Nontarget effects of host-specific biological control agents

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NONTARGET EFFECTS OF HOST-SPECIFIC BIOLOGICAL CONTROL AGENTS

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Presented in partial fulfillment of the requirements for
the degree of Doctor of Philosophy
The University of Montana
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Nontarget effects of host-specific biological control agents

Exotic plant invasions threaten the biological diversity of natural ecosystems around the world. Classical biological control, the introduction of exotic organisms to control exotic invasive species, is a promising strategy that has proven effective at controlling exotic pests once they become widely established in natural ecosystems. However, the introduction of exotic organisms for biological control entails risks to nontarget species. For example, control agents with broad host ranges sometimes attack native species causing deleterious nontarget effects. To reduce this threat, rigorous screening for host-specificity is conducted before introduction of weed biological control agents to ensure control agents do not directly attack nontarget species. However, this does not prevent control agents from indirectly impacting nontarget organisms through food web interactions. I demonstrate that two host-specific biological control agents (*Urophora* spp.), widely established across western North America to control spotted knapweed (*Centaurea maculosa*), provide food subsidies that double or triple populations of a native generalist consumer, the deer mouse (*Peromyscus maniculatus*). This direct effect of gall flies on mice results in indirect effects on other nontarget species through food-web interactions. I show that deer mouse seed predation can reduce emergence and establishment of native grass and forb species, and the strength of seed predation impacts appears to be a density driven process. This suggests that as spotted knapweed invades native plant communities and directly impacts native plants through competition, it may also indirectly impact native plants through a form of second-order apparent competition by increasing seed predation on native plants through gall fly subsidies to mice. Moreover, the prevalence of Sin Nombre hantavirus, the etiological agent of the deadly hantavirus pulmonary syndrome, is three times higher in deer mouse populations subsidized by gall fly larvae. Host specificity alone does not ensure safe biological control. Host-specific biocontrol agents that establish, but fail to reduce the densities of their hosts may facilitate bottom-up effects that link the target weed to other organisms through food webs, thereby expanding the impacts of the invasive weed. Biological control agents must suppress pest populations enough to reduce their own numbers in order to minimize risks to nontarget species.
ACKNOWLEDGEMENTS

It is a long journey from a high school dropout living in a broken-down trailer on a dusty Montana back road to the place where I now sit writing this document. Although there are many people to thank, I must begin by acknowledging my own journey and my primary source of strength along this path. The wild places of Montana have always been my spiritual sanctuary, my font of strength, and the very essence of who I am. Without this source, my path would be very different than it is today. I am grateful for the stunning beauty that is springtime in the Bridgers and the Crazies, the relentless wind that screams down the east front off the backbone of the continental divide, the mysteries of the ancient cedar-hemlock forests of the McDonald Valley, the serenity of the Sweetgrass Hills, the sheer might of the Anaconda Pintlers and Bitterroots, the gentle grandeur of the Pioneers, the desert solitude of the Priors, and the peacefulness that is the Blackfoot Valley and was once the Bitterroot Valley. Finally, I acknowledge the greatest asset of Montana - all those places which I have not yet seen, but that still await my discovery and admiration.

There are many people to thank. I am exceedingly grateful to Ray Callaway who served as my spiritual mentor even more than my academic advisor. John Maron has far exceeded his role as a committee member by repeatedly taking the time to provide the critical feedback that has consistently elevated a good product to something much more. Len Ruggiero, my boss and friend, made this feat both necessary and possible by providing the incentive and also the flexibility needed to accomplish my job and a Ph.D. Len has also reminded me of who I am at times when I was weary of the battle. Kerry Foresman and Diana Six were extremely flexible and understanding in responding to my sometimes crazy time lines. The Callaway lab, the wildlife unit at RMRS, and the DBS staff have provided invaluable support. I am tremendously thankful to Yvette Ortega and Kathryn Socie for their help and friendship. Yvette has unfailingly coddled my statistical questions and ecological conundrums. Kathryn has single-handedly maintained my many research projects in my mental absence. I thank my mother for her unflattering love and support, which is a tribute to the confidence I bear today. My wife Ellen has heroically raised our amazing children while I attempted to work and fulfill a Ph.D. Finally, I am indebted to my children Alex, Anya, and Rachel who sacrificed much time without their father over the past four years so that I could accomplish this goal.
PREFACE

The purpose of this dissertation is to examine the assumption implicit in classical biological control that host-specificity is a sufficient safeguard against nontarget effects of introduced biological control agents. The dissertation is divided into five chapters. Each chapter is written as a standalone paper for a specific scientific journal and audience, and as such, there is some redundancy in reintroducing basic concepts and background information among chapters. However, each chapter addresses a key question and each sequentially builds upon the previous ones to ultimately provide a comprehensive treatment of the question. The chapters are broken down as follows.

