso	COC.5	38.675	-104.78	1828	0.58	24	0.50	0.33	18	0.49	n/a	0	
C. undulatum var. undu	CO	El Paso	COC.6	38.708	-104.782	1828	0.93	14	0.27	n/a	0		n/a	0	
C. undulatum var. undu	CO	Jefferson	COJE2.2	39.875	-105.118	1767	0.06	17	0.24	n/a	0		n/a	0	
C. undulatum var. undu	CO	Jefferson	COJE3.2	39.732	-105.237	1767	0.60	5	0.55	n/a	0		n/a	0	
C. undulatum var. undu	CO	Jefferson	COJE3.6	39.743	-105.235	2194	0.36	14	0.50	0.00	2	0.00	n/a	0	
C. undulatum var. undu	CO	Jefferson	COJE7.3	39.667	-105.252	2011	0.20	5	0.45	1.00	2	0.00	n/a	0	
C. undulatum var. undu	CO	Jefferson	COJE7.4	39.667	-105.252	2011	0.50	2	0.71	n/a	0		n/a	0	
C. undulatum var. undu	CO	Jefferson	COJE7.5	39.667	-105.252	2011	0.67	3	0.58	0.00	1	0.00	n/a	0	
C. undulatum var. undu	NE	Dawes	NEDA.2	42.727	-103.111	1092	0.16	25	0.37	0.00	1	0.00	n/a	0	
C. undulatum var. undu	NE	Dawes	NEDA.3	42.677	-103.186	1164	0.29	17	0.47	0.00	1	0.00	n/a	0	
C. undulatum var. undu	NE	Dawes	NEDA.7	42.686	-103.473	1238	0.82	11	0.40	n/a	0		n/a	0	
C. undulatum var. undu	NE	Sheridan	NESH.3	42.791	-102.718	1106	0.30	20	0.47	0.41	17	0.51	n/a	0	
C. undulatum var. undu	NE	Sheridan	NESH.6	42.758	-102.695	1170	0.11	19	0.32	0.00	6	0.00	n/a	0	
C. undulatum var. undu	NE	Sioux	NESI.2	42.785	-103.801	1227	0.30	20	0.47	0.00	3	0.00	n/a	0	
C. undulatum var. undu	NE	Sioux	NESI.4	42.913	-103.82	1171	0.06	17	0.24	0.14	7	0.38	n/a	0	

Species	**State**	**County**	**Pop.**	**Lat.**	**Long.**	**Elev. (m)**	**Avg. Ovip Freq. 1˚ Capitula**	**N (1˚)**	**SD (1˚)**	**Avg. Ovip Freq. 2˚ Capitula**	**N (2˚)**	**SD (2˚)**	**Avg. Ovip Freq. 3˚ Capitula**	**N (3˚)**	**SD (3˚)**
C. undulatum var. undu	NE	Sioux	NESI.5	42.917	-103.869	1193	0.39	33	0.50	0.38	26	0.50	0.22	9	0.44
C. undulatum var. undu	NE	Sioux	NESI.6	42.437	-103.788	1406	0.82	39	0.39	0.71	70	0.46	0.40	15	0.51
C. undulatum var. undu	NE	Sioux	NESI.7	42.411	-103.791	1369	0.31	29	0.47	0.20	54	0.41	0.00	16	0.00
C. undulatum var. undu	NE	Sioux	NESI.9	42.422	-103.769	1348	0.94	32	0.25	0.46	41	0.50	0.20	5	0.45
C. undulatum var. undu	SD	Custer	SDC.1	43.737	-103.817	1713	0.35	23	0.49	n/a	0		n/a	0	
C. undulatum var. undu	SD	Custer	SDC.2	43.841	-103.391	1416	0.58	24	0.50	0.47	19	0.51	n/a	0	
C. undulatum var. undu	SD	Custer	SDW.1	43.581	-103.485	1306	0.77	13	0.44	n/a	0		n/a	0	
C. undulatum var. undu	SD	Custer	SDW.10	43.629	-103.437	1430	0.14	21	0.36	0.00	2	0.00	n/a	0	
C. undulatum var. undu	SD	Custer	SDW.11	43.627	-103.377	1206	0.47	15	0.52	0.43	7	0.53	0.50	2	0.71
C. undulatum var. undu	SD	Custer	SDW.12	43.628	-103.375	1270	0.07	14	0.27	0.00	1	0.00	n/a	0	
C. undulatum var. undu	SD	Custer	SDW.13	43.624	-103.377	1270	0.31	13	0.48	n/a	0		n/a	0	
C. undulatum var. undu	SD	Custer	SDW.2	43.581	-103.483	1296	0.07	15	0.26	n/a	0		n/a	0	
C. undulatum var. undu	SD	Custer	SDW.3	43.56	-103.491	1313	0.65	23	0.49	0.67	6	0.52	n/a	0	
C. undulatum var. undu	SD	Custer	SDW.4	43.561	-103.493	1314	0.63	16	0.50	n/a	0		n/a	0	
C. undulatum var. undu	SD	Custer	SDW.9	43.593	-103.489	1291	0.06	18	0.24	0.00	3	0.00	n/a	0	
C. undulatum var. undu	WY	Campbell	WYCA1.1	44.301	-105.499	1383	0.38	16	0.50	n/a	0		n/a	0	
C. undulatum var. undu	WY	Converse	WYCO15.3	42.835	-105.494	1496	0.68	25	0.48	0.29	21	0.46	0.00	1	0.00
C. undulatum var. undu	WY	Converse	WYCO15.4	42.854	-105.5	1500	0.81	26	0.40	0.28	25	0.46	0.51	61	0.50
C. undulatum var. undu	WY	Converse	WYCO5.1	42.825	-105.172	1556	0.38	39	0.49	0.20	40	0.41	0.00	2	0.00
C. undulatum var. undu	WY	Converse	WYCO5.2	42.825	-105.21	1584	0.11	36	0.32	0.24	21	0.44	n/a	0	
C. undulatum var. undu	WY	Crook	WYCR4.1	44.583	-104.724	1196	0.74	19	0.45	0.80	10	0.42	0.00	2	0.00
C. undulatum var. undu	WY	Crook	WYCR4.3	44.599	-104.724	1280	0.56	18	0.51	0.33	6	0.52	n/a	0	
C. undulatum var. undu	WY	Goshen	WYGO2.1	41.638	-104.211	1458	0.08	25	0.28	0.00	3	0.00	n/a	0	
C. undulatum var. undu	WY	Platte	WYPL1.2	41.874	-104.789	1543	0.50	46	0.51	0.14	21	0.36	n/a	0	
C. undulatum var. undu	WY	Platte	WYPL1.3	41.902	-104.789	1585	0.19	21	0.40	0.60	5	0.55	n/a	0	
C. undulatum var. undu	WY	Platte	WYPL1.4	41.887	-104.789	1563	0.67	24	0.48	0.31	29	0.47	0.00	1	0.00
C. undulatum var. undu	WY	Platte	WYPL5.1	42.105	-104.907	1402	0.10	21	0.30	0.00	5	0.00	n/a	0	

Species	**State**	**County**	**Pop.**	**Lat.**	**Long.**	**Elev. (m)**	**Avg. Ovip Freq. 1˚ Capitula**	**N (1˚)**	**SD (1˚)**	**Avg. Ovip Freq. 2˚ Capitula**	**N (2˚)**	**SD (2˚)**	**Avg. Ovip Freq. 3˚ Capitula**	**N (3˚)**	**SD (3˚)**
C. undulatum var. undu	WY	Platte	WYPL5.2	42.105	-104.907	1402	0.71	7	0.49	0.00	10	0.00	n/a	0	

Chapter 2: Demographic effects of pre-dispersal seed predation by two biocontrol agents on Tracy's thistle (*Cirsium undulatum var. tracyi*)

ABSTRACT

The risks of weed biocontrol agents to native plant species continue to be controversial and ecological data demonstrating the risks remains scarce. We quantified capitulum-level, plant-level, and population-level effects of pre-dispersal seed predation by two introduced biocontrol agents (*Larinus planus* and *Rhinocyllus conicus*) on the native Tracy's thistle (*Cirsium undulatum* var. *tracyi*). We asked specifically, how does floral herbivory by introduced and native seed predators affect seed production and what is the relationship between insect herbivory and host plant recruitment? The study provides substantial evidence that *R. conicus* is responsible for demographic changes in Tracy's thistle and also shows that *L. planus* has an effect similar or greater to that of *R. conicus* on the native thistle's seed production. *Larinus planus* and *R. conicus* were responsible for an estimated 70% and 60% reduction in seed production respectively while a native seed predator, the tephritid fly, *Orellia occidentalis*, showed a much smaller effect. Further, seed predator exclusion in thistle populations hosting *R. conicus* responded with increased seedling recruitment. We also present evidence suggesting negative competitive effects weevils on *O. occidentalis*. Results contribute to ecological theory regarding top-down control of plant populations as well as risks of the deliberate introduction of exotic phytophagous insects for biocontrol.

INTRODUCTION

Plant populations have been traditionally viewed as being resource limited (Hairston et al. 1960), yet this paradigm has been challenged given the evidence of the importance of herbivory on plant performance. However, relatively few studies to date have evaluated more than direct effects of herbivory to a single generation. Despite the fact that the great majority of herbivore species are insects, published studies outlining population-level effects of herbivory on native plants are typically with vertebrate herbivores. Studies of insect herbivore effects of plant population remain sparse (Louda 1982, 1983, Parker 1985, Crawley 1989a, Louda 1989a, Louda and Potvin 1995, Crawley 1997b, Maron and Simms 1997, Kelly and Dyer 2002). There is some evidence for the limitation of exotic plants by exotic insect herbivores (e.g. McEvoy et al. 1991), but experimental data are generally lacking even for the evaluation of biocontrol releases (Kok 1986, Nechols 2000).

The risks of weed biocontrol agents to native plant species continue to be controversial (see Follett and Duan 2000, Louda et al. 2003). The release of biocontrol agents for control of weeds has been, in effect, a large experiment testing the ability of phytophagous insects to control plant populations – minus adequate controls (Crawley 1997b). Biocontrol of weeds theory is founded on the premise that weed populations grow at elevated rates in part because they have been released from top-down controls that regulated populations in their native ecosystems. In effect, exotic species can outperform natives due to lack of herbivore pressure (Crawley 1997a, Keane and Crawley 2002). The use of phytophagous insects in controlling exotic weed populations is increasing, but so is the predictability of risk (Barratt et al. 2000, Pemberton 2000) and the evidence demonstrating nontarget effects.

At least 153 exotic insects have been introduced to control exotic weeds in the United States (Julien and Griffiths 1998), and two studies have shown clear demographic consequences for native plants – the effects of the moth *Cactoblastis cactorum* on native cacti in Florida (Johnson and Stiling 1998), and the effects of *Rhinocyllus conicus* on native thistles in the Midwest (Louda et al. 1997, Louda 2000, Louda et al. 2003). Observations of a third introduced insect, *Larinus planus*, feeding on inflorescences of native thistles in Colorado, Oregon, South Dakota, and Washington suggest decreased seed production in these plants due to *L. planus* and the potential for demographic effects (Louda and O'Brien 2002, Dodge et al. in review).

The fact that seed limitation in native plant populations is not uncommon (Turnbull et al. 2000) suggests that seed predators may regularly influence the structure and dynamics of plant populations. Although many annuals and biennials have seed banks, those short-lived plants that rely on current seed production for recruitment will be more susceptible to top-down controls (Louda 1989b, Maron and Vila 2001). Given that research into the relationship of seed production to population structure has not been performed on a random sampling of species and habitats, it is inferred that ecological factors affecting seed production can have indirect consequences for dynamics of many plant populations (e.g. Louda 1982, 1983, Louda and Rodman 1996, Maron and Simms 1997, Bevill et al. 1999, Kelly and Dyer 2002).

The purpose of this study was twofold: first, to address the continued debate of how insect seed predators affect plant population dynamics; and second, to address concerns of nontarget effects by introduced biocontrol agents. I endeavored to determine capitulum-level, plant-level and population-level effects of pre-dispersal seed predation by two introduced biocontrol agents on the native Tracy's thistle (*Cirsium undulatum* var. *tracyi*). I asked specifically: how does herbivory by introduced and native seed predators affect seed production at the inflorescence and plant levels, what is the relationship between seed added to a population and recruitment, and what is the relationship between insect herbivory and recruitment? Results will be used to support or refute ecological theory regarding top-down control of plant populations as well as risks of the deliberate introduction of exotic phytophagous insects for biocontrol.

METHODS

Study species

Tracy's thistle (*Cirsium undulatum* (Nutt.) Spreng. var. *tracyi* (Rydb.) Welsh) (Asteraceae) is a large-flowered monocarpic perennial thistle endemic to the western slope of Colorado and Utah. Monocarpy facilitates investigation of the lifetime fitness of the plant (see Louda and Potvin 1995). The native tephritid fly *Orellia occidentalis* (Snow) (Diptera: Tephritidae) and the introduced weevils *Larinus planus* (Fabricius) (Coleoptera: Curculionidae) and *Rhinocyllus conicus* (Froelich) (*Coleoptera: Curculionidae*) were the most numerous seed predators of Tracy's thistle in the study area. I have also found lepidopteran larva, likely *Homoeosoma impressale* (Lepidoptera: Pyralidae), at a much lower frequency (approximately 0.5 percent of seedheads).

Filled, non-damaged achenes in Tracy's thistle are relatively large; weight (±SE) = 10.98mg (0.36), length = 6.26mm (0.099), width = 2.33mm (0.065), depth = 1.03mm (0.019). Seed dispersal in Tracy's thistle has not been studied in detail, but observation and simulation of seed release by manually releasing seeds with their pappus attached indicate that, due to the large size of the achene and the dehiscent nature of the pappus, the seeds generally land within one meter of the plant.

Larinus planus is a European seed head weevil widely introduced to the western US as a biocontrol agent for Canada thistle (*Cirsium arvense* (*L.*) Scop.) (Asteraceae), an invasive, polycarpic weed species exotic to North America (native to Eurasia). *L. planus* was accidentally introduced in the eastern U.S. and has been established at least since 1968 (White 1972, Wheeler and Whitehead 1985, McClay 1989). It has since been intentionally released in at least 11 states and provinces in western North America (Louda and O'Brien 2002, Dodge et al. in review). The bulk of releases took place in the mid-late 1990s (see Louda and O'Brien 2002). Release sites include public lands, including at least six national parks where conservation of native flora is a priority. Because *L. planus* is adventive in the eastern U.S., it has not been subjected to the same regulations of testing and interstate transfer that accompany other introduced biocontrol agent releases.

Rhinocyllus conicus, another Eurasian seed head weevil, has been released throughout the western states for biocontrol of *Carduus spp. (L.)* (Asteraceae) (Reese et al. 1995), invasive perennial weed species exotic to North America (native to Eurasia). *Rhinocyllus conicus* was originally released in Canada in 1968 and in the U.S. in 1969, and it has since been found in at least 26 states (Louda et al. 1997).

Both weevils are univoltine, oviposit on closed, pre-anthesis buds (stage 2), and complete larval development in developing capitula of thistles. *Larinus planus* oviposits by chewing a small hole in an immature capitulum, placing an egg in the hole and packing the hole with vegetative matter. *Rhinocyllus conicus* lays its eggs on the exterior of developing capitula. Larvae hatch and burrow into the flower head. Both species leave conspicuous, distinctive evidence of oviposition. Damage to the flower head by both weevils is primarily through larval feeding on ovules, developing seeds, and the receptacle. Both weevils pupate inside the flower head in pupal chambers. These chambers are distinct and countable.

Orellia occidentalis is a North American picture-winged fly (Diptera: Tephritidae) native to the western United States (Foote and Blanc 1963). The flies are generally univoltine or bivoltine, overwintering in the pupal stage. Adult females oviposit during early anthesis (stage 3) when phyllaries spread and expose the tops of non-elongated florets. Female flies insert eggs into the mass of florets. Larvae hatch and tunnel down the corolla tube and into developing seeds, feeding on the seeds. Larvae can bore into additional seeds and feed on several leaving distinct entry and exit holes in the seed coats. Larvae then usually drop to the soil to pupate (Lamp and McCarty 1982). The larvae are easily caught in mesh bags placed over the flower head.

Study Sites

I studied six populations of Tracy’s thistle in Gunnison County, Colorado. Two of the populations (Cabins and Bluff) hosted *L. planus*, three populations (Cove, Cut, Pine) hosted *R. conicus*, and a single population (Onemile) hosted neither biocontrol agent. All thistle populations were similar in density, ranging between 1.2 and 2.4 flowering plants per 100m^2. The Cabins, Cut, and Cove populations were located on private property, whereas the other three populations were located on public land. The sites were relatively similar, along moderately disturbed roadsides. All sites were characterized by patchy vegetation and lots of bare ground, often loosely associated with *Artemesia tridentata* and *Chrysothamnus nauseosus*. Populations ranged in altitude between 2255 and 2530 m.

Phenological overlap and initial observations on seed predation

In 2001, I quantified the phenology and effects of *L. planus* on the seed production in Tracy's thistle, by measuring the diameter, developmental stage, and weevil oviposition (yes/no) of each flower bud on five plants every two to three days for the duration of the summer at the Cabins and Bluff sites. I defined inflorescence stages as follows:

1. Bud is obscured by subtending leaves and bracts – not apparent
2. Bud is extended beyond protection of leafy bracts and apparent, but phyllaries have not yet begun spreading (stage of oviposition by both *R. conicus* and *L. planus*)
3. Early anthesis - phyllaries have begun spreading and flowering parts become visible at the bud top (stage of oviposition by *O. occidentalis*)
4. Flowers are open, receptive, and producing pollen
5. Flowers are post pollination and pre-seed release
6. Phyllaries have reflexed and seeds are being released from the receptacle.

If a plant had more than two main stems, then only two stems were studied. This occurred in approximately 20% of the plants. In 2002, I selected plants and stems at random before each visit. I identified flower head buds uniquely by a numbering system based on the plant's architecture, to follow the development and attack rate for a bud throughout the growing season. The data also allow quantification of the rate and pattern of oviposition.

As flower heads matured, they were bagged following pollination and prior to seed dispersal. I collected the capitula that matured and released seed (N = 304) and stored them in one-ounce plastic cups with tight-fitting cardboard caps. The capitula were dissected to establish the relationship between oviposition, weevil development, and seed production. Oviposition prevalence, on each sampling date, was determined as the percentage of all stage 3 inflorescences (early anthesis) with at least one oviposition scar. This group represented the capitula that were susceptible to weevil oviposition, and it eliminated the effect of recounting capitula that had moved to later stages.

To quantify the relationships between thistle seed production and seed predator presence, I conducted a multiple regression analysis of capitulum diameter, number of weevil larvae, and number of fly larvae on number of seed produced per capitulum (square root transformed). I followed the analysis with comparisons of the estimated partial regression coefficients (B coefficients), the standardized partial regression coefficients (Beta coefficients), and the partial correlation coefficients. B coefficients represent the magnitude effect of an *individual* of each species on seed production. Beta coefficients represent the relative effect each *species* has on seed production. Partial correlation coefficients represent a species' contribution to the fit of the model (squared, would be analogous to r^2, the coefficient of determination) (Kleinbaum et al. 1998, StatSoft 2004).

Exclusion of L. planus and R. conicus from Individual Capitula

In order to quantify the direct effects of *L. planus* and *R. conicus* on seed production, I excluded weevils from individual capitula at three sites on Tracy's thistle in 2002 and 2003. Studies in 2002 comprised 17 plants at one site hosting *L. planus*. The design included four treatments: two experimental reductions of "all-insect exclusion", and "weevil exclusion", and two controls, which were "sham control" and "natural control". "All-insect exclusion" (N = 45 capitula) was a chemical exclusion consisting of a weekly application of acephate (Isotox®) painted on capitula in susceptible classes (stages 1-3). This treatment intended to keep both weevils and tephritid flies from the inflorescence, while not affecting foliage, stem, and root herbivores. "Weevil exclusion" (N = 35) was a physical exclusion consisting of wrapping individual capitula with a plastic, self-adhering film that's permeable to oxygen and carbon dioxide (Parafilm M®; Pechiney Plastic Packaging, Inc.), during stage 2 when the capitulum is susceptible to attack by *L. planus*. The intent was to prevent *L. planus* from recognizing the capitulum as a suitable oviposition site or from actually chewing through the wrap into the developing capitulum and ovipositing, while allowing tephritid oviposition in the florets at stage 3. The "sham controls" (N = 53) consisted of handling and painting water on developing capitula to control for both the handling effect of physical exclusion and the application effect of the chemical exclusion. "Natural control" (N = 56) capitula were not handled except for measuring of developmental phenology. Since I applied all four treatments to the same plant, any systemic effect altering stem, leaf, and root herbivory would have been present in all treatments and, thus, I can estimate inflorescence herbivory, albeit in a slightly altered system.

In 2003, treatment techniques changed since use of acephate on capitula in 2002 appeared to alter the adult weevil population, and the Parafilm treatment led to the breakage and destruction of some capitula. New treatments consisted of three bagging treatments and a control that were applied to 11 plants at the Pine population. The first treatment, "all insect exclusion," was designed to exclude both weevils and flies by tying nylon mesh bags over developing capitula until stage 4, when the bag was removed to allow pollination (N = 20 capitula). The second treatment, "weevil exclusion," was designed to exclude the weevils by bagging the capitulum only during stage 2, when it was most susceptible to weevil oviposition (N = 24). The third treatment, "fly exclusion," was to exclude only the flies, so capitula were bagged only for stage 3 (N= 19). The fourth treatment was a sham control, in which capitula were handled and measured, but were never bagged (N = 18).

Seed production per treatment was compared using a Kruskal-Wallace ANOVA and median test. Multiple post-hoc comparisons were performed with p-values adjusted using a Bonferroni adjustment (Sokal and Rohlf 1998). Tests of efficacy of the exclusion treatments were made on both weevil and fly development (yes/no) on the capitula using a chi-square 4x2 table (treatment by insect presence) and contrasts using Fisher's exact test with a Bonferroni adjustment. Direct treatment effects on seed production were tested by comparing seed production across treatment levels but using only those capitula that were not attacked by seed predators in a single factor ANOVA (plant was statistically non-significant as a random blocking factor in both experiments).

Exclusions of Seed Predators from Whole Plants

I conducted whole-plant exclusion experiments at four different populations: Cut (N = 8 plants), Cove (N = 17), and Onemile (N = 12) in 2002, and Pine (N = 20) in 2003. The Onemile population was the only one of the four that did not have either *L. planus* or *R. conicus*. The two treatments were a chemical exclusion and water control. The chemical exclusion consisted of weekly applications of acephate to the entire plant throughout the growing season. The control treatment had water applied at the same rate as in the chemical exclusion. Half of the plants at a site were randomly assigned to each treatment. At Cove, three control plants were trampled and eliminated from the study. Plants were measured for architecture and developmental phenology twice weekly. To estimate seed production from these plants I systematically collected the terminal capitulum on the main stem and terminal capitulum off every other branch along the main stem. All sampled capitula were bagged upon completion of flowering and then collected at the time of seed release. Capitula were dissected either in the field or in the lab.

To estimate the effect of the treatments on recruitment, at Pine and Cut, seeds were released back into the population by scattering them within one meter of the maternal plant. Onemile and Cove were not included in this part of the study for logistical reasons. Onemile is a small population and was used as part of the seed augmentation experiment (outlined below) and Cove is located on private property and I was informed the site was to be used for parking equipment.

To compare seed production among treatments across all populations, I conducted a mixed model ANOVA on seed produced per capitulum averaged per plant with treatment as a fixed factor and population as a random factor. Treatment efficacy was tested by comparing plant-level seed predator intensity across all sites with a Mann-Whitney U test. Seed predator intensities were tested independently for weevils and flies and were defined as proportion of capitula per plant with weevil pupal chambers or fly larvae. Plants from Onemile were not included in the weevil analysis and plants from Cut were not included in the fly analysis due to the fact that these seed predators were never recorded at these sites. I then conducted comparisons among treatment levels at each site to clarify the relationship between seed production and the prevalence of seed predators at each site. I tested the efficacy of the exclusion treatments on frequency of both weevil and fly development (yes/no) on the capitula using a Fisher's exact test. I used the two different non-parametric analyses to conduct plant-level comparisons across populations and capitulum-level comparisons across plants within a population.

To measure natural recruitment around each of the plants at Pine and Cut, I counted all recruits within a circle of two-meter radius centered on the maternal plant stalk in July of the year following the treatments. No circles overlapped in area. Each recruit was measured for number of leaves and length of the longest leaf. I compared the square root transformed counts of recruits per plant between treatments across both sites in a single-factor ANOVA. The original mixed model was modified due to the non-significant effect of using population as a random blocking factor. I investigated site-level differences with comparisons of treatment effect at each site. Size of recruits per plant per treatment was also analyzed using a size index (number of leaves times the length of longest leaf) and compared between treatments using a single-factor ANOVA.

Seed Addition Experiment

To investigate localized seed limitation, I collected undamaged seeds and distributed them around randomly selected plants as a seed augmentation experiment at two sites in 2002 (Onemile and Pine). The plants selected were not part of the exclusion experiment. Treatment values were determined by seed availability and number of available plants to treat as well as trying to incorporate the estimated seed rain in the population. At the Pine site I had four treatments (0, 10, 25, 50 seeds) per one-meter radius circle centered on the main stalk, and at the Onemile site I used three treatments (0, 50, 100). I placed seeds uniformly in the one-meter radius circle around the flowering stalk and buried them to a depth of 3mm. The following summer, I located, tagged, and measured number of leaves and length of longest leaf on seedlings within one meter of plants. I revisited both sites again in 2004 to measure growth and survivorship of the seedlings.

I conducted one-way ANOVA analyses of number of recruits (square root transformed) to determine differences in the recruitment rates of the treatments. I also conducted one-way ANOVA analyses on a size index (length of longest leaf multiplied by number of leaves) to investigate size differences in recruits per treatment.

Statistical Analyses

All statistical analyses were performed using Statistica v. 6 (Statsoft 2004). In cases where data violated parametric ANOVA assumptions, I employed non-parametric alternatives. I also dropped random blocking factors from mixed-model ANOVAs when they were not significant to increase sensitivity of the ANOVA. Significance was determined at $\alpha = 0.05$. Multiple, between-group post-hoc contrasts were performed with a Bonferroni adjustment (Statsoft 2004).

RESULTS

Phenological overlap and initial observations on seed predation

In 2001, oviposition by *L. planus* coincided with the near entirety of the flowering period of Tracy's thistle at the Cabins site. More than 50% of the capitula were attacked during the periods with the highest flower production. Peak oviposition intensity of about 92% of the capitula was measured on July 8 (Figure 2-1a).

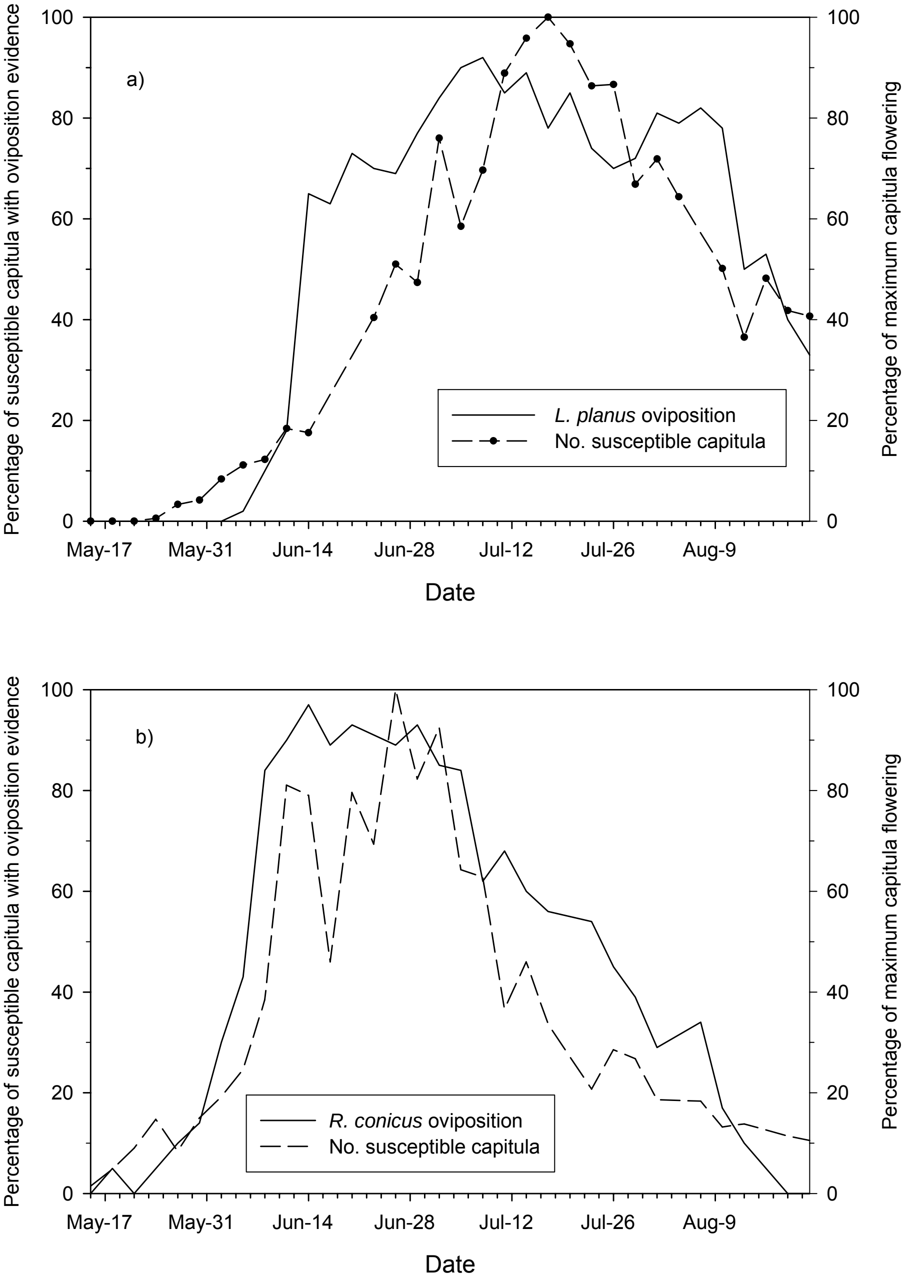

Figure 2-1. Percentages of stage 2 capitula with oviposition scars from two separate populations of Tracy's thistle, one hosting *L. planus* (a), and one hosting *R. conicus* (b). Data for *L. planus* were measured in the summer of 2001 and for *R. conicus* in 2002, prior to any manipulations to the populations. The right axis indicates (as a percentage of maximum) the number of stage 2 capitula in the population to give an indication of the flowering phenology.

Both *L. planus* and *O. occidentalis* had significant negative effects on seed production at the Cabins site in 2001 (Table 2-1). The estimated effect of *L. planus* on seed production per individual is more than four times greater than that of *O. occidentalis* (see B coefficients) and the estimated effect of *L. planus* as a group is over twice that of *O. occidentalis* (see Beta coefficients). Finally, the overall contribution of *L. planus* to the system is twice that of *O. occidentalis*. Restated, the estimated negative effect of *L. planus* is twice that of *O. occidentalis* at both the capitulum and system levels.

Table 2-1. Multiple regression. Partial regression coefficients (B), standardized coefficients (Beta), and partial correlation coefficients for *L. planus* and *O. occidentalis* on seed production (square root transformed) at the Cabins and Bluff sites in 2001 (N = 304). $F_{3,\ 300} = 56.65$; $p < 0.01$; Adjusted $R^2 = 0.36$.

	B	SE of B	Beta	SE of Beta	Part. Corr.	t (300)	p
Intercept	- 4.758	0.639				- 7.448	< 0.01
Diameter	0.430	0.039	0.532	0.048	0.540	11.109	< 0.01
L. planus larvae	- 0.743	0.153	- 0.230	0.048	- 0.270	- 4.851	< 0.01
O. occidentalis larvae	- 0.177	0.076	- 0.111	0.048	- 0.133	- 2.322	0.02

In 2002, I found similar use of Tracy's thistle by *R. conicus* at the Pine site. Oviposition occurred on over 50% of capitula for an extended period, including peak flowering. Peak oviposition reached 97% on June 14 (Figure 2-1b). *R. conicus* was significantly and negatively related to seed production. In contrast, *O. occidentalis* did not have a significant impact on seed production (Table 2-2). The estimated effect of an individual *R. conicus* was approximately nine times the estimated effect of *O. occidentalis*, the standardized negative effect per capitulum of *R. conicus* was three times that of *O. occidentalis*, and finally the contribution of *R. conicus* is nearly three times that of *O. occidentalis* to the fit of the model.

Table 2-2. Multiple regression. Partial regression coefficients (B), standardized coefficients (Beta), and partial correlation coefficients for *R. conicus* and *O. occidentalis* on seed production (square root transformed) at the Pine site in 2002 (N = 89). $F_{3,\,85} = 9.71$; $p < 0.01$; Adjusted $R^2 = 0.23$.

	B	SE of B	Beta	SE of Beta	Part. Corr.	t (210)	p
Intercept	0.265	1.222				0.217	0.83
Diameter	0.263	0.068	0.363	0.094	0.387	3.867	< 0.01
R. conicus larvae	- 0.936	0.252	- 0.349	0.094	- 0.374	- 3.719	< 0.01
O. occidentalis larvae	- 0.105	0.086	- 0.116	0.094	-0.133	- 1.230	0.22

Exclusion of L. planus from Individual Capitula

Capitula in the all-insect and weevil exclusion treatments produced more seeds than the controls (Table 2-3, Fig. 2-2a). There were no measurable differences between the all-insect and weevil exclusions and no differences between the sham and natural controls. Frequency of weevil development was reduced in the all-insect and weevil exclusions compared to the controls ($X^2 = 36.07$, df = 3, $p < 0.01$ between all groups, $p < 0.01$ for contrasts) (Fig. 2-2b). Weevil development did not differ in either all-insect versus weevil exclusions (contrast $p > 0.05$), or in sham versus natural controls ($p > 0.05$).

Table 2-3. Multiple comparisons z-values (and adjusted 2-tailed p values) comparing number of seeds per capitulum among treatments for the Cabins individual exclusions conducted in 2002. Kruskal-Wallis test: H (3, N = 189) =18.25862; p < 0.01. Bolded comparisons are significant using Bonferroni adjustment for multiple comparisons with an experiment-wise error rate α = 0.05. Note: this is the relevant excerpt from the four by four table of all comparisons.

	Weevil exclusion	Sham control	Natural control
All-insect exclusion	0.33 (0.370)	3.46 (< 0.001)	2.95 (0.002)
Weevil exclusion		2.88 (0.002)	2.40 (0.008)
Sham control			0.58 (0.282)

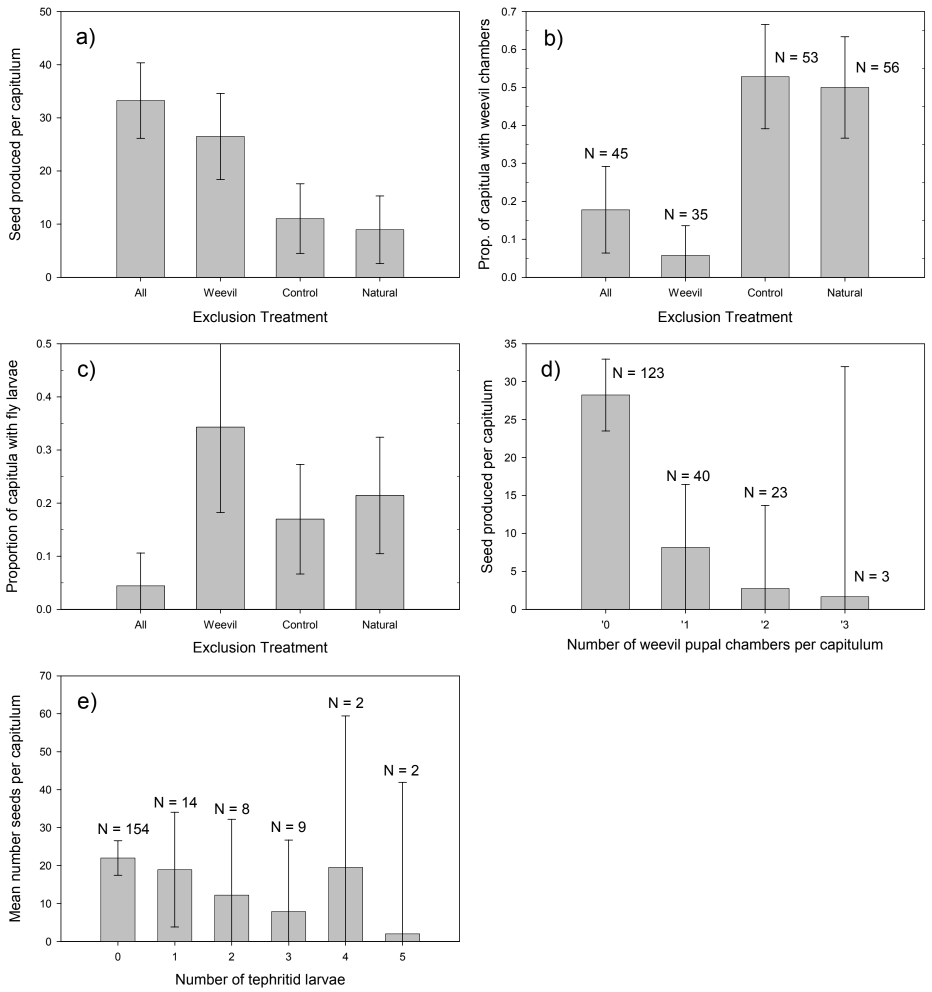

Figure 2-2. Results from individual capitulum exclusions. Seed production increased approximately 150% in capitula treated to exclude weevils (a). Exclusions resulted in a significant (although not complete) reduction in weevil development in the capitula (b). Tephritid fly use was reduced by the all-insect exclusion but not by the weevil exclusion(c). There is a strong negative relationship between the number of pupal chambers per capitulum and the mean number of seeds produced across all treatments (d). There also is a detectable negative relationship between the number of tephritid larvae per capitulum and the number of seeds produced across all treatments in capitula with no weevil development (e). All error bars indicate two standard errors.

Frequency of tephritid development was reduced in the chemical treatment but not, as expected, in the weevil exclusion compared to both the controls using chi-square analysis and Fisher's exact tests ($X^2 = 17.85$, df = 3, $p < 0.01$ between all groups, $p < 0.01$ for contrasts) (Fig. 2-2c). Tephritid development in the three non-chemical treatments did not differ when compared among each other (contrast $p > 0.05$). The reduction in seed production was attributable to seed predators as it was significantly negatively related to both number of *L. planus* chambers and *O. occidentalis* larvae (Table 2-4, Figs. 2-2d and e). I found no evidence of direct treatment effects through comparing seed production across all treatments in capitula with no weevil or fly development ($F_{3, 91} = 1.01$, p = 0.39).

Table 2-4. Multiple regression. Partial regression coefficients (B), standardized coefficients (Beta), and partial correlation coefficients for *L. planus* and *O. occidentalis* on seed production (square root transformed) at the Cabins site in 2002 (N = 189). $F_{3, 185} = 49.18$; $p < 0.01$; Adjusted $R^2 = 0.43$.

	B	SE of B	Beta	SE of Beta	Part. Corr.	t (185)	p
Intercept	-0.701	0.603				-1.163	0.24
Diameter	0.373	0.041	0.502	0.055	0.556	9.091	< 0.01
L. planus chambers	-1.743	0.220	-0.438	0.056	-0.504	-7.937	< 0.01
O. occidentalis larvae	-0.746	0.174	-0.238	0.056	-0.300	-4.285	< 0.01

Exclusion of R. conicus from Individual Capitula

Capitula from the two treatments in which *R. conicus* development was prevented (all-insect and weevil exclusions) produced more seeds than those that were allowed to host weevil larvae (Table 2-5, Fig 2-3a). There were no measurable differences in seed production between the all-insect and weevil exclusions or between the fly exclusion and the sham control. The frequency of weevil development per treatment was reduced in the all-insect and weevil exclusions compared to the fly exclusion and control ($X^2 = 28.45$, df = 3, $p<0.01$ between all groups, $p < 0.01$ in four contrasts comparing either all-insect and weevil exclusions with fly exclusion or the sham control) (Fig. 2-3b). There was no difference in weevil development between the all-insect and weevil exclusions ($p > 0.05$) and no difference between the fly exclusion and control ($p > 0.05$). Frequency of tephritid development per treatment was reduced only when I compared the fly exclusion to the control and to the weevil exclusion ($X^2 = 8.11$, df = 3, $p=0.04$ between all groups, $p = 0.02$ and 0.03 in contrasts respectively) (Fig. 2-3c). Seed reduction was attributable to *R. conicus*. Seed production was significantly and negatively related to the number of weevil chambers but not to the number of tephritid larvae and puparia (Table 2-6, Figs. 2-3d and e). I found no direct treatment effects on seed production across all treatments in those capitula with no weevil or fly development ($F_{3,78} = 0.35$, $p = 0.79$).

Table 2-5. Multiple comparisons z-values (and adjusted 2-tailed p values) comparing number of seeds per capitulum between treatments for the Pine individual exclusions conducted in 2003. Kruskal-Wallis test: H (3, N= 113) =19.53; $p < 0.01$. Bolded comparisons are significant using Bonferroni adjustment for multiple comparisons with an experiment-wise error rate $\alpha = 0.05$. Note: this is the relevant excerpt from the four by four table of all comparisons.

	Weevil exclusion	Fly exclusion	Sham control
All-insect exclusion	0.87 (0.193)	3.71 (< 0.001)	3.13 (< 0.001)
Weevil exclusion		2.98 (0.001)	2.39 (0.0083)
Fly exclusion			0.47 (0.318)

Table 2-6. Multiple regression. Partial regression coefficients (B), standardized coefficients (Beta), and partial correlation coefficients for *R. conicus* and *O. occidentalis* on seed production (square root transformed) at the Pine site in 2003 (N = 113). $F_{2, 110} = 36.22$; $p < 0.01$; Adjusted $R^2 = 0.39$.

	B	SE of B	Beta	SE of Beta	Part. Corr.	t (185)	p
Intercept	7.364	0.332				22.161	0.24
R. conicus chambers	-1.715	0.206	-0.622	0.075	-0.504	-8.303	< 0.01
O. occidentalis larvae	0.195	0.336	0.043	0.075	-0.300	0.580	0.56

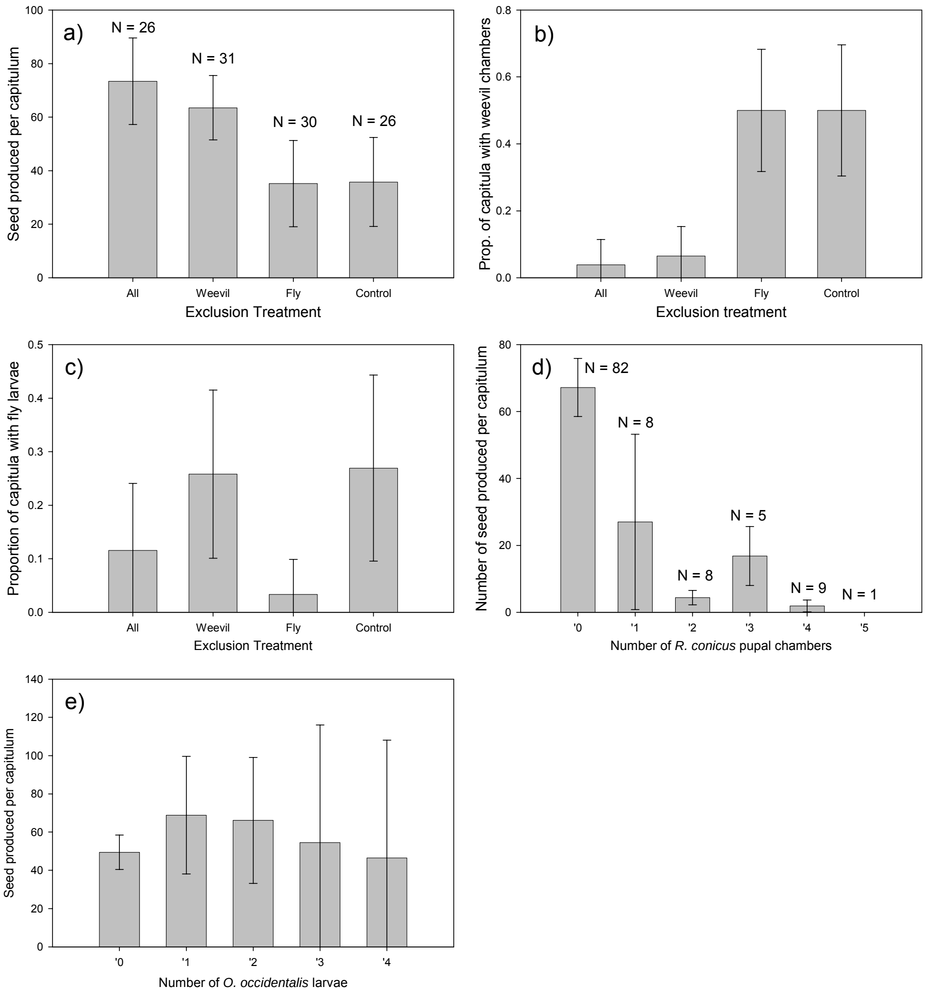

Figure 2-3. Results from individual capitulum exclusions of *R. conicus* at Pine in 2003 indicate that *R. conicus* is responsible for an approximate 50% reduction in seed production per capitulum. There was a significant increase in seed production in capitula treated to exclude weevils (a). The exclusions resulted in a significant (although not complete) reduction in weevil development in the capitula (b). *O. occidentalis* use of the capitula was reduced by the exclusions but not completely (c). There was a strong, negative relationship between the number of *R. conicus* pupal chambers and the number of seeds produced per capitulum across all treatments (d). There was no detectable relationship between the number of *O. occidentalis* larvae and seed production per capitulum (e). The All error bars indicate two standard errors.