Chapter 1 provides an introduction to the theory and practice of classical biological control, and then challenges a basic tenet of biological control by arguing from recent theory and empirical examples that host specificity does not ensure the safety of biological control agents, because host-specific biological control agents can affect nontarget species if they subsidize native consumers. This chapter also introduces the study system as a gall fly (Urophora spp.) biological control agent introduced for the control of spotted knapweed (Centaurea maculosa) that is exploited by a generalist consumer, the deer mouse (Peromyscus maniculatus). A condensed version of this chapter was published in Trends in Ecology and Evolution (Pearson and Callaway 2003).

Chapter 2 examines the direct effect of host-specific biological control agents on a native generalist consumer, the deer mouse. This chapter builds on a previous autecology study showing that deer mice effectively exploit the gall fly biocontrol agent as an important winter food resource (Pearson et al. 2000), and a previous observational study that suggests that exploitation of the biological control agents
may increase deer mouse populations (Ortega et al. 2004). This study expands on the previous observational study to experimentally quantify the effect of gall flies on deer mouse population abundance and survival by manipulating gall fly larvae using herbicide to kill the host plant. This research provides the basis for examining the indirect effects gall flies have on other native organisms by way of food subsidies to deer mice.

 Chapter 3 examines the indirect effects of the gall fly biocontrol agents on native plants by evaluating whether manipulation of gall fly populations using herbicide to kill the host plant alters deer mouse seed predation and thereby recruitment for two dominant native plants, arrowleaf balsamroot (Balsamohriza sagittata) and bluebunch wheatgrass (Pseudoroegneria spicata). This study overlays the deer mouse population study in Chapter 3 to provide an explicit link between gall fly direct effects on deer mouse populations and gall fly indirect effects on native plant recruitment by way of deer mouse seed predation.

 Chapter 4 evaluates the indirect effects of gall flies on the Sin Nombre hantavirus by examining the relationship between gall fly abundance, deer mouse abundance, and hantavirus prevalence in deer mouse populations using an observational study that compares these relationships between plots with high and very low spotted knapweed abundance at eight replicate sites scattered across western Montana. This chapter highlights the fact that nontarget effects of host-specific biological control agents can even have significant ramifications for human health when they elevate the etiological agent of a deadly zoonotic disease like hantavirus.

 Chapter 5 assesses the implications of nontarget effects of biological control agents for the future practice of biological control. This chapter attempts to reevaluate the practice of biological control in light of the results from the previous
chapters that show that host-specificity does not ensure the safety of biological control agents, because a biological control agent can still impact nontarget species through food-web interactions even if it does not directly attack nontarget species. This chapter emphasizes improving biocontrol agent efficacy as a means of both guarding against nontarget effects that arise from food-web interactions and improving the success of biological control. The paper is published in *Biological Control* (Pearson and Callaway 2005).

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CHAPTER 1

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CHAPTER 5

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Arrow direction indicates direction of the dominant interaction and the weight indicates the strength of the interaction. Lines without arrows in (a) simply indicate some sort of dependency.
CHAPTER 1
INDIRECT EFFECTS OF HOST-SPECIFIC BIOLOGICAL CONTROL AGENTS

Abstract. Biological control is a crucial tool in the battle against biological invasions, but biocontrol agents can have a deleterious impact on native species. Recognition of risks associated with host shifting has increased the emphasis on host specificity of biocontrol agents for invasive weeds. However, recent studies indicate host-specific biocontrol agents can also exhibit substantial nontarget effects through indirect interactions and food-web subsidies. Based on an evaluation of these studies, I conclude that the interaction strength between biocontrol agents and their hosts is at least as important as host specificity for determining the outcome of biocontrol introductions. Host-specific biocontrol agents that establish, but fail to reduce the densities of their hosts may facilitate bottom-up effects that link the target weed to other native organisms through food webs, thereby expanding the impacts of the invasive weed. I believe that indirect nontarget effects of host-specific biocontrol agents arising from food-web subsidies could prove more deleterious to native species than the direct nontarget effects currently recognized from host shifting.

Key words: biocontrol, biological invasions, compensatory response, ecological replacement, exotic species, food web subsidies, indirect effects, invasive species, nontarget effects

INTRODUCTION

Biological invasions increasingly threaten the biological diversity and ecological integrity of natural ecosystems (Mack et al. 2000). Classic biological
control (referred to henceforth as biocontrol), the importation of natural enemies for the control of exotic, invasive species, is a powerful management tool that has proven effective at controlling numerous invasive species (DeLoach 1991, McFadyen 1998). However, biocontrol also poses significant risks to nontarget, native taxa (Howarth 1991, Simberloff and Stiling 1996, Strong and Pemberton 2000, Louda et al. 2003). The most notable examples of nontarget effects arise when biocontrol agents expand their host range to attack native organisms (host shifting) (McFadyen 1998, Simberloff and Stiling 1996, Louda et al. 1997, Boettner et al. 2000, Henneman and Memmott 2001, Louda and O’Brien 2002). Recognition of the risks associated with host shifting has spawned an intense debate over the importance of host specificity of biocontrol agents (DeLoach 1991, McEvoy 1996, Secord and Kareiva 1996, Simberloff and Stiling 1996, 1998, Frank 1998, McFadyen 1998, Thomas and Willis 1998, Strong and Pemberton 2000). I believe this debate has improved the field of biocontrol of exotic plants by establishing host specificity-requirements for biocontrol agents that reduce risks to nontarget species (DeLoach 1991, McEvoy 1996, McFadyen 1998). However, recent studies examining insect biocontrol of invasive plants indicate that even host-specific biocontrol agents can incur significant nontarget effects on native species (Callaway et al. 1999, Pearson et al. 2000, Ortega et al. 2004). Yet, indirect threats have not been considered in biocontrol theory or practice. Here, I discuss the indirect effects of biocontrol agents in the context of the current theory and practice of biocontrol and recent advances in community ecology to illustrate that host-specific biocontrol agents can exhibit nontarget effects on native species and ecosystems. I focus my discussion on insect biocontrol of invasive plants, but the general concepts also apply to biocontrol of invertebrate pests (Schellhorn et al. 2002).
BIOCONTROL IN THEORY AND PRACTICE