Exclusions of Seed Predators from Whole Plants

More seeds were produced per capitulum averaged per plant in the treated plants compared to the control plants across all sites (Table 2-7) (Fig. 2-4a). Significantly more seed was produced in the exclusion treatments at both the Pine site (contrast $p = 0.01$) and at the Cove site ($p = 0.01$) but not at either the Cut site ($p = 0.31$) or the Onemile site ($p = 0.31$) (Fig 2-4b).

Table 2-7. ANOVA. Effect of population and whole plant treatment on seed production per capitulum per plant from the Cove, Cut, Onemile, and Pine sites combined (N = 57 plants).

	Effect	SS	df	MS	F	p
Intercept	Fixed	44354.15	1	44354.15	242.64	< 0.01
Site	Random	528.40	3	176.13	1.41	0.39
Treatment	Fixed	3051.11	1	3051.11	22.47	0.01
Site * Treatment	Random	375.07	3	125.02	0.48	0.70
Error		12692.89	49	259.04		

The frequency of weevil development was reduced in chemically treated plants across all sites (Mann-Whitney U = 47.5; $N_{treat} = 24$, $N_{control} = 21$; adjusted $z = 5.06$; $p < 0.01$) (Fig. 2-4c). Population-level analysis revealed that fewer capitula in the chemical exclusion group hosted weevil development than those in the control group at the Pine site ($X^2 = 39.18$, df = 1, Fisher's exact $p < 0.01$) and at the Cove site ($X^2 = 18.57$, df = 1, Fisher's exact $p < 0.01$) (Fig. 2-4c). However, the difference was less pronounced and only significant at the Cove site ($X^2 = 4.54$, df = 1, Fisher's exact $p = 0.07$). I found no evidence of weevil seed predators at the Onemile site.

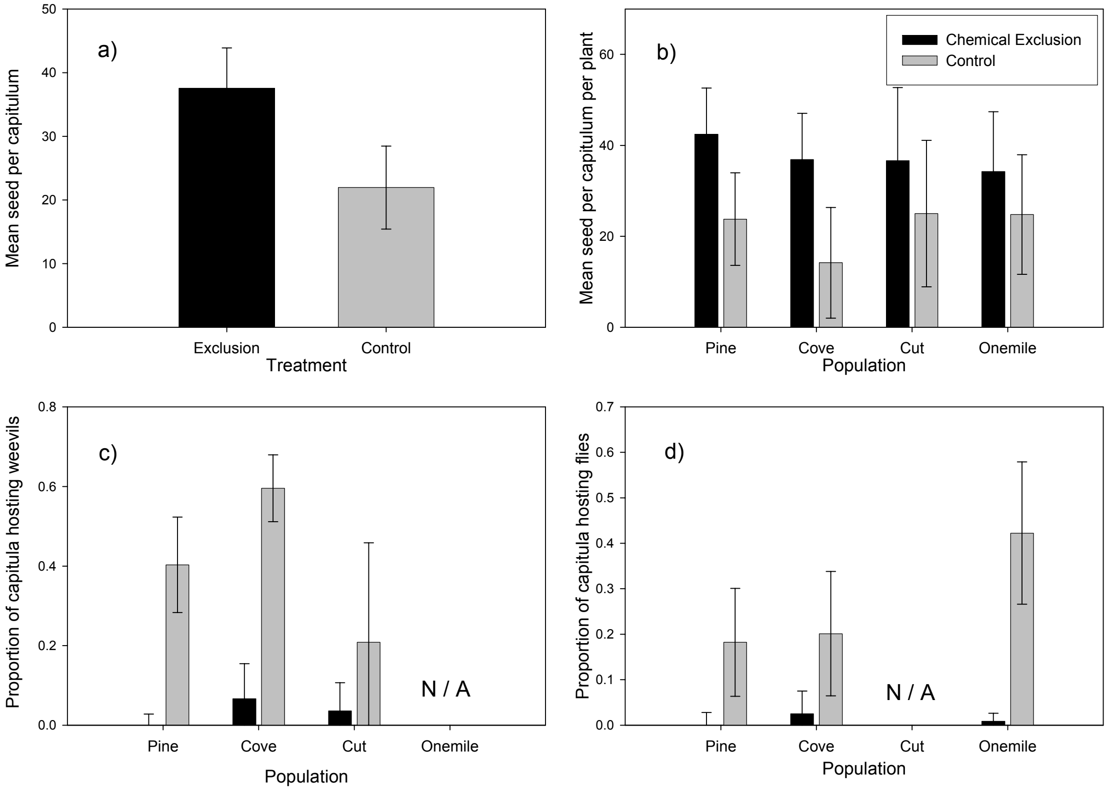

Figure 2-4. Exclusion of seed predators from whole plants resulted in a 71% increase in seed production across the four study populations (a). Two of the three populations (Pine and Cove) hosting *R. conicus*, responded with significant increased seed production while the third (Cut) and the single population not hosting *R. conicus* or *L. planus* (Onemile) did not respond significantly (b). The two responding populations had a high rate of ambient weevil use – as noted in the control populations compared to the non-responding populations (c). Onemile did not respond significantly to seed predator exclusion despite a high ambient rate of fly use (d). All error bars indicate two standard errors.

Similarly, the frequency of fly development was reduced in chemically treated plants (Mann-Whitney U = 78.0; $N_{treat} = 26$, $N_{control} = 23$; adjusted z = 4.97; $p < 0.01$) (Fig. 2-4d). Fewer capitula in the chemical exclusion hosted fly larvae than those in the control group at the Pine site ($X^2 = 16.55$, df = 1, Fisher's exact $p < 0.01$) and at the Onemile site ($X^2 = 26.86$, df = 1, Fisher's exact $p < 0.01$). I found only marginally significant differences at the Cove site ($X^2 = 3.19$, df = 1, Fisher's exact p = 0.08). I found no evidence of fly seed predators at the Cut site.

Average number of recruits around the chemically excluded plants was greater than around control plants across both sites $F_{1, 25} = 5.0907$, p = 0.03). Post-hoc comparisons at each site indicated a significant increase in number of recruits at the Pine site ($F_{1,17} = 5.176$, p = .04) but a non-significant difference at the Cut site ($F_{1, 6} = 0.301$, p = 0.60) (Figure 2-5a). There was no difference in size of the recruits between the exclusion and control plants across all sites ($F_{1,52} = 0.77$, p = 0.38) (Figure 2-5b). Planned comparisons at each site indicated no difference at Pine ($F_{1,36} = 1.5479$, p = 0.22) or at Cut ($F_{1,14} = 0.64273$, p = 0.44) (Figure 5b).

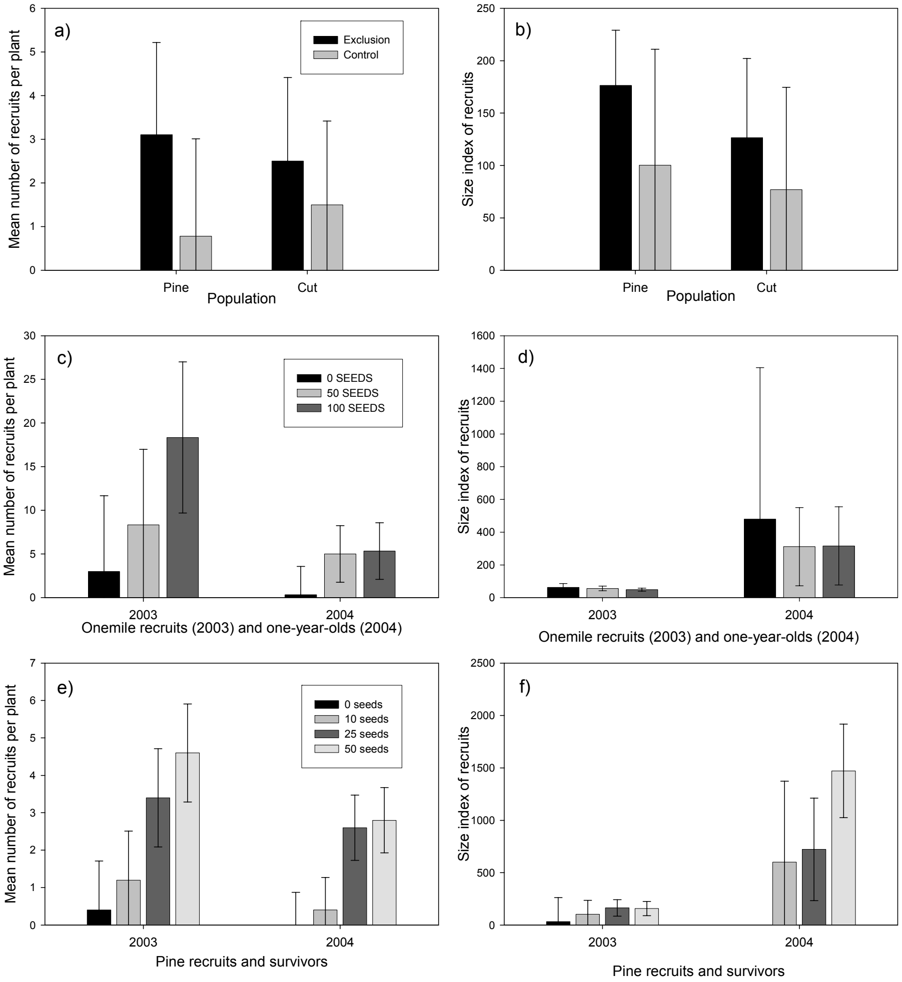

Figure 2-5. Comparisons of thistle recruits from whole plant exclusions and seed addition experiments. There was a significant increase in recruitment around exclusion plants at Pine but not Cut (a). Although the data for Cut are not significantly different the trend is similar to that of Pine. There is no detectable difference in the size of the recruits (b). At Onemile, there was an increase in recruits with increased seed added (c), but after one year, there were no detectable differences between the 50 and 100 seed treatments. There were no differences in the sizes of these recruits and one-year-olds (d). Results were similar at Pine with recruitment per increase in seed added (e) and no detectable differences in size of recruits and surviving one-year-olds (f). All error bars represent two standard errors.

Seed additions

At the Onemile site there was a marginally significant increase in recruitment from adding 50 seeds and a significant increase in recruitment with 100 seeds ($F_{2,6}$ = 5.13, p = 0.05 between all groups; p = 0.06 in contrast comparing 0 and 50; and p = 0.02 contrasting 0 and 100) (Fig. 2-5c). There were no detectable differences in size of the recruits per treatment in 2003 or the same plants in 2004 ($F_{2,28}$ = 0.06, p = 0.94) (Fig. 2-5d).

At the Pine site there was a larger number of recruits at both the 25 and 50 seed treatments than at the 0 and 10 seed treatments and no differences between 25 and 50 and no difference between 0 and 10 (Overall effect $F_{3,16}$ = 9.6984, p < 0.01; Tukey comparisons are significant at p < 0.05) (Fig. 2-5e). The differences in recruitment extended into 2004 – there were more one-year-olds in both the 25 and 50 seed treatments than the 0 and 10, and no differences between 25 and 50 or between 0 and 10 (overall effect $F_{3,16}$ = 11.456, p <0.01; Tukey - Kramer comparisons are significant at p < 0.05). There were no detectable differences in the size index of the recruits per treatment in 2003 ($F_{3,44}$ = 0.57113, p = 0.64) or in the one-year-old survivors in 2004 (there were no surviving recruits from the 0 seed treatment) ($F_{2,23}$ = 3.3586, p = 0.05) (Fig. 2-5f).

DISCUSSION

The results strongly suggest native thistles are being impacted by exotic insect herbivory. First, *L. planus* and *R. conicus* have extended periods of high oviposition rates that overlap with the majority of the flowering season in Tracy’s thistle. Observational and experimental studies indicate that that both *L. planus* and *R. conicus* have large effects on seed production. Finally, the populations studied responded to increased seed input with increased recruitment.

Phenological overlap and initial observations on seed predation

The phenological overlap of both *L. planus* and *R. conicus* oviposition and flowering by Tracy's thistle is such that very high rates of weevil use coincide with the peak of the flowering season. In both cases, I found the majority of the flowering season was characterized by oviposition on over 50% of the inflorescences and the peaks of the flowering season by oviposition on over 80% of the inflorescences. The observed direct effects of seed predation by both *L. planus* and *R. conicus* greatly outweighed the observed effects of the native *O. occidentalis*. The standardized effect *L. planus* was more than double and the standardized effect of *R. conicus* more than triple that of the tephritid fly.

Exclusion of L. planus and R. conicus from Individual Capitula

I found a significant and large effect of seed predator herbivory on seed production in both the Cabins and the Pine site. More specifically, I found no significant differences in seed production when comparing "weevil" with "all insect" exclusions at both sites, and I found no difference in the "fly" exclusion from the control groups at the Pine site. Further, I found no difference between the sham and natural controls. The effect of the weevils and flies on a capitulum level, however, is likely much more pronounced than these results suggest for two reasons. First, the exclusions were not complete – in both sites and for both weevils and flies, exclusions reduced frequency of insect attack, but did not eliminate it. Second, weevils attacked only about 50% and flies about 25% of those capitula available to them (Figures 2-2b and c, 2-3b and c). Additionally, I am confident the treatments themselves had little direct effect on the capitula because at both sites there was no difference in seed production per treatment when including only those capitula that were not attacked by either weevils or flies.

The effects of *L. planus* and *O. occidentalis* larval herbivory on seed production per capitulum can be isolated better by comparing all capitula (across treatments) that hosted the insects and those that did not. Isolation of the effects of *L. planus* herbivory on seed production using experimental exclusions of *L. planus* and *O. occidentalis* from individual capitula indicated that the weevil is responsible for approximately a 70% reduction in seed production per capitulum with one developing larva and even greater reductions when more than one larva develops in the capitulum (Fig. 2-2d). *Orellia occidentalis* has a much smaller influence on seed production (14% per larva), which was only detectable when looking at only those capitula with no weevil development. Additionally, the standardized coefficient for *L. planus* (-0.438) in the multiple regression is nearly twice that of *O. occidentalis* (-0.238) (Table 2-4). Note that, in the analysis, the data are square root transformed, thus, the cumulative effect of *L. planus* on the square root of seed production is nearly twice that of *O. occidentalis*. The lack of significant difference in seed production between the weevil-only exclusion and the all-insect exclusion coupled with the lack of change in seed production with fly presence (when including all capitula) indicate that currently, in this system, the native tephritid fly, *O. occidentalis*, has little impact.

A similar pattern of seed reduction is seen with *R. conicus* - a 60% reduction in seed production with a single *R. conicus* larva and much greater reduction with more than one larva (Fig. 2-3d). The decrease in seed production is attributable to *R. conicus.* There are no measurable effects from *O. occidentalis* in this experiment either via the multiple regression where the estimated coefficient was 0.04 and the p-value 0.56, or in the analysis of the relationship between number of larvae per capitulum and seed production.

Whole plant exclusions

In two of the four populations in which I conducted experimental exclusions of insect herbivores I found that insect herbivory resulted in a greater than 50% decrease in seed production at the plant level (Fig. 2-5b). The two populations that showed significant responses to insect exclusion (Pine and Cove) were the two that hosted both *R. conicus* and *O. occidentalis*. The Cut population did not host *O. occidentalis* and had a low frequency of weevil attack, 21% of all capitula compared with 40% and 60% for Pine and Cove respectively, and the Onemile population did not host *R. conicus* at all. Despite higher frequency of tephritid attack, the seed production at Onemile was not significantly affected by the treatment whereas the Cut population had low weevil frequency and was still close ($p = 0.07$) in significance.

The increase in recruitment associated with exclusion of herbivores at the Pine population had a similar but not statistically significant trend at the Cut population are evidence of insect limitation of Tracy’s thistle (Fig. 2-6a). The increased recruitment at both sites was not associated with a measurable decrease in recruit quality – the sizes index of the recruits was no different than the control recruits (Fig. 2-6b).

Seed additions

I found a clear and positive response in recruitment by adding seeds at two of the populations (Figs. 2-6a-d). Although the difference in seed added was small compared to the estimated seed rain of the populations, by burying the seeds I removed post-dispersal seed predators and long distance dispersal. The seed addition experiment demonstrated that even in the immediate proximity of the maternal plant the seed rain does not saturate the habitat and even these very localized sites are seed limited. It would be expected from seed dispersal curves and field observations that these sites would receive a large proportion of the seed rain. The disparity between this hypothesized seed rain and the results implies one to three conditions: first, seed dispersal continues after the seed lands and is released from the pappus, and/or second, that post-dispersal seed predators are eating all the seed, and/or third, that the seeds never manage to find suitable microhabitat (i.e. three millimeters under the soil surface).

Ecological implications

I have presented strong evidence that the exotic insect, *R. conicus*, is limiting the population growth of native thistles at the Pine site. The whole plant exclusions indicate that insect herbivory is limiting recruitment, and the individual capitulum exclusions viewed with the percentage of capitula used by *R. conicus* indicate that *R. conicus*, alone, is responsible for an approximate 30-50% reduction in seed output over the course of the growing season. I speculate analogously that *L. planus* is also limiting populations from two pieces of evidence. First, oviposition rates and larval herbivory are similar to that of *R. conicus* (perhaps even more damaging); and second, and I found no population that did not respond positively to added seed.

I can also speculate on the effects of competition of *L. planus* and *O. occidentalis* with a single piece of evidence. When *L. planus* was excluded from the individual capitula at Cabins in 2002, the frequency of fly attack increased significantly over the controls (Fig. 2-3c). It has been shown that *O. occidentalis* selects oviposition sites non-randomly (Lamp and McCarty 1982) and that it oviposits after the weevils. We assume that the flies can sense either the presence of weevil larvae in the capitulum or that the capitulum quality is somehow lowered.

The seed addition results and the increased recruitment from the whole-plant exclusions both suggest that even at the much localized habitat within two meters of the maternal plant the population is seed limited. Because this is the area that would generally be more prone to intraspecific competition we might expect more drastic effects of reduced seed production in the ability of the plant or population to colonize more distant sites (Harper 1977). Thus populations may be able to persist, but colonization and recruitment decreases or ceases.

Conservation implications

Although much has been written about negative effects of introduced and invasive species, few studies have demonstrated cases of reduced performance of native species caused by deliberate introduction of exotic insects. Louda has compiled the most complete study to date of how an introduced insect (in this case *R. conicus*) has drastically influenced population dynamics of native plants and the native phytophagous insects that depend on it (see Louda 2000, Gassmann and Louda 2001).

The current known regional distribution of *L. planus* on native plants consists of disjunct sites in Colorado, Oregon, South Dakota, and Washington and it has been recovered from four native taxa (Dodge et al. in review). *R. conicus* on the other hand has been described on approximately 30 taxa in 26 states (Louda et al. 1997, Pemberton 2000, Herr 2004, Dodge et al. in review). The little we know of invasion dynamics suggests that exotic species may coexist at low-level apparent equilibria for many years prior to undergoing a large-scale population expansion (Crooks and Soulé 1999). Research in biocontrol suggests analysis of population-level effects should occur only after 10-20 years have elapsed since introduction (McFadyen 1998). For example, it took *R. conicus* 20 years from its introduction in Nebraska to the time it colonized some native thistle patches in the sand prairie (Louda 2000). Most introductions of *L. planus* in the western US took place in the early 1990s (Louda and O'Brien 2002, Dodge et al. in review) – so current distribution is not necessary an accurate indication of future distribution.

Both *L. planus* and *R. conicus* fed on and oviposited on native *Cirsium* species during lab tests for their suitability as biocontrol agents (Zwolfer and Harris 1984, McClay 1989, Arnett and Louda 2002). New world *Cirsium* species comprise at least 96 taxa indigenous to North America (Jordon-Thaden and Louda 2003, USDA and NRCS 2004). Most of the species diversity in the genus occurs west of the continental divide and includes several taxa recognized as endangered. Past and current redistributions of these insects clearly flirt with compromising the native ecosystems as much as or more than the exotic weeds that the insects are released to control.

Conclusion

The current study provides substantial evidence that *R. conicus* is responsible for demographic changes in Tracy's thistle and has also shown that *L. planus* has an effect similar to that of *R. conicus* on seed production. Furthermore, the native seed predator *O. occidentalis* has a comparatively much-smaller effect on seed production. The study has tested four population sites of similar habitat and found each to be seed limited. Together this evidence provides a strong example of top-down control in Tracy's thistle in the Gunnison Valley.

This study also contributes to a clearer picture of the non-target effects of these biocontrol agents. Ecological implications of biocontrol and the role of ecological theory in its application are still not thoroughly understood (Murdoch and Briggs 1996, Strong and Pemberton 2000). When biocontrol agents are approved, released, or redistributed in the future, we present an argument that the natural history of species closely related to the target should be included in the decision-making process.

Chapter 3: Predispersal seed predation in Rocky Mountain thistle (*Cirsium perplexans*): evidence and conservation implications.

ABSTRACT

Evidence of ecological nontarget effects of weed biological control agents has been increasing but quantification of the effects is still deficient. It has been speculated that the greatest threat to the overall survival of *Cirsium perplexans*, a rare native thistle, is the use of exotic insects as biocontrol agents for invasive thistle species. The current study incorporated experimental exclusion of insect herbivores, a seed augmentation experiment, and a survey of populations to quantify the effects of native and introduced seed predators on seed production and implications for recruitment. Herbivory by *Rhinocyllus conicus*, a biological control weevil, *Orellia occidentalis*, a native fly, and *Homoeosoma impressale*, a native moth, was responsible for a multi-fold decrease in seed production in a population that showed indications of seed limitation. *R. conicus* has a much larger effect relative to the other insects. Further, seven of ten populations visited in a survey hosted *R. conicus* and one hosted *Larinus planus*, another weevil introduced to control exotic thistles. *R. conicus* oviposition intensity was shown to be correlated with size and proximity of exotic thistle populations. These results help understand the risk of biological control of weeds using oligophagous insects and potential indirect effects of exotic species.

INTRODUCTION

Biological control of invasive weeds is, by nature, a delicate balance between introducing effective biological control agents and not introducing another invasive species. A disconcertingly similar suite of traits is used to describe invasive insect species and to identify appropriate biological control agents (or candidates) (Goeden 1983, Crawley 1989b, Williams and Meffe 1998, Schooler et al. 2004). Good control agents and invasive exotic species have high rates of dispersability, they are good colonizers (a single gravid female will colonize), and they have high reproduction rates. It shouldn't come as a surprise that two previously released weed biological control agents can be characterized as invasive species (see Johnson and Stiling 1998, Louda 2000).

Host specificity seems to be the singular exception in separating favorable weed biological control agent traits from invasive species traits. Host specificity is a primary criterion in determining suitability as a released agent (McEvoy 1996), however the threshold for acceptable levels of specificity is hotly contested. Use of strictly monophagous insects, while optimal, is not a viable solution if biological control is to continue, because truly monophagous herbivores are relatively rare. Selecting insects that have genus-level specificity is risky if there are native congeneric species or other closely related species (Simberloff and Stiling 1996, Louda and Arnett 1999, Pemberton 2000).

The magnitude of nontarget herbivory on native species is based, theoretically, on more than just direct interaction between the agent and the native host. In addition to the direct effects of herbivory, populations of native plants can be affected by competition and herbivore-mediated apparent competition from other plant species (Futuyma and

Wasserman 1980, Parker and Root 1981, Rand and Louda 2004). The shared predation model (Holt and Hochberg 2001) and two prey model (Harmon and Andow 2004) both predict that the abundance of the biocontrol agent, being sustained by a suite of hosts, is greatly affected by the abundance and productivity of all hosts, and that agents with multiple hosts will be more abundant. Thus, in biological control of weeds, risk to a nontarget plant is affected by the abundance and proximity of the target weed plant as well as the agent's ability to control that target weed.

Nontarget ecological effects of weed biological control have been shown in an increasing number of cases (Louda et al. 2003, Coombs et al. 2004b), but quantification of the effect and risk is still "a scientific frontier" (Louda et al. 2005). Study of the ecological effects of nontarget herbivory on rare and endangered plant species is especially valuable in terms of establishing management regimes that are conducive to protecting the vulnerable species. *Rhinocyllus conicus*, a biocontrol weevil introduced to control exotic thistles, has been documented feeding on more than 30 species of native *Cirsium* thistles including at least seven that are considered rare or endangered (Pemberton 2000, Herr 2004, Dodge et al. in review). Little work has been done, however, investigating actual direct and indirect effects on rare or endangered taxa.

Cirsium perplexans has been identified as globally and locally imperiled (G2, S2) (CNHP 2004, NatureServe 2005) with 25 known occurrences – all in western Colorado. Nontarget use of *C. perplexans* by insect biological control agents was first observed in 2001 (personal observation), and recently it has been speculated that the greatest threat to the overall survival of this species is the use of exotic insects as biocontrol agents for invasive thistle species (Spackman-Panjabi and Anderson 2004).

This study has two primary aims, both fundamental to key aspects of conservation biology; first, to investigate the ecological implications of herbivory by invasive insects for native and rare plant species and, second, to quantify the ecological ramifications of biocontrol efforts on native species. In addressing the above questions we designed the current study to address the relationship between predispersal seed predation and seed production in *C. perplexans*, investigate the relationship between seed production and seedling establishment in *C. perplexans*, and determine whether nontarget herbivory by biological control insects presents a localized or generalized threat for *C. perplexans*.

METHODS

Study species

Cirsium perplexans (Rydb.) Petrak (Asteraceae), Rocky Mountain thistle, is a perennial, polycarpic member of the Cardueae tribe. It has been previously described as both a biennial, obligate biennial, and perennial (see Spackman-Panjabi and Anderson 2004). The classification of polycarpy is based on examination of rosettes and flowering individuals that had remnants of previous years' flowering stalks still attached. *Cirsium perplexans* has been classified as imperiled at the state level by the Colorado Natural Heritage Program (CNHP 2004) and as globally imperiled by NatureServe (2005). There are approximately 25 known occurrences of *C. perplexans* in Delta, Garfield, Gunnison, Mesa, Montrose, and Ouray counties in western Colorado (Spackman-Panjabi and Anderson 2004).

Cirsium perplexans is generally found in sparsely populated or barren disturbed clay and shale soils. In the populations I studied, it is loosely associated with pinyon-juniper forests and sagebrush scrublands. The soils were generally disturbed either by human activity (roadsides and access roads) or by natural heaving and cracking resulting from swelling of the shale soils.

The two populations used in the present studies on seed predation, seed augmentation, and demography were located in the Cimarron State Wildlife Area (SWA) in Montrose County, Colorado and were referred to as the Sign and View populations. Population locations are on record with the CNHP and are not presented here because of the sensitive status of the species.

Rhinocyllus conicus (Froelich) (*Coleoptera: Curculionidae*) is a Eurasian seed head weevil, released throughout the western states for biocontrol of plumeless thistles (*Carduus spp.* (L.)) (Rees et al. 1996). *R. conicus* was originally released in the U.S. in 1969, and it has since been found in at least 26 states (Zwolfer and Harris 1984, Turner et al. 1987, Louda et al. 1997, Gassmann and Louda 2001). *R. conicus* is univoltine and completes its larval development within developing capitula of thistles. *R. conicus* lays its eggs on the exterior of the bracts surrounding developing flower buds, covering them with an easily seen cap layer of masticated plant tissue. Larvae hatch and burrow into the flower head. Damage to the flower head by both weevils is primarily through larval feeding on ovules, developing seeds and receptacle tissues. Larvae undergo metamorphosis in distinctive pupal chambers inside the flower heads, and then emerge as adults.

Orellia occidentalis (Snow) (*Diptera: Tephritidae*) is a North American picture-winged fly native to the western United States (Foote and Blanc 1963). The flies are generally univoltine or bivoltine, overwintering in the pupal stage. Adult females oviposit during early anthesis when phyllaries spread and expose the tops of non-elongated florets. Females insert eggs into the mass of florets. Larvae hatch and tunnel down the corolla tube and into developing seeds, feeding on the seeds. Larvae can bore into additional seeds and feed on several leaving distinct entry and exit holes in the seed coats. Larvae then usually drop to the soil to pupate (Lamp and McCarty 1982). The larvae are easily caught in mesh bags placed over the flower head.

Homoeosoma impressale (Hulst) (*Lepidoptera: Pyralidae*) is a seed-eating moth native to North America and found on various *Cirsium* and closely related genera. *H. impressale* larvae feed on seeds and seed coats and their presence is characterized by the accumulation of frass in the flower head. Unlike *R. conicus*, which oviposits on closed flower buds, and *O. occidentalis*, which oviposits on early anthesis inflorescences, *H. impressale* oviposits among the florets when the inflorescence is in bloom (Lamp and McCarty 1979 and personal observation).

Pre-dispersal seed predation

In July 2001, I conducted observational studies of pre-dispersal seed predation on Rocky Mountain thistle at the Sign population – with the goal of identifying which seed predators were influential and their prevalence. Over a period of two weeks, I collected up to five flowering heads from 15 plants (totaling 65 capitula), selecting capitula that were post-flowering and close to seed release – at a stage when seeds have matured and filled, and seed coats have hardened. During dissection, I collected data on capitulum diameter, larval development, and seed production. For weevils, I counted larvae and pupal chambers, for the tephritid flies I counted larvae and puparia, and for the pyralid moths I noted only the presence and absence based on finding frass and/or moth larvae. Some fly puparia and moth larvae were reared for identification purposes.

I compared the relative effects of the seed predators using a multiple regression with ln-transformed number of seeds as the dependent variable and weevil pupal chambers, tephritid larvae, and moth presence or absence as the dependent variables. I made comparisons of the B coefficients to quantify the relationship between individuals of each species and seed production, comparisons of the Beta coefficients to quantify the relationship between the species as a group on seed production, and the partial correlation coefficients to quantify the magnitude of effect on the model fit (Kleinbaum et al. 1998, StatSoft 2004) . The model began with the inclusion of capitulum diameter as an additional variable, but was removed as it was insignificant.

In order to quantify the collective effects of predispersal seed predation in *C. perplexans*, in June 2002 I conducted a plant-level exclusion experiment that incorporated 40 plants at two sites (20 at the Sign site and 20 at the View site). Plants were randomly selected to one of two treatment levels. An insecticide treatment consisted of weekly spraying of the entire plant with acephate (Isotox ®), and a sham control consisted of spraying with water. I measured the diameter of all capitula as the widest part of the phyllaries (not including the spines) and bagged the inflorescences in nylon mesh bags at the termination of flowering. I collected the capitula after the phyllaries had reflexed to release seed. Capitula were dissected in the lab. I noted the number and stage of insects as well as counted puparia and pupal chambers in the capitulum.

To test the direct effects of spraying insecticide on the plants I compared flower production between the sham control and treatment with two statistical analyses. First, I tested the sum of flowers produced by an individual throughout the entire season using a single factor ANOVA on flowers produced. Second, I tested flower production at weekly intervals using repeated measure analysis.

I quantified the exclusion efficacy through analysis of insect development prevalence per treatment at each site by comparing the proportions of collected capitula per plant that had been used (presence or absence) by each of the seed predators between treatments at each site as well as for all seed predators combined. For these comparisons I used non-parametric Mann-Whitney U-tests due to continued violations of ANOVA assumptions (even after using arcsine-square root transformation) with parametric analyses.

To test cumulative effects of all insect predispersal seed predation on seed production I compared mean number of undamaged seeds (ln-transformed) per capitulum per plant for treatments versus controls in a mixed model ANOVA with population and the interaction term as random blocking factors. I used the average seeds per capitulum per plant as the dependent variable for two reasons: first, to control for number of capitula per plant; and second, to satisfy assumptions of variable normality. To clarify direct effects of species of seed predators on seed production, I also conducted a three-way ANOVA analysis with the log-transformed number of seeds as the dependent variable and *R. conicus*, *O. occidentalis*, and *H. impressale* (presence or absence) per capitulum as the independent variables to estimate each seed predator's cumulative impact in the presence of the others and used the standardized parameter estimates (β) to compare the relative contribution of each seed predator to seed production.

Seed limitation

I installed a seed augmentation study of five treatments (0, 5, 10, 20, 40 seeds) buried at 3mm in 0.25m x 0.25m squares on two grids in July 2003 at the Sign site in Montrose County, Colorado. The first grid measured 2.5m by 2m and replicated each treatment four times and the second grid measured 2m by 5m and replicated each treatment eight times. Grids were placed within the population and in areas that were representative of densities of pre-existing flowering and non-flowering plants. Treatments were randomly assigned to cells.

Treatment levels were selected to ensure covering the maximum density seed rain at the population. Maximum density seed rain was estimated at 23 seeds per cell by multiplying the greatest observed number of flowers on a single plant by the greatest number of seeds observed from a single capitulum in the population and estimating the seed density given that all seeds fall within one meter of the maternal plant (14 flowers times 82 seeds divided by 3.14 m^2 = 366 seeds per m^2 or 23 seeds per 0.25m x 0.25m). The best estimate of seed rain is actually 2.01 seeds per cell (7.10 flowers times 14.25 seeds divided by 3.14 m^2 = 32.22 seeds per m^2 = 2.01 seeds per 0.25m x 0.25m).

In July 2004 I counted and mapped recruits. Recruits were distinguished from other plants by the persistent cotyledons (sometimes dried). I counted number of leaves and measured the longest leaf as an indicator for size. Recruitment was analyzed using a mixed linear model with number of recruits (log transformed) as the response variable and treatment (fixed, continuous), plot (random), and plot*treatment (random) as the independent variables. I also analyzed germination rate per treatment (excluding the 0 seeds added treatment) to investigate the relationship between number of seeds added and germination. I again used a mixed linear model with germination rate (the ratio of recruit number divided by seed input, arcsine-square root transformed) as the dependent variable and treatment (fixed, continuous), and plot (random) and plot*treatment (random) as the independent variables. Recruit size as a function of recruit density was analyzed in a mixed linear model analyses with size as the dependent variable and recruit density per cell (fixed, continuous), plot (random), and plot*recruit density (random) as independent variables.

Population survey

In late July 2004 I visited 11 of the 25 known occurrences of Rocky Mountain thistle. I also visited the reported sites of four more that I never found. Occurrences are defined as a single population or a group of sub-populations, each within 1.6 km of another sub-population (criteria currently used by CNHP). In addition I encountered a single new occurrence. At each site I counted flowering individuals and in cases where there were no flowering plants, I counted vegetative basal rosettes. I randomly chose five flowering individuals and inspected each capitulum for evidence of oviposition by *R. conicus* and *L. planus* by checking for external evidences as well as cracking the receptacle open and looking for pupal chambers. I visited the populations post seed-release so no seed production data were collected. For the Cimarron SWA Sign and View populations, I used data collected in 2001 from before the populations were manipulated in the exclusion experiments.

Following this I inspected the population for remnants of the previous year's flowering plants – either dried, standing stalks or larger rosettes with the base of the broken stalk still attached. I selected five of these and searched the ground within a circle of one-meter radius centered on the old flowering stalk and counted vegetative rosettes and recruits. Recruits were still discernable from vegetative rosettes by the dried yet still attached cotyledons. I conducted statistical analysis of the relationship between recruits and *R. conicus* oviposition intensity per plant using a correlation between the average, log transformed number of recruits (ln+1) per population and the average oviposition prevalence in the plant population. I did not conduct the same analysis for *L. planus* oviposition intensity for lack of adequate sample size.

Following the investigation of the Rocky Mountain thistle, I then searched the surroundings for the nearest populations of exotic thistles - specifically *Carduus nutans* and *Cirsium arvense* out to a distance of 1000 meters. I estimated the size of the *C. arvense* populations in area (square meters), and the size of *C. nutans* populations in number of flowering individuals. I then measured the distance to Rocky Mountain thistle populations, and inspected the populations for use by *R. conicus* and *L. planus*.

To investigate a relationship between oviposition intensity on *C. perplexans* and availability of exotic hosts I conducted a correlation analysis between *R. conicus* oviposition intensity (primary capitula) and an index of exotic floral resources. This index was constructed as the sum of a *C. arvense* index and a *C. nutans* index. These species indices were defined as the nearest population size divided by the distance (both numerator and denominator were log transformed).

Notes on statistical analyses

In all cases where I performed parametric analyses, the procedure was checked to conform to the assumptions of homogeneity of variance and normality of residuals. Checking for homogeneity of variance involved visual inspection followed by Levine's, Hartley F-max, Cochran C, and Bartlett chi-square tests (StatSoft 2004). Normality was confirmed through visual inspection of histograms and normal probability plots.

The presence of zero values in the seed production leads to potential violation of the assumption that variables are normally distributed. I proceeded with the analysis despite this issue because the truncation of the residuals and resulting skewed residual distribution occurred only at very low predicted seed production. Furthermore, general linear regression models are considered robust when dealing with minor violations of this assumption (StatSoft 2004). For multiple regression models I also looked for correlation between the independent variables as an indicator of multicollinearity. Linear models were analyzed using Type III sums of squares.

RESULTS

Predispersal seed predation

The observational studies of predispersal seed predation identified three primary phytophagous insect species as major players: two native insects, *O. occidentalis*, and *H. impressale,* and *R. conicus*. 90% of all capitula had been attacked by insect herbivores. 71% were attacked by *R. conicus* (0-4 larvae per capitulum), 31% by *O. occidentalis* (0-3 larvae per capitulum), and 9% by *H. impressale*. Capitula hosting any seed predator produced fewer seeds than those not hosting a seed predator (Mann-Whitney U Test; $p < 0.01$). Overall plant intensity per seed predator species and across all species are presented in Table 3-1.

Table 3-1. Seed predator prevalence calculated as the average proportion of capitula per plant hosting seed predators (SE).

Seed predator	2001 Sign Observed	N	2002 Sign Exclusion	N	2002 Sign Control	N	2002 View Exclusion	N	2002 View Control	N
R. conicus	0.699 (0.070)	15	0.030 (0.043)	16	0.138 (0.096)	13	0.000 (0)	8	0.081 (0.103)	7
O. occidentalis	0.312 (0.069)	15	0.021 (0.036)	16	0.021 (0.039)	13	0.005 (0.025)	8	0.024 (0.058)	7
H. impressale	0.141 (0.043)	15	0.021 (0.026)	16	0.077 (0.074)	13	0.000 (0)	8	0.012 (0.041)	7
All species	0.861 (0.046)	15	0.066 (0.033)	16	0.231 (0.084)	13	0.005 (0.005)	8	0.117 (0.075)	7

Multiple regression results of effects of each species on seed production indicate a significant negative effect for both *R. conicus* and *H. impressale* on seed production and no significant effect for *O. occidentalis* (Table 3-2). Examination of the standardized beta coefficients indicated that *R. conicus* had almost three times the effect on seed production than *H. impressale* and approximately ten times that of *O. occidentalis* (Figure 3-1). Further, the relationship with *R. conicus* contributed to the model fit more than twice that of *H. impressale* and eight times that of *O. occidentalis*.

Table 3-2. Multiple regression. Partial regression coefficients (B), standardized regression coefficients (Beta), and partial correlation coefficients for *R. conicus*, *O. occidentalis*, and *H. impressale*, on seed production (ln-transformed) from observations in July 2001 (N = 65). $F_{3, 61} = 21.78$; $p < 0.01$; Adjusted $R^2 = 0.49$.

	B	SE of B	Beta	SE of Beta	Part. Corr.	t(61)	p-level
Intercept	3.676	0.283				13.012	< 0.01
R. conicus larvae	-1.204	0.169	-0.699	0.098	-0.674	-7.120	< 0.01
O. occidentalis larvae	-0.138	0.209	-0.065	0.099	-0.085	-0.663	0.51
H. impressale presence	-1.089	0.401	-0.243	0.089	-0.328	-2.714	0.01

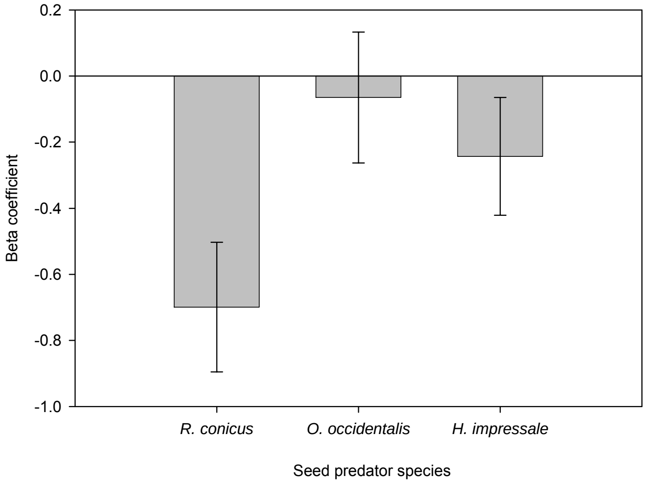

Figure 3-1. Estimated standardized coefficients (Beta coefficients) for each of three seed predator species from observational studies conducted in July 2001. The estimated effect for *R. conicus* is nearly three times that of *H. impressale* and ten times that of *O. occidentalis*. Error bars represent two standard errors.

There was no evidence for direct effects of spraying acephate or water on flower production. Single factor ANOVAs showed no difference on the seasonal sums of flowers per plant at the Sign site ($F_{1,31}$ = 1.84977; p = 0.18) or the View site (F = 1.368; p = 0.26). There also was no difference in flower production between the treatment and control using the repeated measures analysis of weekly floral production throughout the summer at either site (Table 3-3a and b). Further, there was no indication of treatment efficacy; there were no differences among seed predator intensities (percent of capitula per plant utilized by any seed predator) at both sites (Mann-Whitney U-test corrected z = 1.59; p = 0.11 for Sign; and z = 0.91, p = 0.36 for View). *Rhinocyllus conicus* intensity did not differ significantly between treatments (z = 1.50; p = 0.13 for Sign; and z = 1.56, p = 0.12 for View), nor did use by *O. occidentalis* (z = 0.15, p = 0.88 for Sign; z = 0.20, p = 0.84 for View) or *H. impressale* (z = 0.20, p = 0.84 for Sign; z = 1.07, p = 0.29 for View). Seed predator intensity averaged per plant is presented in Table 3-1.

Table 3-3. Repeated measures analysis. Results of flower production as a function of treatment (chemical exclusion and control) and date at the Sign site (a) and View site (b). There are two error terms per analysis – the first is the appropriate error term for between subject effects and the second is the term for within subject effects.

a.					
	SS	df	MS	F	p
Intercept	205.84	1	205.84	42.76	< 0.01
Treatment	10.90	1	10.90	2.26	0.14
Error – between subjects	144.40	30	4.81		
Date	94.24	8	11.78	5.49	< 0.01
Date * treatment	20.93	8	2.62	1.22	0.29
Error – within subjects	515.05	240	2.14		

b.					
	SS	df	MS	F	p
Intercept	1903.45	1	1903.45	29.41	< 0.01
Treatment	88.45	1	88.45	1.37	0.26
Error – between subjects	1164.81	18	64.71		
Date	787.51	9	87.50	5.77	< 0.01
Date * treatment	91.31	9	10.15	0.67	0.74
Error – within subjects	2457.49	162	15.17		

Mixed model analysis of seed production across populations indicated that the insecticide treatment affected seed production in the populations differently – note the significant interaction term (Table 3-4). Subsequent analysis of simple effects indicated that treated plants produced more seeds than control plants at the Sign site (Tukey HSD, $p < 0.01$) but not at the View site ($p = 0.78$) (Figure 3-2).

Table 3-4. Mixed model ANOVA. The effects of insecticide treatment on mean seed (square-root transformed) per capitulum per plant. The significant interaction indicates a difference in treatment effects at the two sites.

	Effect	SS	df	MS	F	p
Intercept	Fixed	820.27	1	820.27	3.73	0.304002
Site	Random	219.65	1	219.65	14.0877701	0.165823
Treatment	Fixed	6.77	1	6.77	0.43405	0.629136
Site*Treatment	Random	15.60	1	15.60	10.21165	0.002724
Error		61.12	40	1.53		

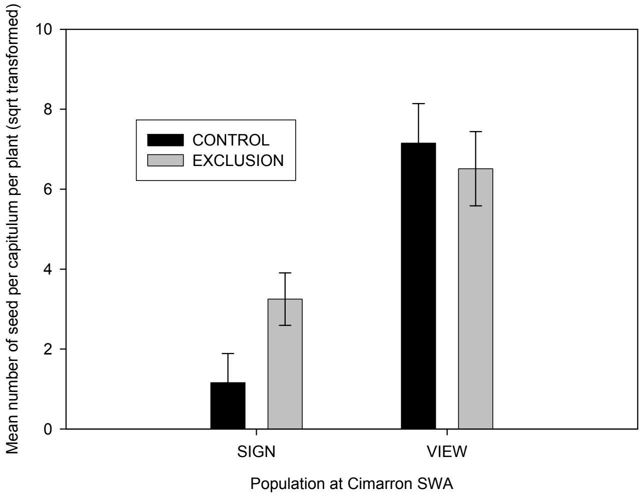

Figure 3-2. Seed production per capitulum of insecticide-treated plants and control plants at two populations of *C. perplexans* in the Cimarron SWA. Mixed-model ANOVA indicated a significant interaction between the treatment and the site. Tukey HSD tests indicate a difference in the control and exclusion seed production at the Sign site ($p < 0.01$) but not at the View site ($p = 0.78$). Error bars represent two standard errors.

Results of a three-way ANOVA comparing the effects of the presence of *R. conicus*, *O. occidentalis*, and *H. impressale* on seed production indicated that only *R. conicus* significantly lowered seed production (Table 3-5). Comparison of the standardized estimates (β) indicated that the estimated effect of *R. conicus* was approximately three times that of *H. impressale* and several times more than that of *O. occidentalis* (Fig. 3-3).