The conceptual model underlying classic biological control was derived from predator-prey theory (Fig. 1) (Smith and van den Bosch 1967, van Driesche and Bellows 1996). This model is based on the notion that exotic species become invasive by escaping the controlling influence of their natural enemies (Williamson 1996, Crawley 1997, Keane and Crawley 2002). I refer to this model as the ‘natural enemies model’. In this model, control of the invasive species is achieved when the introduction of its natural enemies reduces its mean equilibrium density below some economically or ecologically defined threshold (Smith and van den Bosch 1967, van Driesche and Bellows 1996). The model predicts a direct negative effect of the biocontrol agent on its intended host that translates into an indirect positive effect on native species and a negative feedback that reduces and regulates its own populations (Fig. 2).

When biocontrol is successful, it is truly elegant. Cases of successful biocontrol demonstrate that top-down control can be achieved over invasive species using natural enemies when the biocontrol behaves as a keystone species (an organism with community effects that are disproportionately large relative to its abundance) (Power et al. 1996). This point is well illustrated by the control of Klamath weed *Hypericum perforatum* by the chrysomelid beetle *Chrysolina quadrigemina* (Huffaker and Kennett 1959). Klamath weed is an exotic forb that had invaded nearly 1 million ha of range land in northern California by the mid 1900s, causing dramatic reductions in the biomass and diversity of native species. The introduction of *C. quadrigemina* reduced Klamath weed to <1% of its peak invasion densities and facilitated recovery of the native system within 12 years of the initial
release. These two species currently persist at densities well below the threshold of ecological impact. Presumably, experimental removal of *C. quadrigemina* would result in Klamath weed recovering its former range at great expense to the native community. This keystone phenomenon is also demonstrated by other successful biocontrol agents in natural systems (DeLoach 1991, McEvoy 1996) and illustrates the importance of interaction strength (the population-level impact of one species on another; Wootton 1997) for achieving successful control. Although the host-specificity of *C. quadrigemina* may have contributed to its success, host-specificity alone was not sufficient to attain this success. Most plant biocontrol agents remain host-specific, yet fail to control their target pest (Williamson 1996, Julien and Griffiths 1998). The key to the success of *C. quadrigemina* was its interaction strength, i.e., its strong negative effect on Klamath weed populations.

Although the natural enemies model predicts that biocontrol agents will successfully control their target species, most biocontrol agents fail to achieve successful control (Julien and Griffiths 1998, McEvoy and Coombs 1999) so the outcomes of most introductions are unknown. Introduced biocontrol agents can host shift and attack nontarget species (e.g., Howarth 1991, Simberloff and Stiling 1996, Louda et al. 1997, Henneman and Memmott 2001), and host shifting can result in biologically significant negative impacts on nontarget species (Simberloff and Stiling 1996, Louda et al. 1997, Boettner et al. 2000, Henneman and Memmott 2001, Louda and O'Brien 2002). Recognition of the risks associated with host shifting has led to an increased emphasis on host specificity in screening prospective biocontrol agents to reduce this problem (McEvoy 1996). However, most biocontrol introductions result in the establishment of host-specific biocontrol agents that exhibit weak negative effects on their host (Julien and Griffiths 1998), and weak biocontrol agents
are presumed safe because host-specificity is believed to ensure their neutrality
toward nontarget species (DeLoach 1991, van Dreische and Bellows 1996, McFadyen
1998). This assumption has contributed to the ‘multiple release’ strategy in
introducing multiple agents for each target species with little regard for interaction
strength. The ironic result of the multiple release approach is that exotic biocontrol
insects now far outnumber the exotic plants that they were introduced to control
(Julien and Griffiths 1998, McEvoy and Coombs 1999), and newly emerging research
(Callaway et al. 1999, Pearson et al. 2000, Ortega et al. 2004) suggests that host-
specific biocontrol agents can incur strong nontarget effects through indirect
interactions associated with ecological replacement, compensatory responses and
food-web subsidies.