Table 3-5. Three-way ANOVA. The effects of the presence of *R. conicus*, *O. occidentalis*, and *H. impressale* on seed production from an insect exclusion experiment in July, 2002. *R. conicus* was the only seed predator that had a significant effect on seed production and had an estimated effect of greater than three times that of the other species.

	SS	df	MS	F	p	Beta coefficient	SE (Beta)
Intercept	58.25	1	58.25	20.21	< 0.01		
R. conicus	14.41	1	14.41	5.00	0.03	-0.137	0.061
O. occidentalis	2.37	1	2.37	0.82	0.37	0.056	0.061
H. impressale	1.80	1	1.80	0.62	0.43	-0.048	0.061
Error	752.48	261	2.88				

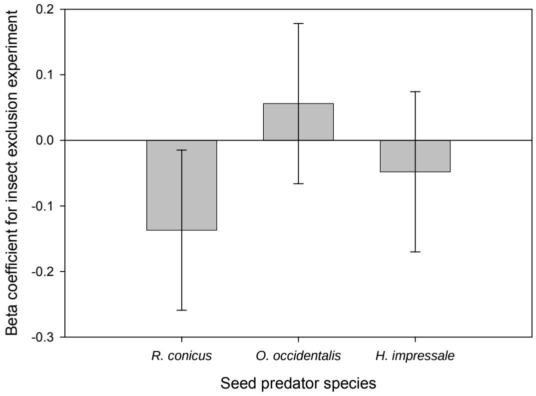

Figure 3-3. Estimated standardized coefficients for each of three seed predator species from studies conducted in July 2002. The only significant effect was that of *R. conicus*. Note that the estimated effect for *R. conicus* is nearly three times that of *H. impressale*. Error bars represent two SE.

In 2001, capitula hosting no *R. conicus* produced an average (± SE) of 37 (± 5.1) seeds, those hosting one *R. conicus* produced an average of 7 seeds (± 1.6) seeds, and capitula hosting more than one *R. conicus* produced an average of 2 seeds (± 1.1) (Fig. 3-4). At the prevalence levels of 2001, *R. conicus* was responsible for a 2.6-fold decrease in seed production across all capitula. In 2002 the relationship was similar with capitula hosting zero, one, and more than one *R. conicus* producing on average 30 (± 2.1), 17 (± 8.0), and 3 (± 10.6) seeds respectively. The marked difference between the two years was due to the prevalence of *R. conicus*. In 2001, capitula hosting zero, one, and more than one *R. conicus* comprised 29%, 42%, and 29% of all capitula respectively, while in 2002 the prevalences changed to 90%, 6%, and 4% respectively.

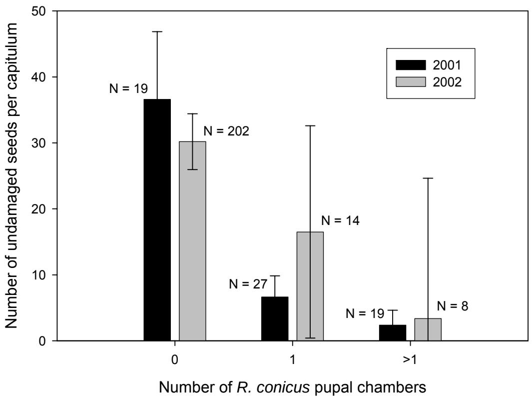

Figure 3-4. Relationship between seed production and number of *R. conicus* pupal chambers per capitulum. Estimates indicated that capitula hosting no *R. conicus* produced at least twice as many seeds as those hosting one larva, and approximately eight times as many seeds as those hosting more than one larvae. Error bars represent two SE

Seed limitation

Number of seeds added to the grid cells significantly affected the number of recruits in the cell when blocked by plot (Table 3-6). The results indicate a clear increase in recruits per seed added in each of the plots (see Figure 3-5). The relationship appeared linear with no clear indication of density effects even at the forty seed treatment. Further, there was no indication of any relationship between seed added and germination rate (Table 3-7) or between recruit density and recruit size (Table 3-8).

Table 3-6. Mixed model ANOVA. The effects of seed added (treatment) to recruitment. Plots were included as a random blocking factor.

	Effect (F/R)	SS	Df	MS	F	p
Intercept	Fixed	7.62	1	7.62	8.43	0.21
Plot	Random	0.90	1	0.90	2.20	0.14
Seed density	Fixed	9.01	1	9.01	527.06	0.03
Plot*Seed density	Random	0.02	1	0.02	0.04	0.84
Error		22.96	56	0.41		

Table 3-7. Mixed model analysis. Germination rates across levels of seed density. There was no apparent effect from seed density on the rate of germination across all treatment levels.

	Effect (F/R)	SS	Df	MS	F	P
Intercept	Fixed	3.70	1	3.70	6.34	0.24
Plot	Random	0.58	1	1.85	6.86	0.01
Seed density	Fixed	0.18	1	0.18	1.02	0.50
Plot*Seed Density	Random	0.17	1	0.17	2.03	0.16
Error		3.74	44	0.08		

Table 3-8. Mixed model linear analysis. Recruit size as a function of recruit density. There was no indication that the number of recruits in a cell influenced recruit size.

	Effect (F/R)	SS	Df	MS	F	P
Intercept	Fixed	111449.8	1	11149.8	18.24	0.15
Plot	Random	6111.6	1	6111.6	2.73	0.10
Recruits per cell	Fixed	555.1	1	555.1	0.25	0.70
Plot*Recruits per cell	Random	2209.2	1	2209.2	0.99	0.32
Error		311654.4	139	2242.1		

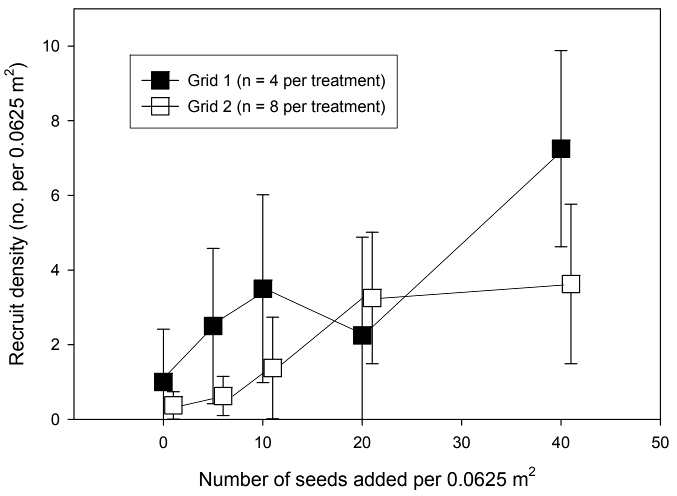

Figure 3-5. Number of recruits per cell as a function of seed added per cell. Recruit density was significantly related to seed density across both grids. There were also significantly more recruits at Grid 1 than Grid 2 across seed densities.

Survey

The populations ranged in size from zero flowering individuals to 2000 flowering individuals. Of the 10 occurrences visited with flowering individuals seven hosted *R. conicus* and one hosted *L. planus* (Table 3-9, Figure 3-6). Oviposition rates by *R. conicus* in the 10 distinct populations where it occurred were generally high (Figure 3-7), ranging from 4% to 100% of primary capitula and from 0% to 85% of secondary capitula across the populations. Oviposition rates of *L. planus* at the three populations were less drastic, ranging from 15% to 42% of primary capitula and from 0% to 11% of secondary capitula (Fig. 3-8).

Table 3-9. Summary of Rocky Mountain thistle populations visited in the survey and utilization by biocontrol weevils. All but two of the population locations were provided by the CNHP – the other two were found during research. Population size of Rocky Mountain thistle is measured in number of flowering plants except in the cases of zero flowering plants in which case I also include number of vegetative basal rosettes (v.b.r.).

Population	**Occurrence**	**County**	**Date visited**	**Flowering phenology**	**Population size**	**Herbivory by biocontrol weevils**	**Exotic thistles and biocontrol weevils**
Lands End Mountain	Lands End Mountain	Delta	7/27/2004	None	0 (15 v.b.r.)	*L. planus* – No *R. conicus* – No	*C. arvense* – None *C. nutans* – None
Paonia Cedar Hill	Paonia Cedar Hill	Delta	7/27/2004	Late flowering	1	*L. planus* – No *R. conicus* – No	*C. arvense* – None *C. nutans* – 1 flowering plant at 10 meters hosting *R. conicus*
De Beque North	De Beque North	Mesa	7/27/2004	Post seed dispersal	300	*L. planus* – No *R. conicus* – No	*C. arvense* – None *C. nutans* – None
Pyramid Rock East Bench	Pyramid Rock East Bench	Mesa	7/26/2004	Post seed dispersal	2000	*L. planus* – No *R. conicus* – No	*C. arvense* – None *C. nutans* – None
Winter Flats 2	Winter Flats	Mesa	7/27/2004	Post seed dispersal	1500	*L. planus* – No *R. conicus* – Yes	*C. arvense* – None *C. nutans* – None
North of Cimarron Campground	Cimarron Campground	Montrose	7/27/2004	Late flowering, some post-dispersal	15	*L. planus* – No *R. conicus* – Yes	*C. arvense* – 5m^2 at 200 meters hosting *R. conicus* *C. nutans* – 5 flowering plants at 200 meters hosting *R. conicus*
Cimarron SWA - Sign	Cimarron SWA – Sign	Montrose	7/2/2001	Mid-flowering, pre-dispersal	47	*L. planus* – No *R. conicus* – Yes	*C. arvense* – 200m^2 at 5 meters hosting *R. conicus* *C. nutans* – 90 flowering plants at 15 meters hosting *R. conicus*
Cimarron SWA - View	Cimarron SWA – View	Montrose	7/2/2001	Mid-flowering, pre-dispersal	20	*L. planus* – No *R. conicus* – Yes	*C. arvense* – 500m^2 at 200 meters hosting *R. conicus* *C. nutans* – None

Population	Occurrence	County	Date visited	Flowering phenology	Population size	Herbivory by biocontrol weevils	Exotic thistles and biocontrol weevils
Doug Creek 1	Doug Creek	Montrose	7/30/2004	Post seed dispersal	32	*L. planus* – No *R. conicus* – Yes	*C. arvense* – 50m^2 at 700 meters hosting *L. planus* and *R. conicus* *C. nutans* 30 flowering plants at 10 meters hosting *R. conicus*
Doug Creek 2	Doug Creek	Montrose	7/30/2004	Late flowering through seed dispersal	2	*L. planus* – Yes *R. conicus* – Yes	*C. arvense* – 25m^2 at 5 meters hosting *L. planus* and *R. conicus* *C. nutans* – 10 flowering plants at 350 meters hosting *R. conicus*
Doug Creek 6	Doug Creek	Montrose	7/30/2004	Post seed dispersal	16	*L. planus* – Yes *R. conicus* – Yes	*C. arvense* – 25m^2 at 10 meters hosting *L. planus* and *R. conicus* *C. nutans* – 5 flowering plants at 300 meters hosting *R. conicus*
Doug Creek 7	Doug Creek	Montrose	7/30/2004	Late flowering through post dispersal	2	*L. planus* – Yes *R. conicus* – Yes	*C. arvense* – 500m^2 at 15 meters hosting *L. planus* and *R. conicus* *C. nutans* – 500 flowering plants at 20 meters hosting *R. conicus*
State Tunnel Dam	State Tunnel Dam	Montrose	7/26/2004	None	0 (200 v.b.r.)	*L. planus* –No *R. conicus* – No	*C. arvense* – None *C. nutans* – None
West of Landfill	West of Landfill	Montrose	7/26/2004	Post seed dispersal	22	*L. planus* – No *R. conicus* – Yes	*C. arvense* – None *C. nutans* – None
Chaffee Gulch	Chaffee Gulch	Ouray	7/26/2004	Post seed dispersal	9	*L. planus* – No *R. conicus* - Yes	*C. arvense* – 50m^2 at 100 meters hosting *L. planus* and *R. conicus* *C. nutans* – 5 flowering plants at 400 meters hosting *R. conicus*

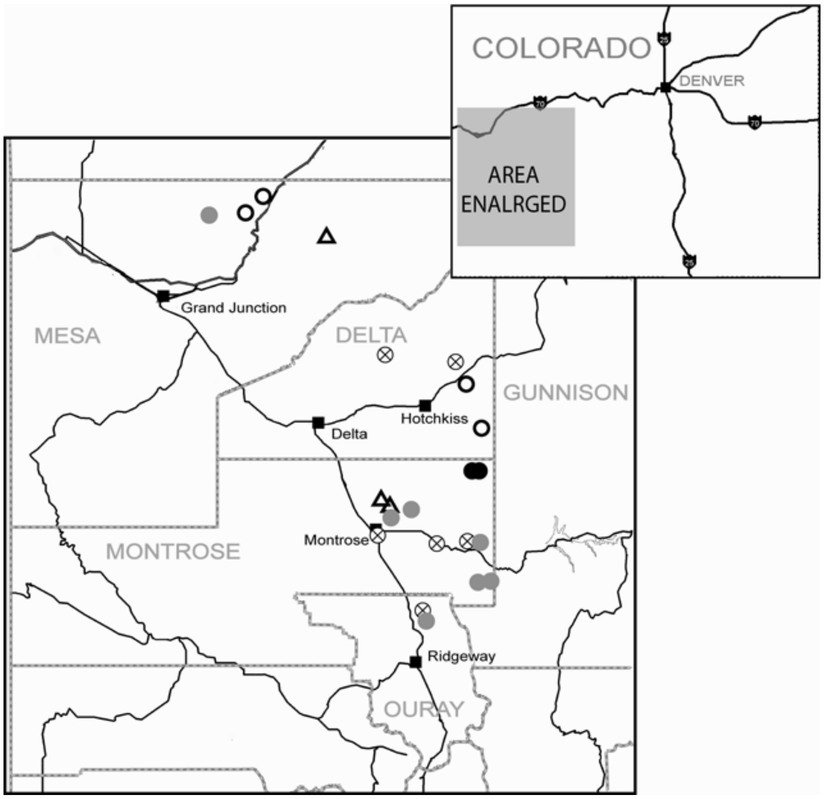

Figure 3-6. Populations and reported populations of *C. perplexans* visited on the survey. A population marked with a closed circle (●) signifies hosting both *L. planus* and *R. conicus*, a gray circle (●) signifies hosting *R. conicus*, an open circle (O) signifies a population hosting neither *L. planus* nor *R. conicus*, and a hatched circle (⊗) signifies a reported population that I searched for but never found. Open triangles (Δ) represent reported populations in the area I did not visit.

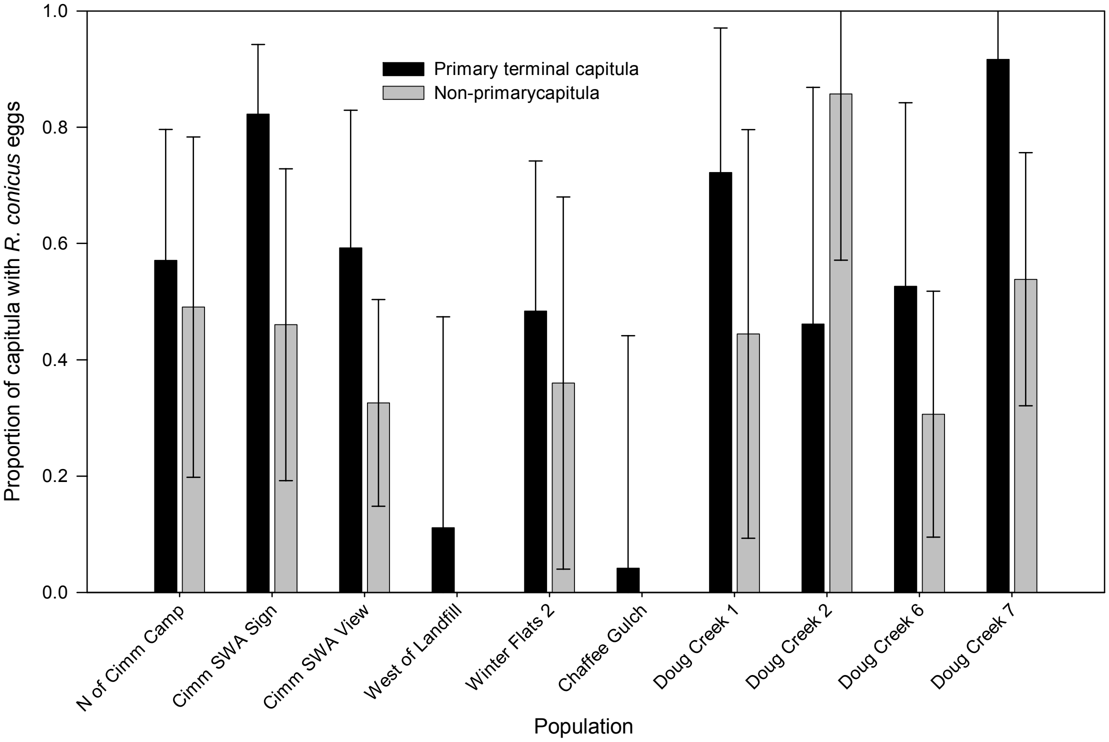

Figure 3-7. Proportion of primary and non-primary capitula with *R. conicus* egg casings at the 10 populations in 7 occurrences where *R. conicus* was found on plants. Data for the first three populations (North of Cimarron Campground, Cimarron SWA- Sign, and Cimarron SWA – View) were collected in July 2001. All other data were collected in 2004.

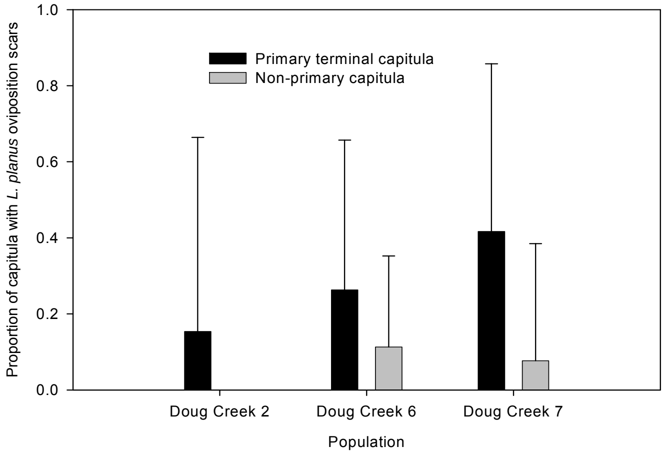

Figure 3-8. Proportion of primary and non-primary capitula with *L. planus* oviposition scars at the three populations where *L. planus* was found on plants.

Average, per population recruitment in one-meter radius circles surrounding the stalks of the previous year's flowering plant was significantly associated with the current *R. conicus* oviposition intensity (n = 11; $r = -0.73$; p = 0.01) (Figure 3-9). Higher oviposition prevalence was associated with lower numbers of recruits.

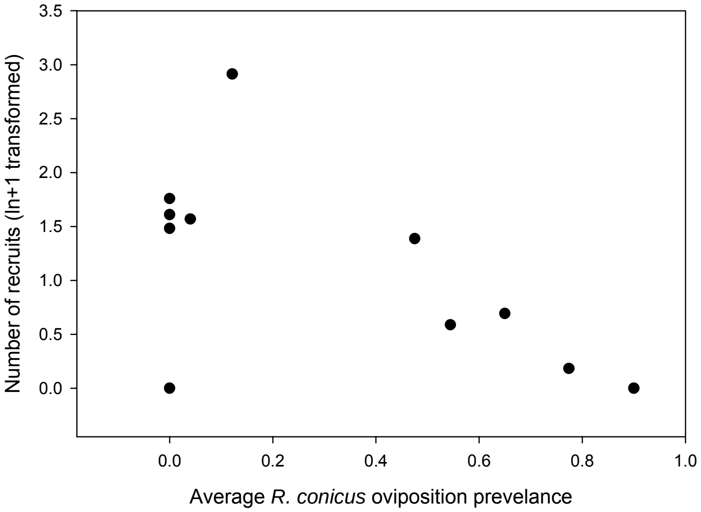

Figure 3-9. The significant association between recruit density and *R. conicus* oviposition intensity (n = 11; *r* = -0.73; p = 0.01).

Rhinocyllus conicus oviposition prevalence was significantly associated with the size and proximity of exotic floral resources (n = 13; $r = 0.78$; $p < 0.01$). More exotic floral resources was associated with higher oviposition intensity on *C. perplexans* (Figure 3-12). *Larinus planus* oviposition intensity was not significantly associated with exotic floral resources (n = 13; $r = 0.53$; $p = 0.07$).

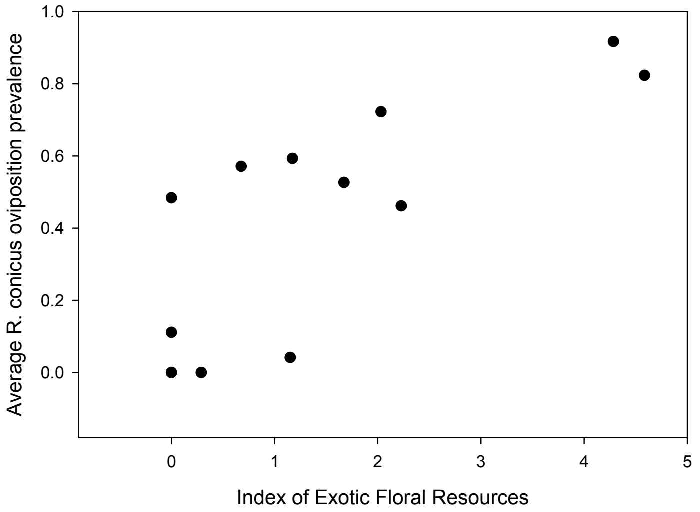

Figure 3-10. Association between average *R. conicus* oviposition intensity per population and the exotic floral resources proximate to the population resources (n = 13; *r* = 0.78; p < 0.01).

DISCUSSION

Predispersal seed predation

Observational studies in 2001 indicated that *C. perplexans* hosted three common seed predators – *R. conicus*, *O. occidentalis*, and *H. impressale*. Utilization of the resource by the seed predators was high – 90% of all capitula were attacked by at least one of the insects. *R. conicus* and *H. impressale* presence both significantly lowered seed production, however the effects of these were unequal. Comparison of the beta coefficients indicates an estimated effect of *R. conicus* to be approximately twice that of *H. impressale* and *O. occidentalis* (Figure 3-1).

Spraying insecticide had measurable effects on seed production per plant at the Sign site, but not at the View site. Seed production at the Sign site increased from about one seed per capitulum in the control group to about nine seeds per capitulum in the insecticide treatment. These results are likely underestimates of the actual effect because seed predators utilized a small number of insecticide treatment capitula. Because there were no changes in flower production between treatments at either site, the effect at the Sign site is likely due to removal of herbivory and not direct effects of spraying acephate. The lack of difference in seed production in the presence or absence of seed-predators at the View site can be attributed to a marked population-level decrease in seed predators - presumably due to application of pesticide to the treatment group and the mortality of the insect seed predators.