NONTARGET EFFECTS OF HOST-SPECIFIC BIOCONTROL AGENTS

Ecological replacement

Indirect nontarget effects can occur through ecological replacement when a
biocontrol is used against a pest that has become integrated into the native community
by physically or functionally replacing native species. Although biocontrol in cases
involving ecological replacement can result in indirect nontarget effects on native
species, this is not a failure of the biocontrol or the natural enemies model. The
natural enemies model predicts that those organisms directly interacting with the pest
species will be affected by its control (Fig. 2), and well established exotic species can
be expected to develop interactions with native organisms. For example, biocontrol
of exotic European rabbits Oryctolagus cuniculus in Great Britain is believed to have
resulted in the extirpation of the large blue butterfly Maculina arion through a series

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of indirect effects that fatally linked this species to the rabbits (Moore 1987). The large blue required nests of the ant *Myrmica sabuleti* for the development of their larvae. These ants in turn were dependent upon rabbit grazing to maintain open habitat for their nests, so biocontrol of the rabbits with *Myxoma* virus initiated a cascade of interactions believed to have lead to the extinction of the large blue. Recognition of the risks associated with indirect nontarget effects from ecological replacement has helped to avoid repeating the story of the large blue. For instance, proposed biocontrol of saltcedars *Tamarix* spp. in the southwestern USA was rejected because of risks to the endangered subspecies of the southwestern willow flycatcher *Empidonax traillii extimus* (Myers et al. 2000). This flycatcher currently relies on saltcedars for nesting sites in areas where these exotics have replaced its native nesting habitat (USFWS 1993). The concern was that biocontrol of saltcedars would remove nesting habitat before the native vegetation could be restored. The problem of ecological replacement is likely to increase as biological invasions proliferate and exotics become increasingly entrenched within native communities over time (van Reil et al. 2000). Therefore, it will become increasingly important to effectively assess the extent of ecological replacement by invasive species to determine the potential for unintended indirect nontarget effects arising from the biocontrol of well-established invaders.

*Compensatory responses*

Herbivory does not always result in direct negative effects on plants. Plants can alter the outcome of biocontrol herbivory through compensatory growth or increased production of secondary compounds. For example, field and greenhouse experiments indicate that herbivory by the root-boring biocontrol moth *Agapeta*
zoegana on the invasive forb Centaurea maculosa may deleteriously affect native grasses such as Festuca idahoensis through an indirect effect (Callaway et al. 1999, Ridenour and Callaway 2003). Application of A. zoegana did not significantly decrease C. maculosa biomass and actually stimulated small but significant decreases in F. idahoensis reproduction and trends towards lower F. idahoensis biomass. The mechanism for this unusual indirect effect is not clear, but there are three non-mutually exclusive hypotheses. First, C. maculosa exhibits a very strong compensatory growth response to herbivory (Müller-Schärer 1991, Kennett et al. 1992, Ridenour and Callaway 2003), and resource competition might intensify with increased resource uptake. Second, herbivory can stimulate increased production of harmful root exudates (Callaway et al. 1999, Bais et al. 2002). Finally, the negative effect of biocontrol herbivory on C. maculosa could be mediated by mycorrhizal fungi (Marler et al. 1999, Callaway et al. 2001). These studies illustrate the potential for unpredictable indirect effects of host-specific biocontrol agents to impact negatively the very native species that they were intended to help.

Food-web interactions

Introduced biocontrol agents that become established have the potential to become superabundant within the host environment because they encounter plentiful food, little competition and few natural enemies of their own. If biocontrol agents are strong enough to control their host populations, their superabundance will be ephemeral because the biocontrol will decline as it depletes its food resource. However, if an established biocontrol is ineffective at reducing its host densities, populations of the biocontrol are likely to remain abnormally high. High resource concentrations present a lucrative opportunity for native consumers, and native
consumers are commonly observed preying upon biocontrol agents (Goeden and Louda 1976, Kluge 1990, Müller et al. 1990, Story et al. 1995, Dray et al. 2001). However, predation on biocontrol agents has simply been viewed as a source of interference with the biocontrol, and there has been little regard for the potential for deleterious indirect nontarget effects within the native system. In fact, biocontrol subsidies to native consumers have been interpreted as a ‘means by which previously little-used resources benefit various food chains in the environment’ (Harris 1990). This view is dangerously over simplistic.

Recent studies on food subsidies to communities provide convincing evidence that many organisms are food limited and that food subsidies to key organisms at various trophic levels can serve to restructure community interactions, whether inputs come from native or exotic sources (Power 1990, Spencer et al. 1991, Polis and Strong 1996, Huxel and McCann 1998, Jefferies 2000, Roemer et al. 2001). For example, the introduction of exotic kokanee salmon *Oncorhynchus nerka* to Flathead Lake in western Montana (Spencer et al. 1991) resulted in annual fall salmon runs up the Flathead River system into Glacier National Park. This spawning activity created a concentrated food resource that was exploited by a variety of native consumers including bald eagles *Haliaeetus leucocephalus*, gulls *Larus* spp., grizzly bears *Ursus arctos*, and coyotes *Canis latrans*. Although the indirect effects associated with exotic subsidies to native predators were not quantified in this system, they have been quantified elsewhere. On the northern Channel Islands off the coast of California, exotic feral pigs *Sus scrofa* are facilitating the extirpation of the endemic island fox *Urocyon littoralis* by subsidizing recently established golden eagle *Aquila chrysaetos* populations (Roemer et al. 2001). Energy budgets demonstrate that the exotic pigs are key to this interaction because the eagles cannot sustain their populations on
native prey alone (Roemer et al. 2001). Negative impacts on native species have also been documented from exotic crop subsidies to snow geese Chen caerulescens and exotic insect subsidies to parasitoids (Settle and Wilson 1990, Jefferies 2000). These studies indicate that exotic food resources can subsidize important native consumers with unpredictable and potentially significant indirect effects for native species through food webs. Thus, it is reasonable to expect that exotic biocontrol agents could subsidize native consumers with similar outcomes.

Although native consumers commonly exploit biocontrol agents (Goeden and Louda 1976, Kluge 1990, Müller et al. 1990, Story et al. 1995, Dray et al. 2001), studies examining the outcomes of such interactions virtually always focus on the consumer’s effects on the biocontrol, while ignoring the potential effects of the biocontrol on the native consumer (Goeden and Louda 1976, Kluge 1990, Müller et al. 1990, Story et al. 1995, Dray et al. 2001). However, recent studies (Pearson et al. 2000, Ortega et al. 2004) of gall fly Urophora spp. biocontrol agents introduced to control spotted knapweed indicate that biocontrol agents can have significant direct and indirect nontarget effects on native species through food web interactions when biocontrol agents provide food subsidies to generalist consumers like native deer mice Peromyscus maniculatus (Box 1).

The knapweed-Urophora-deer mouse case study serves to illustrate the importance of interaction strength and the direction of energy flow between the biocontrol and the invasive plant. If the Urophora biocontrol had a strong negative effect on the target plant as intended, it would have initiated top-down control over the invader that would have resulted in an indirect positive effect on the native plants and a negative feedback on its own populations. This outcome would have restricted the set of interactions to those recognized by the natural enemies model and
effectively prevented both exotics from significant interactions with the native community. However, because the biocontrol produced only weak negative effects on its highly prolific host, biocontrol populations increased and instead of restricting interactions to those associated with the natural enemies model, the biocontrol serves to facilitate the bottom-up flow of energy from primary producers into the larger community through food-web interactions (Box 1). How far these effects carry out into the system will depend on interaction strength, particularly the strength of the interaction between the pest and the biocontrol. If *Urophora* had a very weak positive effect on deer mice, the potential for indirect effects would be limited. However, it is difficult to imagine that doubling or tripling populations of a native consumer such as the deer mouse, which so effectively infiltrates native food webs as an aggressive generalist predator, a prominent prey item, and a vector for zoonotic disease, will not translate into significant impacts on native species, and potentially humans. When a biocontrol fails to serve as a keystone predator that maintains top-down control over the system, it has the potential to serve as an 'ecological bridge' for bottom-up effects that links the invasive species to other native organisms, thereby expanding the impacts of the invasive species further into the native community through indirect effects. Such a biocontrol could qualify as a keystone species if its impact on the system is large relative to its biomass (Power et al. 1996).

**Conclusions**

Biocontrol theory focuses on simple predator-prey relationships, but ignores more complex community interactions. As a result, biocontrol programs encourage the release of multiple host-specific biocontrol agents for each target pest with little regard for indirect nontarget effects (Julien and Griffiths 1998, McEvoy and Coombs
This strategy has been criticized because successful biocontrol agents are being sought through a lottery rather than through ecological understandings of natural enemy-host relationships (Julien and Griffiths 1998). The best way to minimize the potential for nontarget effects is to minimize the number of biocontrol agents introduced whilst maximizing the potential for control. McEvoy and Coombs (1999, 2000) have discussed applying rules of parsimony to biocontrol programs to introduce the minimum number of agents necessary for control. This approach involves selecting biocontrol agents that represent different functional groups proven to disrupt transitions in the life cycle of the plant McEvoy and Coombs (1999). This strategy holds ecological merit because it focuses on emphasizing interaction strength and avoiding redundancy when attempting to construct effective ‘natural enemy complexes’ or ‘biocontrol guilds’. However, deploying biocontrol guilds implicitly assumes that multiple biocontrol agents will be more effective than individual control agents. For this to occur, biocontrol agents must either have additive effects or achieve synergistic effects through interactions that increase their collective impact on pest populations (Losey and Denno 1998). Although constructing biocontrol guilds comprised of distinct functional groups of biocontrol agents will favor parsimony in multiple release programs, it does not ensure additive or synergistic effects that increase control. For example, synergistic effects that increase the overall effectiveness of biocontrol may arise from behaviors of biocontrol agents (Losey and Denno 1998) or pest responses that are independent of biocontrol functional group. More research on community aspects of multispecies predator-prey interactions (Losey and Denno 1998, Denno et al. 2000, Eubanks and Denno 2000) is needed to understand how predator complexes function as natural enemies if we are to deploy multiple biocontrol agents effectively. Additionally, comparative studies examining
invasive pests in their native and introduced ranges would help to understand when escape from natural enemies is the mechanism for invasiveness versus other mechanisms (Callaway and Aschehoug 2000, Keane and Crawley 2002, Klironomos 2002, Mitchell and Power 2003) to determine which invaders are most susceptible to control by natural enemies and which natural enemies are most likely to be effective biocontrol agents. Host-specificity is an important attribute for safe, effective biocontrol (McEvoy 1996, Secord and Kareiva 1996, Pemberton 2000). However, host-specific biocontrol agents can impact nontarget species through indirect effects arising from ecological replacement, compensatory responses, and food-web subsidies, and nontarget effects arising from food-wed subsidies can profoundly impact native systems. Strong, host-specific biocontrol agents should be the paradigm for future biocontrol.