Both populations indicated a reduction in seed predator abundance. Plants in the Sign control population averaged 23% seed predator intensity in 2002, while in 2001 they averaged 86%. Although I don’t have data for all seed predator intensity from the View population for 2001, *R. conicus* oviposition rates in 2001 were high (60% of all capitula – see Chapter 1, Appendix D) and in 2002 seed predator intensity in the control group was very low at 12%.

The difference in seed predator intensity between the control populations at the View and Sign sites was likely due to the proximity of source pools of alternative hosts for the insects. Not only was the Sign *C. perplexans* population larger (36 flowering plants vs. 21 flowering plants) but more importantly, the Sign population was within 15 meters of large populations of both *C. arvense* and *C. nutans* - both of which host *R. conicus*. Additionally, both *H. impressale* and *O. occidentalis* feed on multiple *Cirsium* species. The nearest population of *C. arvense* to the View population was 200 meters and there was no *C. nutans* within 500 meters. Thus, spraying the View population exclusion plants likely wiped out the local populations of seed predators but only lowered them at the Sign site, where the exotic thistles were not sprayed.

Rhinocyllus conicus stood out as the most important seed predator in the system. Studies from 2001 showed not only that the weevil had more than double the effect on seed production than the others, and also it was by far the most prevalent - attacking more than 70% of available capitula. In 2002, abundance of *R. conicus* was reduced, but its relative effect was not – in 2002 the relative effect of *R. conicus* was approximately three times that of *H. impressale* and multiple times more than *O. occidentalis*.

Interestingly, in 2002, *O. occidentalis* had what appeared to be a positive effect on the seed production (note the positive beta coefficient). This association with capitula that produce more seed can be explained as an artifact of two things. First, female Tephritid flies have been shown to be choosy when selecting oviposition sites (Bateman 1972, Lalonde and Roitberg 1992), and they may be avoiding capitula already hosting weevil larvae. *Orellia occidentalis* egg loads per capitulum were negatively correlated with number of pupae of a different Tephritid fly (Lamp and McCarty 1982). As in that system, *O. occidentalis* oviposited later in the floral development than did *R. conicus*. Second, and perhaps more to the point, it suggests that *O. occidentalis* had a smaller negative effect on seed production. This is consistent with findings from other authors and congeners.

Seed limitation

The data show that the population is subject to local-scale seed limitation (Fig. 3-7). The population responded with a positive growth rate proportional to the number of seeds added. Additionally, we found no evidence of density effects on germination and growth for seed densities ranging from 0 to 40 seeds per 0.0625 m^2 (0.25 m x 0.25 m).

The implications are that more seed released locally in the population changes the population structure and leads to increased recruitment. Since the maximum treatment value of 40 seeds per cell was nearly double the estimated maximum seed rain of *C. perplexans*, and approximately 20 times the estimated actual seed rain, it is unlikely that the population is anywhere near the point of seed saturation. Recall also that the added seed was in addition to the background seed rain and the grids were place in sections of the population that were representative of pre-existing flowering and non-flowering plants.

The results are not yet conclusive that the population of mature flowering plants will change. Seedling mortality is high following winter heaving of soils, and seedling mortality is size dependent. If plants respond to density effects with differential growth, we can expect to see differential survival at different densities.

Survey

Rhinocyllus conicus is apparently a major threat throughout the limited extent of *C. perplexans*, as has been predicted by researchers (Spackman-Panjabi and Anderson 2004). Seventy percent of the occurrences with flowering individuals hosted *R. conicus*, and oviposition rates at most populations were generally high enough to warrant concern (Fig. 3-3). This is a new taxon from which *L. planus* has been recovered, and although *L. planus* oviposition rates were lower, they were still high enough to be of concern (up to 42% of primary capitula).

The negative association of *R. conicus* oviposition rates and recruitment is strong evidence for population-level effects of *R. conicus* herbivory. Ascribing population-level effects of *R. conicus* herbivory is contingent upon the assumption that oviposition rates are related year to year – thus we can estimate last year's oviposition rates from this year's quantification of demography. Although we have no historic data of *R. conicus* use in any of these populations, *R. conicus* oviposition rates have been shown to be related across years in Platte thistle populations (Louda 2000). Whether or not the recruits or non-flowering rosettes came from the maternal plant around that we measured, the results still indicate a relationship in population density associated with *R. conicus* use.

The association of *R. conicus* oviposition prevalence and the proximity and size of exotic floral resources is alarming but not unexpected. The relationship between the magnitude of herbivory and interspecific floral resource availability has been described as an indirect effect or apparent competition (Holt 1977). The results are corroborated by similar results of interspecific floral resources indirectly influencing negative effects on native plants (Rand 2003) and by recent results of herbivory levels mediated by exotic plants (Rand and Louda 2004).

Population tendencies in Cirsium perplexans

It is important to note an interesting aspect in the population tendencies of *C. perplexans*. Some populations may experience episodic recruitment (i.e. boom years) followed by gradual decline (see notes in Spackman-Panjabi and Anderson 2004). This is not uncommon, especially in arid environments (e.g. Keeler 1991, Menges and Dolan 1998, Wiegand et al. 2004). At the Cedar Mesa site, the population reported by CNHP in 1997 was “thousands of plants” (Lyons 1997). In 2004, the site was visited and held no plants. Location of the former population was verified by the landowner, who also stated the population of flowering plants and rosettes established quickly and then gradually declined after the CNHP visit until it disappeared completely (personal communication, Wayne Wolf, landowner).

Further evidence of the population boom is from Winter Flats where a population was reported as five flowering plants in 1996 (Lyons 1996). The population could not be found in 2004, but in a broad wash below the reported population a “new” population (Winter Flats 2) of approximately 1,500 flowering plants was located. Given that one would need to walk through the wash to reach the slope of the original population, it is highly unlikely that this population of flowering plants and rosettes existed at the time of the first visit in 1996. Lastly, and most surprisingly, was a population of over 500,000 plants (no estimates were made on numbers of flowering individuals) reported growing in the path of a proposed natural gas pipeline (pers. comm. Richard Alward, plant ecologist, and Mike Klish, plant ecologist, WestWater Eng., Grand Junction, Colorado) in 2004 that had been documented in years prior as a much smaller population. The dynamics behind the population booms are not clear and could include a persistent seed bank.

Conservation implications

Exotic plant mediated insect attack is of concern for two reasons. First, the increasing spread of exotic plant species facilitates the spread of the exotic insects. Populations of *C. perplexans* depend on somewhat disturbed habitat that may also be optimal for colonization by the exotics, *C. nutans* and *C. arvense*. Second, the ability of a plant population to purge itself of seed predators is nullified when proximate alternate hosts exist. The life stages of *C. perplexans* include years of non-flowering. Hypothetically, a population could purge itself of *R. conicus* or *L. planus* in years when there are no flowering individuals. The fact that both *L. planus* and *R. conicus* have been shown to coexist regularly with, instead of reduce the size of, populations of their intended targets (Gassmann and Louda 2001, Louda and O'Brien 2002) implies that the target populations of exotic plants can persist and serve as a source pool of the biological control agent in native populations.

Cirsium perplexans is not the only *Cirsium* species of conservation concern that has been documented hosting with biocontrol weevils. Rare taxa in California that host *R. conicus* include three varieties of *C. fontinale* (Greene) Jepson, two varieties of *C. hydrophilum* (Greene) Jepson, and *C. ciliolatum* (Henderson) J.T. Howell (Herr 2004).

Cirsium ownbeyi Welsh was reportedly used by *R. conicus* in the Cross Mountain Canyon of Colorado (personal communications, Thomas Grant, Denver Botanical Gardens), and studies have been conducted as to potential ecological effects of the federally listed *Cirsium pitcheri* (Torr. ex Eat.) Torr. & Gray (Louda et al. 2005) but currently the weevil has yet to invade extant populations.

An additional concern is the discovery of *L. planus* on *C. perplexans* at three sub-populations at one of the occurrences. *Larinus planus* has been shown to reduce seed production greatly in *C. undulatum* var. *tracyi* (Dodge et al. in preparation) with implications for population-level effects. Establishment of a biological control agent may take 10 or more years before control can be measured (McFadyen 1998). *R. conicus* was redistributed throughout the western U.S. in the early 1970s (Rees 1977, Louda 2000), but nontarget herbivory wasn't reported until 1977 (Rees), and ecological effects weren't reported until 1997 (Louda et al.). *Larinus planus* has been found to date on at least five native taxa in Colorado, Oregon, South Dakota, and Washington (Dodge et al. in preparation, and pers. comm. with E. Coombs, Oregon Department of Agriculture). Currently, it appears to be much less of a threat than *R. conicus*, but it is quite plausible that *L. planus* is in the early stages of a broad population increase. The role *L. planus* will play in *Cirsium* ecology remains to be seen.

The mechanisms for preventing future approvals of risky biological control agents are for the most part in place. Emphasis protecting against nontarget herbivory has grown, but even in its early stages was criticized as being "rigid, ultracautious, and stultifying" (Goeden 1983). Foolish or not, the current requirements for approval include passing a Technical Advisory Group (TAG) (Cofrancensco and Shearer 2004) which includes a section explicitly on assessing risks to native plants and addresses impacts for endangered species. Furthermore, the International Code of Best Practices for Classical Biological Control of Weeds contains language addressing nontarget impacts (Balciunas and Coombs 2004). However, there have been six other exotic insects released to control *Cirsium* thistles, of which five have been shown to feed on native species and the sixth was not tested on native species (Gassmann and Kok 2002, McClay 2002, Dodge et al. in review). The issue is compounded by biological control practitioners who continue to release insects long after they have been shown to pose high risk to native plant species. *R. conicus* was still being redistributed on public lands as of 2000, long after evidence of its effects on native species. No group has the capacity to remove a problematic agent once it has established.

Summary of conclusions

Herbivory by *R. conicus*, *O. occidentalis*, and *H. impressale* is responsible for a multi-fold decrease in seed production. Of these three, *R. conicus* has a much larger relative effect than either *O. occidentalis* or *H. impressale*. The *C. perplexans* population at the Cimarron SWA – Sign site is seed limited and thus is likely being markedly altered by the herbivory from *R. conicus*.

A survey of 10 of the 25 known occurrences of *C. perplexans* indicates that a similar story may be in place throughout the range of *C. perplexans*. The relationship between oviposition intensity and recruitment is strong evidence that the populations in general are responding to *R. conicus* herbivory with decreased recruitment.

Concerns are growing regarding the biocontrol weevil, *L. planus*. It has been recovered from native *Cirsium* taxa and also from the exotics *C. arvense* and *Onopordum acanthium* (pers. comm. Gary Piper, Department of Entomology, Washington State University). Both *L. planus* and *R. conicus* seem to have a broad oligophaguous diet comprising members of the thistle tribe.

Chapter 4: Density effects on recruitment and seedling establishment in Tracy’s thistle (*Cirsium undulatum var. tracyi*)

ABSTRACT

Predispersal seed predation by two biological control agent weevils, *Larinus planus* and *Rhinocyllus conicus*, has been shown to reduce seed production in the native Tracy’s thistle (*Cirsium undulatum var. tracyi*). The purpose of the current study is to explore the effects of seed density on the population-level dynamics of Tracy’s thistle with the overall objective of elucidating population-level effects of predispersal seed predation by the biocontrol weevils. Seeds were sown in densities varying from zero to 40 seeds in replicated cells in two plots at a single site in 2002. Recruits were measured and tagged in 2003 and 2004. At both sites, the number of seeds added positively affected recruit density in 2003 and non-flowering rosette density in 2004. One site showed a positive response in flowering plants in 2004. There were no detectable density effects on emergence, growth, and survival at all treatment levels. These preliminary results illustrate that the first indications of density effects on growth and survival of Tracy’s thistle seedlings do not appear until densities much greater than what has been found naturally in Tracy’s thistle, even after removing pre-dispersal seed predators. These results provide further support that herbivory from the biocontrol weevils is having population-level effects on Tracy’s thistle in the study area.

INTRODUCTION

Density dependence and intraspecific competition have explained population density in resource-poor environments in plants (Yeaton and Cody 1976, Yeaton et al. 1977). Negative effects from density dependence has been shown in seed germination (Inouye 1980, Grace et al. 2002), recruit survival (Webb and Peart 1999), survival of plants (Wright 1982), and reproductive output (Silander and Pacala 1985). In short, density dependence has been used to explain most stages of plant survival, development, and reproduction (Watkinson 1997).

Seed limitation in a population is defined as an increase in population size following seed addition (Turnbull et al. 2000). Thus, a population can be seed limited with a simple increase in recruitment, and a corresponding change in vegetative population structure, when seed numbers are increased. However, more important is the effect on adult establishment because seedlings that do not survive to reproductive age will change the structure of a population but will do nothing to change the effective population size. Further, ecological effects such as providing pollen and nectar for pollinators and a food source for native seed predators are also unaffected, if recruits perish. Seed introduction experiments are effective and commonly used tools for measuring population mechanics (population growth rate, density effects on survivorship, growth, and fecundity) if recruits are followed at least until they reach reproductive age (Turnbull et al. 2000).

Predispersal seed predation by two biological control agent weevils, *Larinus planus* and *Rhinocyllus conicus*, has been shown to reduce seed production in the native

Tracy's thistle (*Cirsium undulatum var. tracyi*) in the high desert of Gunnison county, Colorado (Dodge et al. in preparation). The densities of Tracy's thistle populations reported in Chapter 2 ranged between 0.012 and 0.024 flowering plants per square meter (Dodge et al. in preparation). After chemical exclusion of predispersal seed predators from two populations hosting *R. conicus*, recruitment increased from 0.24-0.46 to a range of 0.80-0.95 recruits per square meter. The ecological ramifications of the increased recruitment are dependent on the survival and growth of the recruits and their ability to mature to reproductive adults.

The purpose of the current study is to explore the effects of seed density in population dynamics of Tracy's thistle with the overall objective of elucidating population-level effects of predispersal seed predation by the biocontrol weevils. A seed introduction experiment allows opportunity to quantify the relationships between seeds, seedlings, non-flowering rosettes, and flowering plants in Tracy's thistle. By following the fates of seedlings for multiple years, population mechanics such as seed banks, density dependent germination, density dependent seedling mortality, and size-dependent flowering can be investigated.

In the study, I ask three specific questions. What are seed density effects on emergence, recruitment, plant density, and flowering plant density? What factors, including age, size, and density affect plant survival? What factors, including age, size, and density affect plant growth and flowering? The information gathered in this study will provide ecological evidence addressing risks and nontarget effects of introduction of non-native species as biological control agents and will further theory regarding density effects on plant population dynamics.

METHODS

Study species and study sites

Tracy's thistle is a short-lived monocarpic perennial. Vegetative reproduction in the study area is rare. Occasionally a single plant will form multiple rosettes that will flower at the same time, producing multiple stalks. I have documented cases where vegetative reproduction occurred when the non-flowering rosettes were separated, but each had sufficient rootstalk to survive. Populations of Tracy's thistle are generally found in moderately to highly disturbed habitats. Studies have indicated seed limitation in Tracy's thistle (Dodge et al. in preparation) and in a congener, *Cirsium canescens* (Louda and Potvin 1995).

The two study sites chosen for these seed addition experiments were proximate to existing populations, but held no existing rosettes or flowering plants. The Taylor River site had a small population of five flowering plants five meters from the plot. The nearest population with flowering plants to the Fivemile site was approximately 20 meters and held four flowering adults. The plots appeared very similar to the conditions of extant populations, being moderately disturbed roadside habitats, loosely associated with *Artemesia tridentata* and *Chrysothamnus viscidiflorus*.

Experimental methods

In October 2002, I experimentally sowed locally collected seed in differing densities in two 4.5m x 10m plots – one at each site. I divided each plot into a grid of

0.25m X 0.25m cells that were randomly assigned one of five treatments. Treatments were the additions of 0, 5, 10, 20, or 40 seeds to a cell. I dropped seeds into uniformly distributed holes at 3mm depth and covered them with soil. Empty cells surround each treated cell to deter neighbor effects. 71% of seeds sampled from the collected supply of seeds germinated within seven days when placed between moist paper towels and kept at room temperature.

I chose the treatment seed densities in response to observed, natural seed dispersal patterns, which can spread seed in clumped densities of approximately 20 seeds per 0.30m X 0.30m, and in accordance with Louda's seed addition experiments involving Platte thistle (*C. canescens*) (Louda et al. 1990).

On June 14, June 23, July 7, and July 30 in 2003 and on June 11 and July 6 in 2004 I surveyed the plots. The survey included measuring and mapping each plant. I measured length of longest leaf and counted number of leaves longer than 10 mm. A size index was constructed by multiplying the number of leaves by the length of the longest leaf.

Seed density effects on emergence, recruitment, plant density, and flowering plant density

To determine the effects of seed sowing density on emergence frequency, emerged seedlings were counted at the time of emergence and totals were summed per cell over the two years of the study. I compared number of emerged seedlings (ln+1 transformed) as a function of number of seeds added (treatment) and blocked by site using a mixed-model ANOVA.

The effect of seeds added on recruitment in 2003 and 2004 and total plant density in 2004 was measured using a mixed-model ANOVA. The difference in this analysis from the above is that census data included only surviving plants. There was some emergence and some mortality throughout the growing season. I chose the census dates of July 6, 2003 and July 7, 2004 to make comparisons between seed treatments because this was at the height of the growing season and avoided onset of senescence in late July.

The effects of seed density on flowering plant density were tested by comparing number of flowering plants per cell by treatment using a mixed-model ANOVA.

Factors affecting plant survival

To estimate the effect of size on survival during the growing season in 2003 I conducted a logistic regression (logit model) of size index from June 14 on survival (yes/no) until July 30. A similar model was used to estimate the effect of size (July 7) on non-growing season mortality from 2003 to 2004. I chose July 7 because this date was at the height of the growing season and prior to the onset of senescence, which had begun by the July 30 survey. In 2004 the plots were populated by both recruits and one-year olds. To estimate the effect of both age and size (June 11) on growing season survival (until July 6) I used a multiple logistic regression equation with age (categorical, recruit or non-flowering rosette) and size as independent variables. In all cases size was estimated by the size index. Site had no effect on the relationships between size and age on survival and was removed from the two aforementioned logistic regression models.

In order to quantify the effects of plant density on survival, I used a general linear model assessing the effects of density (number of plants per cell) and site on plant survival frequency per cell – using only those cells that held at least one plant. I chose this model instead of a factorial regression on individual plants to ensure independence of experimental units. The interaction term density*site was not significant and dropped from the model.

Factors affecting plant growth and flowering

Effects of density on growth for 2003 were examined in a general linear model by comparing average size index of plants per cell by number of plants in the cell (continuous) and site (categorical). In 2004 effects of density on growth were tested by comparing average size of non-flowering rosette in a similar model with site and number of non-flowering rosettes in the cell. In both cases the interaction term (number of plants*site) was insignificant and excluded from the final model.

To quantify the effects of rosette size on probability of flowering, I used a logistic regression (logit model) with size index of a rosette in July 2003 and site as the independent variables and flowering (yes/no) in 2004 as the dependent variable.

Notes on statistical analyses

Parametric analyses were used despite minor departures from the assumptions associated with normally distributed variables. Zero values at all treatment levels lead to a truncation of residuals, however this generally only happened at the smaller treatment levels. Furthermore, ANOVA’s are robust when dealing with inconsistencies with this assumption. The assumption for homogeneity of variance was met in all parametric analyses after data transformation.

RESULTS

Seed density effects on emergence, recruitment, plant density, and flowering plant density

Densities of seedlings ranged from 0 to 9 per cell at Taylor River and 0 to 16 per cell at Fivemile in 2003. Number of recruits per cell was significantly affected by the number of seeds added at both sites (Table 4-1, Fig. 4-1a). Comparisons between treatment levels indicated a general increase in recruitment with an increase in seed input, however not all combinations differed significantly (Table 4-1b). In 2004, recruitment was significantly affected by number of seed input at Fivemile but not at Taylor River (Table 4-2, Fig. 4-1b) – note that not all treatment comparisons at Fivemile were significant (Table 4-2b); however, at Taylor River there were no significant comparisons (Table 4-2c).

Table 4-1. Mixed linear model. Number of seedlings per cell (ln+1 transformed) in 2003 as a function of number of seeds added (treatment) and blocked by site (a) and p-values for Tukey HSD adjusted multiple mean comparisons across both sites (b). Since there was no significant site or interaction effect, I present the comparisons of treatment values grouped across both sites.

a)

	Effect (F/R)	SS	DF	MS	F	p
Intercept	Fixed	84.11	1	84.11	211.19	0.04
Site	Random	0.40	1	0.40	0.94	0.39
Treatment	Fixed	37.21	4	9.30	22.01	0.01
Site*Treatment	Random	1.69	4	0.42	1.23	0.30
Error		92.56	270	0.34		

b)

Treatment	5	10	20	40
0	0.12	< 0.01	< 0.01	< 0.01
5		< 0.01	< 0.01	< 0.01
10			< 0.01	< 0.01
20				0.99

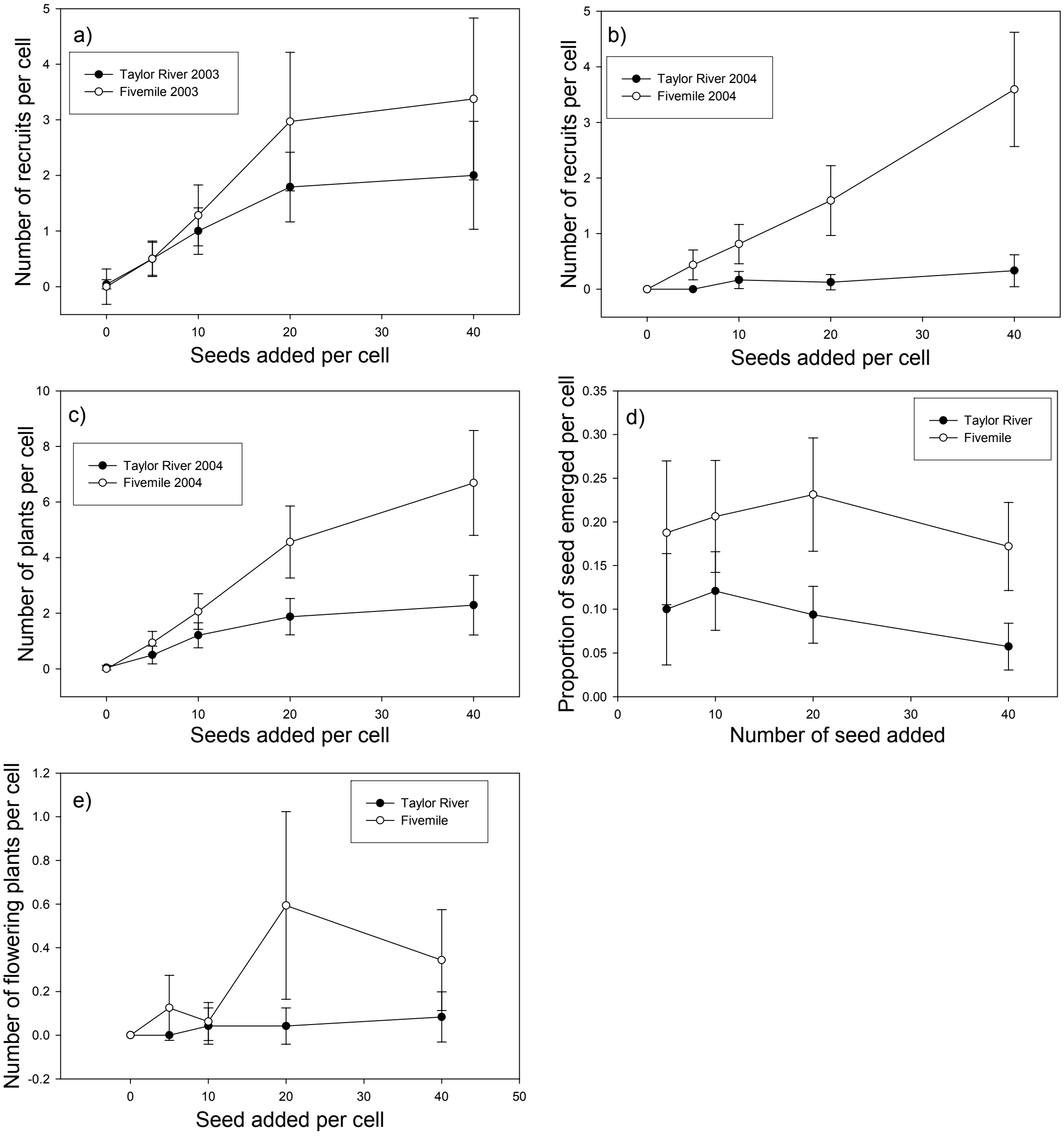

Figure 4-1. Total number of recruits per cell as a function of added seed per cell in 2003 (a). Total number of new recruits in the following year (2004) as a function of seeds added (b). Total number of plants per cell (recruits, non-flowering rosettes, and flowering plants) in 2004 as a function of seed added (c). Proportion of seed that emerged as a function of seed density (d). Number of flowering adults per cell in 2004 as a function of number of seed added in 2003 (e). Note that statistical analyses were performed on ln+1 transformed data. Error bars represent two SE.