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Fig. 1. Theoretical control of pest species using biocontrol agents. Adapted, with permission, from (Smith and van den Bosch 1967). This figure illustrates how pest densities might fluctuate over time before and after the introduction of a successful biocontrol agent. Prior to the introduction of the biocontrol agent, the pest density fluctuates around a mean equilibrium density that is above a threshold of economic or ecological impact. Following the introduction of the biocontrol agent the pest densities stabilize at a new equilibrium level that is below the threshold of impact.

Fig. 2. Theoretical control of pest species using biocontrol agents presented in the context of the natural enemies model. The figure illustrates direct (straight lines) and indirect effects (curved lines) predicted by the model. Line weight indicates interaction strength. Dotted lines indicate empty niche of primary consumer and postulated effects of introducing a biocontrol agent. This figure illustrates how the natural enemies model focuses on the direct negative effect of the biocontrol on the target pest and the resulting indirect positive effect on the native species, but ignores other community interactions that might arise within the system, i.e., they are not addressed by the model.

BOX 1. The Urophora case study

In the early 1970s, two species of gall flies Urophora affinis, and U. quadrifaciata were introduced to western North America for the biocontrol of spotted and diffuse knapweeds Centaurea maculosa and C. diffusa (Julien and Griffiths 1998). The Urophora spp. successfully established and the flies have remained host specific, but have failed to control populations of their host plants (Maddox 1982).
As a result, these biocontrol agents have become superabundant, occurring in North America at densities of 3000 larvae m$^{-2}$ (Harris 1980).

Not surprisingly, this abundant resource has drawn the attention of many native consumers (Story et al. 1995), and recent studies show that the gall flies subsidize populations of at least one very important generalist predator, the deer mouse Peromyscus maniculatus (Pearson et al. 2000, Ortega et al. 2004). Gall fly larvae, which overwinter within knapweed seedheads, are readily exploited by deer mice, and these larvae now comprise 85% of the deer mouse winter diet in grasslands invaded by knapweed (Pearson et al. 2000). The result of this winter food subsidy has been an increase in over-winter survival that has elevated deer mouse populations two- to three-fold in knapweed-invaded grasslands (Ortega et al. 2004). Subsidizing this generalist predator has potentially significant ramifications. Deer mice are aggressive predators of seeds and insects, competitors with other small mammals, and important prey for larger predators (Zimmerman et al. 1996, Pearson et al. 2000, Maron and Simms 2001). They are also the primary vector for the deadly Sin Nombre hantavirus (Childs et al. 1994). Thus, gall fly subsidies to deer mouse populations could disrupt established food webs and elevate the prevalence of a deadly zoonotic disease (Fig. 1). Moreover, these subsidies might exacerbate the impacts of knapweed on the native community through indirect effects. For example, seed predation by deer mice can significantly reduce recruitment in native plant populations (Maron and Simms 2001). Therefore, gall fly subsidies that elevate deer mouse populations in knapweed-invaded habitats could cause reduced recruitment of native plants already impacted by knapweed invasion. A similar case can be made for deer mouse predation on ecologically important invertebrate prey.
BOX 1 - Fig. I. Currently documented (solid lines) and postulated (dotted lines) direct and indirect effects associated with gall fly *Urophora affinis* and *U. quadrifasciata* biocontrol agents introduced for the control of spotted knapweed *Centaurea maculosa*. The *Urophora* biocontrol agents exhibit very weak negative top-down effects on *C. maculosa*. Because of their lack of control over the weed, *C. maculosa* exhibits very strong bottom-up effects on the biocontrol agents. The resulting superabundance of the biocontrol has facilitated the bottom-up flow of energy further out into the native system by subsidizing native predators such as deer mice *Peromyscus maniculatus* (Pearson et al. 2000) that are integrated into native food webs. The extent to which this unintended outcome is likely to carry out into the system is a function of the strength of the various interactions. The most important interaction is that between the biocontrol and the native consumer. In the case of the deer mouse, this interaction has proven to be very strong (Ortega et al. 2004), increasing the likelihood that other postulated nontarget indirect effects will follow. Line thickness indicates interaction strength.
Mean equilibrium density

Threshold density

Mean equilibrium density

Biocontrol release

Time

Fig 1.
Biocontrol insect

Invasive plant ↔ Native plants

Fig 2.
Box 1 - Fig I.
CHAPTER 2
EXOTIC ORGANISMS AS FOOD SUBSIDIES: REMOVAL OF BIOLOGICAL
CONTROL AGENTS REDUCES CONSUMER POPULATIONS

Abstract. Food limitation is thought to be a major factor in regulating animal
populations and structuring communities. Thus, the introduction of exotic organisms
that serve as allochthonous food subsidies may alter food-limited consumers and have
important ramifications for trophic interactions within the affected community. I
examined a system involving two introduced biological control agents that provide a
food subsidy to deer mouse (Peromyscus maniculatus) populations to quantify the
extent to which these exotic subsides influence the demography of this generalist
consumer. I conducted a large-scale, long-term removal of subsidies (by killing the
host plant, Centaurea maculosa) and followed P. maniculatus populations over one
full population cycle. Following treatment, mouse populations in the presence of the
biocontrol agents were on average approximately two times more abundant than
where biocontrol agents had been experimentally removed. Elimination of the food
subsidy did not affect the timing of the population cycle, as both treatment groups
cycled in synchrony, but it did affect the amplitude of the cycle, with both the peak
and trough reduced in the removal treatment. These results support two key
hypotheses derived from food supplementation studies: 1) food subsidies generally
double animal populations, and 2) food subsidies do not prevent population cycles.
However, in contrast to these hypotheses, reproductive output as measured by sex
ratios, reproductive status, pregnancy rates, and juvenile recruitment were unaffected
by the treatment. Multi-state mark-recapture models indicated that survival declined
where food subsidies were removed, but that emigration and immigration did not
affect this outcome. Together, these results suggest that exotic organisms can significantly subsidize populations of a generalist consumer with important trophic implications for the affected community.

**Key words:** food subsidies, allochthonous inputs, biological control, food limitation, food supplementation, population ecology, survival, movement, reproduction, exotic species

**INTRODUCTION**

The notion that food resources limit animal populations is fundamental to animal ecology (Lack 1954), evolutionary theory (Darwin 1859), and community ecology (Hairston et al. 1960). When populations are food limited, allochthonous or autochthonous inputs of material or energy can significantly subsidize animal populations, altering equilibrium states, restructuring communities, and in some cases altering ecosystem function (Spencer et al. 1991, Polis and Hurd 1996, Jefferies 2000, Roemer et al. 2001, Polis et al. 2004, Croll et al. 2005). Recent studies indicate that novel resource subsidies, such as allochthonous agricultural inputs, can profoundly influence populations, communities, and ecosystems (Jefferies 2000). However, little attention has been paid to the potential for exotic species introductions to serve as novel inputs that affect native consumers. When exotic organisms displace native species, they necessarily disrupt, replace, or create new food web linkages. Exotic organisms of low palatability or low food quality can act to reduce food-web complexity. The enemy release hypothesis, the dominant hypothesis explaining invasions, poses that exotic organisms are released from consumer pressures because they escape from natural enemies (Keane and Crawley 2002). This suggests that most
exotic organisms are not readily consumed in their new environments and may therefore reduce food-web linkages. However, in some cases exotic organisms can have higher nutritional value than their displaced counterparts (e.g., Richman and Lovvorn 2004), and they can create new resource opportunities (Roemer et al. 2001). They also commonly occur at higher resource concentrations and availabilities that may increase their relative foraging value (Charnov 1976, Richman and Lovvorn 2004). In such situations, exotic organisms may serve as allochthonous food subsidies with important implications for consumer interactions in food limited systems.

Although the concept of food limitation has defined much of our thinking about competition, trophic interactions, and community organization (e.g., MacArthur 1958, Hairston et al. 1960, Connell 1961), effective tests of food limitation have been elusive. Most good examples of allochthonous inputs into systems are based on observational or natural experiments (Polis and Hurd 1996, Jefferies 2000, Croll et al. 2005), because it is extremely difficult to manipulate large-scale inputs. Food supplementation experiments have been used extensively to evaluate autochthonous inputs on individuals and populations (Boutin 1990, Law 1995, Galindo-Leal and Krebs 1998, Banks and Dickman 2000, Diaz and Alonso 2003) and to a much lesser extent on communities (Brown and Munger 1985, Dickman 1988, Krebs et al. 1995). However, it is often unclear how the outcomes of food supplementation experiments apply to real populations and communities. Food supplementation studies are often unrealistic manipulations which are limited to asking basic questions about the capacity of organisms to respond to different resource states. These experiments commonly involve adding novel resources of abnormally high quality and quantity (e.g., Boutin 1990, Galindo-Leal and Krebs 1998, Banks and Dickman 2000, Eccard
and Ylonen 2001, Diaz and Alonso 2003). Moreover, the distribution of food resources in space and time is by necessity subjectively determined by the researchers; often to the point where it does not reflect realistic resource conditions (e.g., Duquette and Millar 1995, Galindo-Leal and Krebs 1998, Diaz and Alonso 2003). The nature of these resource distributions can greatly affect the outcome of food addition experiments (see Boutin 1990, Galindo-Leal and Krebs 1998).