Table 4-2. Mixed linear model. Number of seedlings per cell (ln+1 transformed) in 2004 as a function of number of seeds added (treatment) and blocked by site (a) and p-values for Tukey HSD adjusted multiple mean comparisons for Fivemile (b) and for Taylor River (c). Since there was a significant interaction effect, I present the comparisons of treatment values within sites.

a)

	Effect (F/R)	SS	DF	MS	F	p
Intercept	Fixed	27.57	1	27.57	1.78	0.41
Site	Random	15.49	1	15.49	6.30	0.07
Treatment	Fixed	18.17	4	4.54	1.85	0.28
Site*Treatment	Random	9.83	4	2.46	13.45	< 0.01
Error		49.35	270	0.18		

b) Fivemile

Treatment	5	10	20	40
0	0.29	< 0.01	< 0.01	< 0.01
5		0.65	< 0.01	< 0.01
10			0.26	< 0.01
20				< 0.01

c) Taylor River

Treatment	5	10	20	40
0	1.00	1.00	1.00	0.86
5		1.00	1.00	0.86
10			1.00	1.00
20				1.00

Total plant density per cell (one-year olds and recruits) in 2004 was significantly affected by seed input at both sites (Table 4-3, Fig. 4-1c); however, they responded differently to the treatments. Comparisons between treatment levels indicated a general increase in plant density with increased seed, however not all combinations differed significantly (Tables 4-3b and c).

Table 4-3. Mixed linear model. Total number of plants per cell (ln+1 transformed) in 2004 as a function of number of seeds added (treatment) and blocked by site (a) and p-values for Tukey HSD adjusted multiple mean comparisons for Fivemile (b) and for Taylor River (c). Since there was a significant interaction effect, I present the comparisons of treatment values within sites.

a)

	Effect (F/R)	SS	DF	MS	F	p
Intercept	Fixed	74.13	1	74.13	26.37	0.12
Site	Random	2.81	1	2.81	2.01	0.23
Treatment	Fixed	42.00	4	10.50	7.50	0.04
Site*Treatment	Random	5.60	4	1.40	4.86	< 0.01
Error		77.69	270	0.29		

b) Fivemile

Treatment	5	10	20	40
0	0.96	< 0.01	< 0.01	< 0.01
5		0.08	< 0.01	< 0.01
10			0.23	< 0.01
20				< 0.01

c) Taylor River

Treatment	5	10	20	40
0	0.97	0.39	< 0.01	< 0.01
5		0.99	0.02	0.06
10			0.30	0.59
20				1.00

There was no detectable effect of treatment level on proportion of seed germinated per cell across all treatment levels across both sites (Table 4-4). Germination was higher at the Fivemile site (Fig. 4-1d).

Table 4-4. Mixed linear model. Total number of seeds germinated per cell (arcsine-square root transformed) through 2004 as a function of number of seeds added (treatment) and blocked by site. There was no significant affect of seed density on germination.

	Effect (F/R)	SS	DF	MS	F	p
Intercept	Fixed	22.16	1	22.16	14.95	0.16
Site	Random	1.48	1	1.48	73.73	< 0.01
Treatment	Fixed	0.33	3	0.11	5.51	0.10
Site*Treatment	Random	0.06	3	0.02	0.32	0.81
Error		13.59	216	0.06		

Flowering plant density ranged from zero to five per cell at Fivemile and zero to one per cell at Taylor River in 2004. A single recruit bolted and attempted to flower in 2003, but the stalk was highly deformed and stunted and no flowers formed. Mixed model analysis of number of flowering plants per cell indicated a significant interaction between treatment and site (Table 4-5). Post-hoc comparisons indicated a significant increase in flowering plants over the control for the 20 and 40 seed treatments at the Fivemile site (Table 4-5b). There were no significant differences in any comparisons at the Taylor River site (Table 4-5c). Visual inspection between treatments revealed a general increase in number of flowering plants per seed added (Fig. 4-1e) at the Fivemile site only.

Table 4-5. Mixed linear model. Total number of flowering plants per cell (ln+1 transformed) in 2004 as a function of number of seeds added (treatment) and blocked by site (a). Since there was a significant interaction effect, I present the comparisons of treatment values within sites. P-values for Tukey HSD adjusted multiple mean comparisons for Fivemile (b) and for Taylor River (c).

a)

	Effect (F/R)	SS	DF	MS	F	p
Intercept	Fixed	1.54	1	1.54	2.09	0.39
Site	Random	0.74	1	0.74	4.27	0.11
Treatment	Fixed	1.10	4	0.28	1.60	0.33
Site*Treatment	Random	0.69	4	0.17	2.67	0.03
Error		17.45	270	0.06		

b) Fivemile

Number of seed added	5	10	20	40
0	0.97	1.00	< 0.01	0.03
5		1.00	0.02	0.53
10			< 0.01	0.20
20				0.92

c)

Number of seed added	5	10	20	40
0	1.00	1.00	1.00	1.00
5		1.00	1.00	1.00
10			1.00	1.00
20				1.00

Factors affecting plant survival

Growing season mortality was 10.4% at Taylor River (n = 125) and 22.0% at Fivemile (n = 250) in 2003. Mortality was attributed to desiccation in every case in 2003. Growing season mortality was 17.4% at Taylor River (n = 115) and 28.7% at Fivemile (n

= 356) in 2004. All mortality at Fivemile was attributed to desiccation. 55% of mortality at Taylor River in 2004 was attributed to lepidopteran feeding and the rest to desiccation.

Size index of rosette in early June was a significant predictor of growing season survival in 2003 (df = 1; Wald stat. = 39.57; $p < 0.01$) with larger plants having a higher probability of survival (Fig. 4-2a). However, size was not a significant factor in overwinter survival from 2003 to 2004 (df = 1; Wald stat. = 0.92; $p = 0.34$). Both age and size were significant predictors in the model with non-flowering recruits surviving better than recruits and larger plants surviving better than smaller in the growing season of 2004 (Table 4-6, Fig. 4-2a).

Table 4-6. Logistic regression. Effect of size and age on growing season survival in both Taylor River and Fivemile in 2004.

	Estimate	Standard Error	Wald Stat.	p
Intercept	-0.87	0.19	20.28	< 0.01
Size	0.00074	0.00035	4.35	0.04
Age	0.89	0.19	22.85	< 0.01

Densities of seedlings ranged from 0 to 9 per cell at Taylor River and 0 to 16 per cell at Fivemile in 2003. Plant density in 2004 ranged from 0 to 5 per cell at Taylor River and from 0 to 15 per cell at Fivemile. Plant density and site had no measurable effect on seedling survival at in the growing season of 2003 (Table 4-7). However, density was positively related to plant survival across both sites and was significantly different between sites in the growing season of 2004 (Table 4-8, Fig. 4-2b). There was no effect of either site or density in overwinter survival from 2003-2004 (Table 4-9).

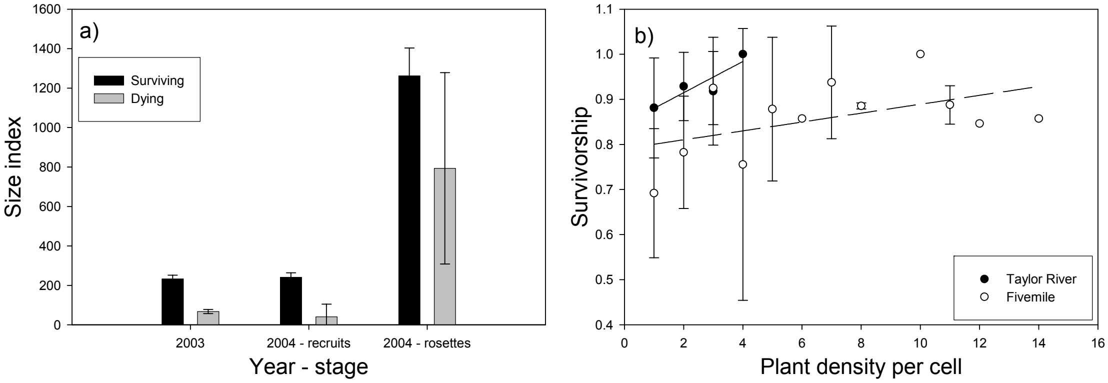

Figure 4-2. Size index had a significant effect on survivorship for recruits in 2003 and 2004, and non-flowering rosettes in 2004 (a). Relationship between density of plants and survivorship of seedlings and one-year olds in the growing season of 2004 (b). Survivorship was significantly better at the Taylor River site than the Fivemile site and survivorship increased with density.

Table 4-7. General linear model. Effect of site (categorical) and density (continuous) on growing season survival in 2003.

	SS	DF	MS	F	p
Intercept	45.535	1	45.535	563.27	< 0.01
Site	0.232	1	0.232	2.87	0.09
Density	0.003	1	0.003	0.03	0.86
Error	10.348	128	0.081		

Table 4-8. General linear model. Effect of site (categorical) and density (continuous) on growing season survival in 2004.

	SS	DF	MS	F	p
Intercept	35.237	1	35.238	649.64	< 0.01
Site	0.517	1	0.517	9.53	< 0.01
Density	0.285	1	0.284	5.25	0.02
Error	6.563	121	0.054		

Table 4-9. General linear model. Effect of site (categorical) and density (continuous) on overwinter survival from 2003-2004.

	SS	DF	MS	F	p
Intercept	13.04	1	13.04	1619.80	< 0.01
Site	0.028	1	0.028	3.53	0.07
density	0.019	1	0.019	2.34	0.13
Error	0.451	56	0.008		

Factors affecting plant growth and flowering

Plants grew larger at the Fivemile site than at the Taylor River site, but plant density did not have an effect on plant size in 2003 (Table 4-10). In 2004 there was no detectable relationship between either site or density on non-flowering rosette size (Table 4-11).

Both size and site had significant effects on flowering (Table 4-12). Larger rosettes in 2003 were more likely to flower in 2004 than smaller rosettes and plants at Fivemile were more likely to flower that plants at Taylor River (Fig. 4-3). Three percent (N = 121) of all one year old Taylor River plants flowered in 2004, while 14% (N = 250) of one year old plants at Fivemile flowered. One recruit bolted to become a flowering plant during its first year. The flowering stalk was very deformed and produced no flowers.

Table 4-10. General linear model. Effect of site (categorical) and plant density (continuous) on plant size in 2003.

	SS	df	MS	F	p
Intercept	4061598	1	4061598	140.98	< 0.01
Site	148123	1	148123	5.14	0.03
Density	51354	1	51354	1.78	0.18
Error	3687759	128	28811		

Table 4-11. General linear model. Effect of site (categorical) and plant density (continuous) on non-flowering rosette size in 2004.

	SS	df	MS	F	p
Intercept	62324772	1	62324772	82.84	< 0.01
Site	1062591	1	1062591	1.41	0.24
Density	366306	1	366306	0.49	0.49
Error	78249083	104	752395		

Table 4-12. Logistic regression. Effect of size in 2003 and site on probability of flowering in 2004.

	Estimate	Standard Error	Wald Stat.	p
Intercept	5.44	0.61	79.45	< 0.01
Size	-0.0084	0.0012	49.38	< 0.01
Age	1.65	0.36	20.56	< 0.01

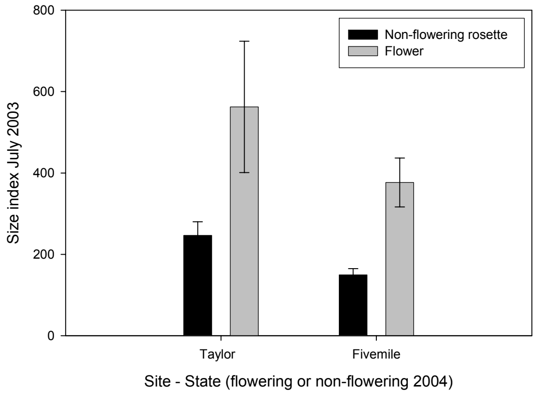

Figure 4-3. The relationship between size in 2003 and flowering or not flowering in 2004.

DISCUSSION

Recruitment was positively affected by seed density at all treatment levels, with the possible exception of density dependent recruitment at the highest levels of seed input (between the 20 and 40 seed treatments) at both sites (Fig. 4-1a). Recruitment in 2004 was also significantly related to seed input, indicating at least a short-term persistent seed bank at the Fivemile site, but not at the Taylor River site. Since we found zero recruitment outside of the cells, we assume there was no persistent seed bank already in the soil. The relationship between emergence and seed density was non-existent in the analyses, providing evidence that at these levels, germination is not density dependent. However, there was a trend, although non-statistically based, that emergence frequency (proportion of seed) may drop with higher densities (Fig. 4-1d). The number of flowering plants was significantly related to seed input at the Fivemile site but not at the Taylor River site (Fig. 4-1e). In summary, there was no conclusive evidence of negative density dependence on emergence, recruitment, plant density, and flowering plant density at the experimental levels of seed input.

The fact that both size and age were related to survivorship is evidence that if growth is negatively affected by density, then it’s likely that density will have negative effects on survivorship. The positive effects of density on survivorship (Fig. 4-2) can be interpreted two ways. It is possible that there is positive density dependence resulting from facilitative effects such as shading, or it is possible that it reflects a situation where the microhabitat of the cell is conducive to both emergence and growth, and that negative density dependence is either overshadowed by the positive effects of the microhabitat, or there are no density dependent effects at this level. While negative density dependence would not be unexpected, facilitative effects have been shown for desert plants (Lortie and Turkington 2002). Regardless, for the purpose of this experiment, it is apparent that seedlings can grow and exist at a much higher density than is found in extant populations.

Although these cursory analyses show little in the way of seedling competition and general axioms of density effects on growth, the results clearly illustrate that in these sites, the populations of Tracy's thistle can grow and mature in densities much higher than what we currently find in the field. Even a seedling density of two per cell translates to 32 per square meter. Here, evidence is presented indicating minimal density effects at greater than 64 per square meter. As discussed in Chapter 2, densities of Tracy's thistle recruits increased to 0.80-0.95 recruits per square meter when chemically excluded from seed predators. It is highly unlikely that seedling and plant competition is affecting plants at this density. Thus, reduced seed production leading to reduced recruitment in those populations is likely having effects on densities of adult, flowering plants. This has important implications for gene flow and effective population size, as well as nectar and pollen resources for native insects.

It is important to continue this study for two primary reasons. First, population-level effects of seed limitation will be most ecologically significant if the number of adult plants changes. As of 2004, only 3% of the original 2003 seedlings at the Taylor River site and 14% at the Fivemile site have flowered. It is very possible that density effects will emerge as average plant size increases.

Chapter 5: Preliminary matrix model projections and elasticity analysis for three *Cirsium* species native to Colorado

ABSTRACT

Population projection matrices are potentially useful tools for conservation studies. Researchers can compute the annual rate of population increase (λ) and can assess how different transitions between stages or ages affect (λ) through elasticity analysis. The purpose of this chapter is to initiate brief and preliminary comparative demographic analyses of three native *Cirsium* thistles in Colorado. The analyses are intended to make preliminary judgment on the key factors and life history stages that most affect population growth trajectories in an effort to understand the effects of pre-dispersal seed predation on population trends. In addition, it was informative to investigate the effects of a hypothetical seed bank. Elasticity analysis of two of the three species indicated that seed production and the resulting recruitment in the spring were important transitions and that the inclusion of seed banks changed the analyses very little. The results will assist land managers in making knowledgeable decisions regarding conservation of these species.

INTRODUCTION

Endeavors in conservation of plant populations or species should include life history analysis in order to direct conservation efforts to life history stages that will produce the largest impacts for a population or group of populations. For example, study of the effects of invasive insects on seed production in a plant may demonstrate population-level effects. Elimination of the insects, however, and the corresponding increase in seed production, may lead to smaller changes in the population dynamics of the plant than other management options.

Population projection matrices are a potentially useful tool for conservation studies. They allow computation of the annual rate of population increase (λ) and can be either age-based (Leslie) or stage-based (Lefkovitch). Sensitivity and elasticity analyses can be used to measure how changes in the elements in the projection matrix affect λ. Further, reproductive values per class can be figured to determine the value of a member in each stage in terms of progeny. These data can assist land managers in making decisions of where to attack problematic weed species, and also can aid in determining where best to focus conservation efforts (Silvertown and Charlesworth 2001, Griffith and Forseth 2005).

The purpose of this chapter is to initiate brief and preliminary comparative demographic analyses of three native *Cirsium* thistles in Colorado. All three species have been shown to host exotic biological control weevils – although only two of the three populations in the analyses do so. The analyses are intended to make preliminary judgment on the key factors and life history stages that most affect population growth trajectories in an effort to understand the effects of different factors on population trends and assist land managers in making knowledgeable decisions regarding conservation of these species. Additionally, the results can be used to evaluate the risks associated with release and redistribution of exotic species as biological control agents.

METHODS

Study species

Cirsium perplexans (Rydb.) Petrak (Asteraceae), Rocky Mountain thistle, is a perennial, polycarpic member of the Cardueae tribe. It has been previously described as both a biennial, obligate biennial, and perennial (see Spackman-Panjabi and Anderson 2004). *Cirsium perplexans* has been classified as imperiled at the state level by the Colorado Natural Heritage Program (CNHP 2004) and as globally imperiled by NatureServe (2005). *Cirsium perplexans* is generally found in sparsely populated or barren, disturbed clay and shale soils. It is loosely associated with pinyon-juniper forests and sagebrush scrublands. *Cirsium perplexans* is found in moderately disturbed habitat, either by human activity (roadsides and access roads) or by natural heaving and cracking resulting from swelling of the shale soils. Observed mortality has been exclusively from desiccation stemming from cracking and heaving of soils.

Cirsium undulatum (Nutt.) Spreng. var. *tracyi* (Rydb.) Welsh, Tracy's thistle, is a large-flowered monocarpic perennial thistle endemic to the western slope of Colorado and Utah. *Cirsium undulatum var. tracyi* is also found in moderately disturbed habitat, along roadsides and paths and can be sympatric with *C. perplexans*. It is loosely associated with sagebrush (*Artemesia tridentata*) and rabbitbrush (*Chrysothamnus viscidiflorus*). Experimental studies have shown Tracy's thistle seeds to flower and produce seeds after two growing seasons, but a more accurate estimation of average vegetative growth is three to four years (personal observation). Observed mortality is mostly from desiccation, and occasionally feeding from caterpillars.

Cirsium scariosum (Nutt.), elk thistle or meadow thistle, is a large-flowered monocarpic thistle found throughout the western states. The stalkless variety of *Cirsium scariosum* has been misapplied as *Cirsium drummondii* Torr. & Gray. and is synonymous with *C. coloradense*. Average time from germination to flowering is estimated at six to ten years (personal observation). Observed mortality at all stages most often is from ground disturbance and possible herbivory by the northern pocket gopher *Thomomys talpoides*.

Cirsium taxonomy is somewhat unresolved. The nomenclature here followed the treatment of USDA and NRCS (2004). Identification was through Weber and Wittmann (2001). *Cirsium undulatum var. tracyi* was treated as *C. tracyi* by Weber and Wittmann.

Matrix models

The transitions represented in the life cycle graphs in Fig. 5-1 are analogous to elements in a stage-based projection matrix **A**, defined by the equation $\mathbf{n}(t+1) = \mathbf{A}\mathbf{n}(t)$. Where $\mathbf{n}(t+1)$ and $\mathbf{n}(t)$ are vectors containing the numbers of individuals per stage as defined in the life cycle graph, and t is a time frame (Caswell 2001). **A** is a square matrix constructed of the survivorship and transition probabilities and fecundities. Of major interest for population projection and conservation are the annual rate of population increase (λ) (determined to be the dominant right eigenvalue of **A)**, sensitivities and elasticities of the matrix elements (Caswell 2001, Griffith and Forseth 2005), and reproductive values (Caswell 2001).

Sensitivities (S_{ij}) of matrix elements measure the magnitude of effect that change in an element will have on λ and thus, quantify the importance of that element in the population growth rate. Elasticities (E_{ij}) of matrix elements are computed in part from the sensitivities and are similar in their function – comparisons between effects of matrix elements for their effects on λ. Sensitivities measure the effect of additive perturbations and elasticities measure the effect of proportional perturbations (Caswell 2001). Elasticities are therefore scaled and all sum to one. Stated otherwise, the elasticity of an element is the proportion of contribution to the population growth rate (Caswell 2001, Silvertown and Charlesworth 2001). I only present elasticities in this study.

Reproductive values are calculated from the left eigenvector of the projection matrix and scaled so that the initial class equals one. Thus, resulting values are relative and in comparison to the first stage (Caswell 2001).

Matrix models can incorporate variation in the parameter estimation and solve for values such as λ using iterative processes (Caswell 2001, Silvertown and Charlesworth 2001). A deterministic model was chosen instead of a stochastic model in order to simplify the preliminary analyses and because the short duration of the study. Future analysis of the data will include variation in the model.

Seed banks and model representation

Four life history graphs are presented in Fig. 5-1. The top two (a and b) represent perennial plants with persistent seed banks, (a) being polycarpic and (b) being monocarpic. Assuming that transitions represent a full year, two clarifications in the life cycle graphs can be illustrated. First, following the logic that flowering plants produce seeds, the transition arrow showing fecundity of a flowering (in these cases F_{14}) plant would go to the seed bin. However, if seeds germinate in the fall following flowering or in the spring prior to survey, then the contribution of a flowering plant to the subsequent year's population is not in seeds produced, but in number of recruits (F_{24}) (Caswell 2001). Inclusion of both flowering individuals and seeds in a projection matrix model implies the existence of a persistent seed bank given that transitions generally represent a full year. A life cycle graph without the transition from flowering plant to recruit implies an obligatory persistent seed bank. Thus, models (c) and (d) represent polycarpic perennials and monocarpic perennials respectively with transient seed banks.

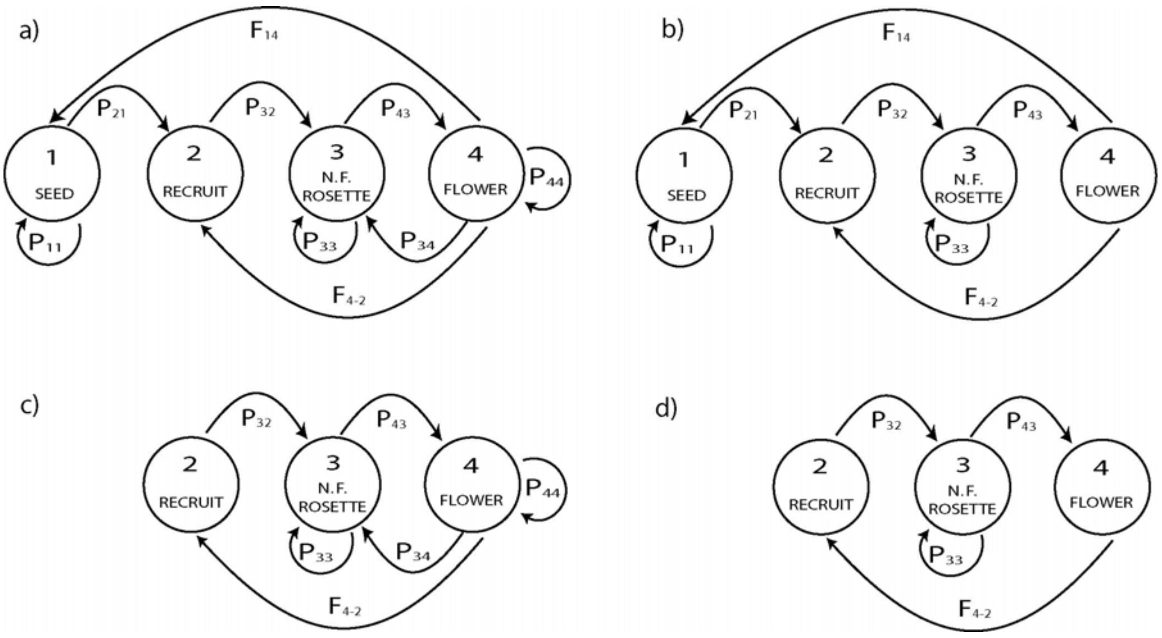

Figure 5-1. Proposed life cycle graphs for the matrix models. A perennial polycarpic plant with a persistent seed bank (a), a perennial monocarpic plant with a persistent seed bank (b), a polycarpic (c) and monocarpic (d) plant with a transient seed bank respectively*. Cirsium perplexans* was simulated using models a and c*. Cirsium undulatum var. tracyi* and *C. scariosum* were simulated using models b and d.

The second clarification represented here is that the transitions from flowers to the persistent seed bank (F_{14}) in models a and b have been referred to as *fecundities* (e.g. Shea and Kelly 1998). However, they are not strict fecundities measured in terms of seed produced. The transition F_{14} represents the number of seeds produced multiplied by the probability that a seed will be a viable seed in the seed bank the following year. By referring to the transition as strict fecundity, one assumes all seeds produced will enter the persistent seed bank thereby eliminating any post-dispersal mortality prior to the next survey date (e.g. post-dispersal seed predation). This is especially important given that post-dispersal seed predation can reduce seeds close to 100% (e.g. Janzen 1971). The transition F_{14} represents the number of viable seeds contributed to the persistent seed bank, measured or estimated at the next survey date.

Transition estimation - general

Transition parameters for the different stages were estimated using maximum likelihood estimates (Caswell 2001) based on tagged individuals for transitions between recruits (R), non-flowering rosettes (N), and flowers (F). Plots were located in extant populations. Plants were marked with small, numbered aluminum tags attached to the ground with nails. Data recorded per plant included number of leaves longer than 10mm and length of the longest leaf. Plants were also examined for cotyledons. Flowering individuals were measured for height and number of inflorescences. Subsequent visits to the plots included finding and measuring all previously marked plants as well as closely inspecting the ground for recruits. Recruits of all three species are quite easily recognized. The flower to recruit transition was based on flower production in the plots and subsequent recruitment the following spring, and adjusted for contribution to and recruitment from the seed bank in the seed bank model.

Parameter estimation – Cirsium perplexans

Transition estimations for *C. perplexans* were gathered from surveys of adjacent demographic plots at the Cimarron State Wildlife Area in Montrose County, Colorado (referred to as the Sign site). Exact location of this site is available from the Colorado Natural Heritage Program (CNHP). I initiated demographic studies by marking and measuring all plants in four adjacent plots in June, 2003. Total area of the plots measured $64m^2$. Plots were visited again in June 2004. A total of 1076 plants have been marked and measured. Seed production per plant estimates come from collections from a different part of the same population in 2001 (Dodge and Inouye In preparation). However, this estimation leads to an overabundance of recruits (approximately double what is observed). Therefore, some sort of post-dispersal seed predation must be responsible for reducing the seed to seedling transition, or the seeds are forming a seed bank. *Cirsium perplexans* is polycarpic and therefore was analyzed using models a) and c) from Fig. 5-1. Transition values are presented in Table 5-1.

Table 5-1. Observed transitions across years and plots per species. These elements were used to construct the matrix model used in analyses with a *transient* seed bank.

	C. scariosum		*C. undulatum var. tracyi*		*C. perplexans*	
Transition	Estimate	SE	Estimate	SE	Estimate	SE
P32	0.447	0.042	0.818	0.039	0.205	0.020
P33	0.73	0.019	0.781	0.024	0.429	0.022
P43	0.015	0.005	0.156	0.021	0.101	0.014
P24	6.64	--	13.67	--	9.16	--
P34	0		0		0.236	0.050
P44	0		0		0.028	0.019

Parameter estimation – Cirsium undulatum var. tracyi

Transition estimations for *C. undulatum var. tracyi* were gathered from surveys of a single demographic plot in the Curecanti National Recreation Area in Gunnison County, Colorado (referred to as the Lake site) (N 38.45296°, W 107.3299°). I initiated demographic studies by marking and measuring plants in June, 2002. Total area of the plot measured $220m^2$. Plots were visited again in July 2002, 2003, and 2004. A total of 339 plants have been tagged and followed. Estimates of seed production per plant come from collections from a similar population (known as the Pine site) located 1.28 km from the plot (N 38.44567°, W 107.3412°) (Dodge et al. in preparation). I found no evidence of a seed bank in a seed augmentation experiment at the Pine site in 2002, although I did find evidence for a short-lived seed bank at one of two sites of a seed introduction experiment 64 km from the plot. *Cirsium undulatum var. tracyi* is monocarpic and therefore was analyzed using models b) and d) from Fig. 5-1. Transition values are presented in Table 5-1.

Parameter estimation – Cirsium scariosum

Transition estimations for *C. scariosum* were gathered from surveys of a single demographic plot at Cumberland Pass in Gunnison County, Colorado (N 38.69351°, W 106.47580°). I initiated demographic studies by marking and measuring plants in August, 2001. Total area of the plot measured 50m^2. The plot was visited again in August 2002, 2003, and 2004. A total of 236 individuals have been marked and measured. Number of seeds per capitulum was quantified from a survey of 14 populations of *C. scariosum* in Colorado, Idaho, Nevada, Oregon, and Utah (Dodge et al. in review). Estimates of seed production per plant were made by multiplying the estimated number of seeds per capitulum by observed number of capitula per plant in the plot itself. I found no data regarding seed banks. *Cirsium scariosum* is monocarpic and therefore was analyzed using models b) and d) from Fig. 5-1. Transition values are presented in Table 5-1.

Seed bank estimation for all species

Entry into the persistent seed bank (Fig. 5-1, F_{14}) was estimated by assuming that one fourth of all marked recruits each year came from the persistent seed bank. This estimate is based solely on the results from *C. undulatum var. tracyi* seed sowing experiments in Chapter 4, where at one site germination of two year old seeds matched that of one year old seeds the year prior and at the other site, I found very little germination of two year old seeds. Using values of observed recruitment per species, the F_{14} transition can be estimated by the equation:

$$F_{14} = \frac{P_{21}}{4R\left(\sum_{t=1}^{5} P_{11}^{t-1}\right)}$$

Where F_{14} is the numeric contribution of a flowering individual into the persistent seed bank, R is the number of recruits observed per flowering plant, P_{21} is the probability of germination for a seed in the persistent seed bank, P_{11} is the proportion of seeds in the persistent seed bank that will remain in the persistent seed bank, and t is the age of the seed in years. Note that the seed is not in the persistent seed bank until it is one year of age.

Estimated survival in the persistent seed bank (P_{11}) was set at 0.25. This estimate is based on reports generally finding survivorship of buried seed to be a negative exponential curve (references in Baskin and Baskin 2001) leading to a constant probability of transition. Studies of seed banks including *Cirsium* have classified the seed banks as either transient or short-term persistent (less than five years) (Thompson et al. 1997). The transition probability of 0.25 represents a short-term persistent seed bank, in which 3.9 per thousand seeds in the seed bank would still be viable and in the seed bank at age five. This parameter is consistent with estimations from seed sowing experiments for *C. undulatum var. tracyi* (see Chapter 4) and estimations for other thistles (Klinkhamer et al. 1988, Popay and Medd 1990, Doucet and Cavers 1996, Grace et al. 2002). Transition values for simulations with a seed bank are presented in Table 5-2.

Table 5-2. Estimated parameters for a matrix model used in analyses with a hypothetical *persistent* seed bank.

	C. scariosum		*C. undulatum var. tracyi*		*C. perplexans*	
Transition	Estimate	SE	Estimate	SE	Estimate	SE
P11	0.25	--	0.25	--	0.25	--
P21	0.086	--	0.086	--	0.086	--
P32	0.447	0.042	0.818	0.039	0.205	0.020
P33	0.73	0.019	0.781	0.024	0.429	0.022
P43	0.015	0.005	0.156	0.021	0.101	0.014
P14	14.48	--	29.81	--	19.98	--
P24	4.98	--	10.25	--	6.87	--
P34	0		0		0.236	0.050
P44	0		0		0.028	0.019

Germination of seeds from the seed bank, P_{21}, for all species was estimated from the second year germination from *C. undulatum var. tracyi* seed sowing experiments to be 0.086. Using the above equation and previously described estimates of P_{21} and P_{11} and recruitments estimates from the previous matrix models (Table 5-1), the F_{14} transitions are estimated to be 19.98, 29.81, 14.48 for *C. perplexans*, *C. undulatum var. tracyi*, and *C. scariosum* respectively (Table 5-2).

RESULTS

Preliminary results for the matrix analyses with transient seed banks revealed λ values of 0.80 for *C. scariosum*, 1.53 for *C. undulatum var. tracyi*, and 0.78 for *C.*

perplexans (Table 5-3). These values are suspect due to the short length of time of the (ongoing) study and the neglect of environmental variation and stochasticity that affect population growth rates. Reproductive values for each species increased with the class of the plant in all cases (Table 5-3). Adding a persistent seed bank to the matrix model had no measurable affect on λ values (Table 5-4). Reproductive values for the model with a persistent seed bank are similar to those with a transient seed bank and are presented in Table 5-4.

Table 5-3. Calculated rate of population increase (λ), reproductive values (V_x), and stable stage distribution (w_x) for the three defined stages (2 = recruit, 3 = non-flowering rosette, and 4 = flowering plant) using a projection model of a *transitional* seed bank.

	C. scariousum	*C. undulatum var. tracyi*	*C. perplexans*
Λ	0.7995	1.5281	0.7821
V_2	1	1	1
V_3	1.7926	1.8681	3.8153
V_4	8.3052	8.9459	13.3402
w_1	0.1327	0.4532	0.5804
w_2	0.8514	0.4962	0.37
w_3	0.016	0.0507	0.0496

Table 5-4. Calculated rate of population increase (λ), reproductive values (V_x), and stable stage distribution (w_x) for the four defined stages (1 = seed, 2 = recruit, 3 = non-flowering rosette, and 4 = flowering plant) using a projection model of a *persistent* seed bank.

	C. scariousum	*C. undulatum var. tracyi*	*C. perplexans*
Λ	0.8047	1.4895	0.7976
V_1	1	1	1
V_2	6.4495	14.4129	6.3671
V_3	11.6359	26.2446	24.7719
V_4	57.911	119.1951	90.3986
w_1	0.2909	0.561	0.6329
w_2	0.1001	0.1929	0.2176
w_3	0.5978	0.2227	0.1322
w_4	0.0111	0.0233	0.0173

The stable stage distribution for a transient seed bank is presented in Table 5-3. Note the abundance of non-flowering rosettes in *C. scariosum* ($w_3 = 0.85$) compared to that of *C. undulatum var. tracyi* ($w_3 = 0.49$) and *C. perplexans* ($w_3 = 0.37$). In all species the non-flowering stages outnumber the flowering plants. The results are similar with a persistent seed bank, especially when considering the non-seed stages. *Cirsium scariosum* had a large proportion of non-flowering rosettes (0.60) compared to both *C. undulatum var. tracyi* (0.22) and *C. perplexans* (0.13).

Elasticity comparison for models with a transient seed bank indicated that for *C. scariosum* the factor that most influences λ is the survivorship of non-flowering rosettes, $E_{33} = 0.78$, while all others were less than 0.08 (Fig. 5-2). The *C. undulatum var. tracyi* model included elasticities that were practically equal for all four transitions with values ranging from 0.25 to 0.26) , and the *C. perplexans* model showed a marked decrease in elasticities for survival of flowering plants (E_{34} and E_{44}) and an equal importance for the other transitions.

Elasticity comparison for models with a persistent seed bank indicated a very similar situation for *C. scariosum* with the most influential transition clearly being survivorship of the non-flowering rosette $E_{33} = 0.74$ (Fig. 5-3). The *C. undulatum var. tracyi* model produced elasticities in two general categories. Influential elasticities included E_{32}, E_{33}, E_{43}, and E_{24}, with values ranging from 0.20 to 0.26. Lesser elasticities were associated with the seed bank, E_{11}, E_{21}, and E_{41} valued in the range from 0.01 to 0.04 (Fig. 5-3). The *C. perplexans* model showed a variable range of elasticities with no clear dominant group. However, $E_{33} = 0.26$, $E_{43} = 0.22$, $E_{32} = 0.20$, and $E_{24} = 0.13$ are the most influential (Fig. 5-3).

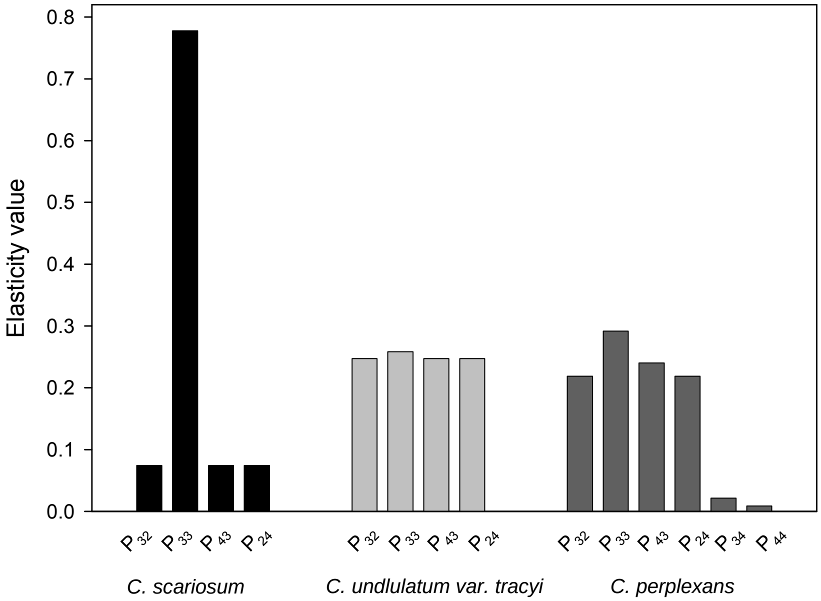

Figure 5-2. Elasticity values for observed transitions from analyses simulating populations with *transient* seed banks.

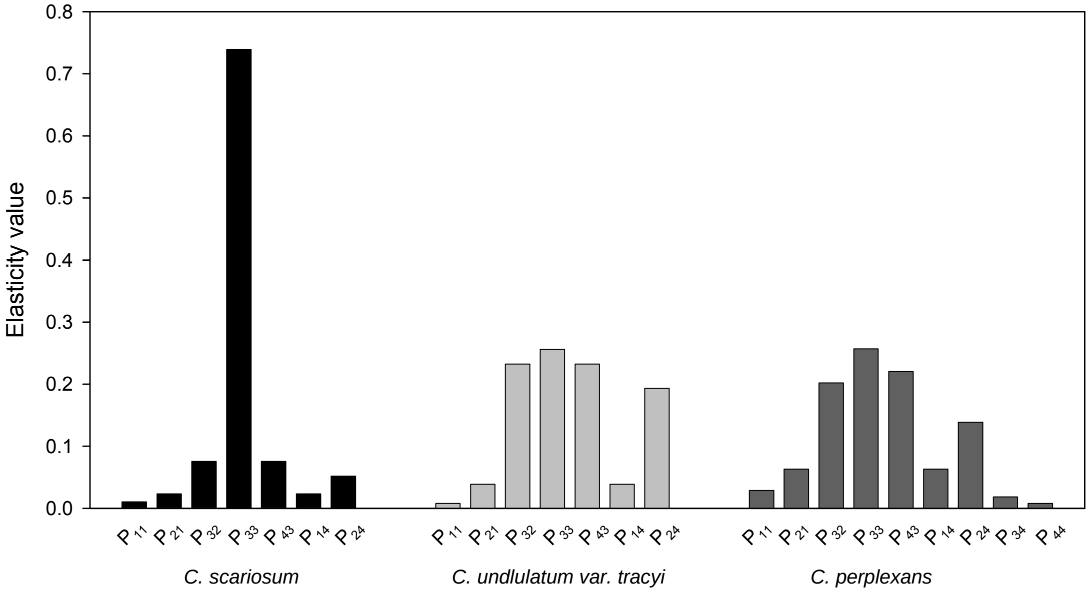

Figure 5-3. Elasticity values for observed transitions from analyses simulating populations with *persistent* seed banks.

DISCUSSION

The lack of effect the seed bank had on λ was not surprising given the deterministic nature of the data set and model. Short-term analyses are limited in their ability to include accurate portrayals of variation and stochasticity. Furthermore, inclusion of seed banks in the matrix model would hypothetically have a greater impact in a stochastic model instead of the deterministic model. Seed banks have been shown to increase generation time in age-structured models and thus dampen fluctuations in λ (Kalisz and McPeek 1992). Generally, greater magnitudes of fluctuation in λ will increase extinction risk (Menges 1998, 2000).

Inclusion of a seed bank also had little effect on the elasticities. In general, those elasticities associated with the seed bank (E_{11}, E_{21}, E_{41}) were less than those not associated with the seed bank. For *C. undulatum var. tracyi* and *C. perplexans*, it is arguable that all non-seed stages would result in similar responses in growth rate. In *C. scariosum*, however, the disproportionate value of E_{33} suggests that vegetative state mortality is having the largest impact on the growth rate. My speculation on the source of this is first, the large proportion of individuals of the population in that state, and second, the relatively high mortality in the non-flowering rosette stage all stemming from the greater number of years (six to ten) it takes to reach maturity and the correspondingly small transition value to a flowering adult ($P_{43} = 0.015$).

Evidence for persistent seed banks in *Cirsium* species is lacking, however this is not an argument for their non-existence. *Cirsium* seeds collected from herbarium specimens up to eight years old have germinated successfully (Moore and Frankton 1963, as reported in Spackman-Panjabi and Anderson 2004). Further studies have shown *C. pitcheri* and *C. vulgare* seeds can form persistent banks at soil depths of 15 cm (Klinkhamer et al. 1988, Rowland and Maun 2001).

I have presented cases in earlier chapters for population limitation from predispersal seed predation in *C. perplexans* and *C. undulatum var. tracyi*, and the results from this analysis do not discount the importance of seed production in those species. From a conservation standpoint, populations are being affected by the biological control agent *Rhinocyllus conicus* and likely *Larinus planus.* The elasticity analysis presented evidence that the transition from flowering plant to seedling was an important transition for both *C. perplexans* and *C. undulatum var. tracyi*. Further, elasticities indicate the relationship in proportional changes of a matrix element to λ. Larger changes in one transition with a small elasticity can have a larger effect on λ than a smaller change on a transition with a large elasticity (Silvertown et al. 1996).

The role of postdispersal seed predation should also be mentioned in these species. In all species the observed number of seeds produced by a flowering plant (following seed release) was many times greater than the estimated transition into the recruit or seed bank – indicating a high rate of post dispersal mortality. Post dispersal seed mortality due to aging is highly unlikely. Seventy-one percent of *C. undulatum var. tracyi* seeds collected for the seed sowing experiment (Chapter 4) germinated within seven days when placed between moist paper towels and kept at room temperature the following spring. One year old seeds of *C. perplexans* have been germinated at rates over 90% in the lab following cold treatment and seed coat manipulation (unpublished data).

Postdispersal seed predation has been shown to be high in *C. canescens* (Louda et al. 1990).

In this paper, I used a deterministic model to elucidate population dynamics. The shortcomings in this approach are well explored and include lack of demographic stochasticity, environmental variation, inflation of mortality estimates (from dormancy), and missing episodic recruitment (Menges 2000). Continuation of data collection of this system is very important to get a clearer picture of the population dynamics and factors that influence λ and population persistence. I intend to return to the demography plots in coming years to collect data on survivorship, growth, seed banks, and seed fate. Future models can more accurately include variation in the transitions and perhaps be modified to an age-structured model that will allow estimation of important life history traits such as generation time.

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