Dominant species or individuals can monopolize subsidies when foods are dispensed at discrete stations (Boutin 1984, 1990, Law 1995, Banks and Dickman 2000). Even at larger scales, novel resource concentrations trigger immigration with the result that the relative role of immigration versus survival or fecundity is often unclear (Gilbert and Krebs 1981, Boutin 1984, 1990, Law 1995, Lofgren et al. 1996, Prevot-Julliard et al. 1999, Banks and Dickman 2000). As a rule, food supplementation studies are simply too short in duration to distinguish the relative importance of longer-term responses such as increased survival and fecundity from potentially short-term responses like immigration (Boutin 1990, Galindo-Leal and Krebs 1998, Banks and Dickman 2000, Eccard and Ylonen 2001, Diaz and Alonso 2003). Thus, food supplementation experiments contribute greatly to understanding the physiological and biological capacity of consumers to respond to increasing food quality and quantity and to changes in the spatial and temporal distribution of food resources, but understanding the effects of food subsidies on population and community dynamics is limited when the manipulated resources and resource states do not emulate natural conditions.

These constraints on food supplementation experiments are well recognized, and food removal experiments have been recommended as more appropriate tests of food limitation (e.g., Boutin 1990, Galindo-Leal and Krebs 1998). Removal
experiments emulate natural concentrations and distributions of the manipulated resource in space and time, thereby avoiding problems associated with novel resource introductions or concentrated resource islands and draw in immigrants from the surrounding landscape. However, food removal experiments are rare (e.g., Todd and Keith 1976, Ewald and Carpenter 1978, Pyke 1989), because removal of natural food resources is logistically challenging. To better understand the ecology of food limitation and consumer interactions in the context of exotic species introductions, I studied a system comprised of two exotic gall fly biological control insects (Urophora affinis and U. quadrijacinta) that have become significant food resources for deer mice (Peromyscus maniculatus). I conducted a large-scale, long-term food removal experiment to evaluate the effects of exotic biocontrol agents on this generalist consumer. Urophora spp. were introduced to North America in the early 1970's to control the exotic invasive plant spotted knapweed (Centaurea maculosa) (Harris 1980). Urophora have since become an important food for P. maniculatus in C. maculosa-invaded habitats from September through May and a critical food during peak winter months from December through April (Pearson et al. 2000). Because resource limitation during winter can inhibit breeding and increase mortality for temperate zone species (Lack 1954, Boutin 1990), the temporal availability of this resource may be especially important for deer mouse ecology. Observational studies comparing P. maniculatus populations between sites with high versus low C. maculosa invasion and correspondingly high versus low Urophora food subsidies suggest that P. maniculatus populations are elevated where this food subsidy occurs (Ortega et al. 2004, Chapter 4). Ortega et al. (2004) argued that this response was due to increased overwinter survival as opposed to increased immigration, but they could not rule out immigration in their experimental design, and inferences about increased
P. maniculatus populations in both studies are limited due to the observational nature of the research. To examine the effect of these naturalized food subsidies on P. maniculatus populations, I experimentally removed gall flies from large replicated treatment areas. My primary objectives were to 1) evaluate whether experimental removal of the Urophora food resource reduces P. maniculatus populations, 2) determine the relative role of survival, reproduction, and movement in effecting any reductions in P. maniculatus populations, and 3) assess the effect of precipitation inputs in terms of a recent drought in determining the above outcomes.

METHODS

Study area

The study was located at Calf Creek Wildlife Management Area approximately 10 km northeast of Hamilton, Montana, in the foothills of the Sapphire Mountains (46° 16' N 114° 5' W). Average annual precipitation is approximately 32 cm mostly in the form of snow in winter and rain in May and June. Mean monthly minimum and maximum temperatures are 1.6 and 8.6 °C during the winter peak in January and 8.6 and 29.3 °C during the summer peak in July. The study area is dominated by extensive grassland benches separated by conifer-lined drainages. Study plots were located on the grassy benches where vegetation is generally sparse and the dominant native plants are bluebunch wheatgrass (*Pseudoroegneria spicata*), june grass (*Koeleria cristata*), and Great Basin sage (*Artemisia tridentata*). *Centaurea maculosa* is the dominant species in these grasslands.
Overall sampling design

Sampling was conducted on four replicate plots from 1999 to 2003. Plots were selected for homogeneous vegetation, microtopography, and soil conditions and were located 500 to > 1000 m apart. Each plot was comprised of three parallel transects 220 m long and 50 m apart running parallel to the slope. One sampling station was located every 10 m along each transect totaling 22 sampling stations per transect. Herbicide treatment was randomly assigned to half of each plot, splitting transects in half. Treatments included large buffer strips 50 to > 500 m wide on each of the 3 exposed sides of each plot. On 5 May 2000, C. maculosa and Urophora were removed from half of each plot by helicopter spraying of the broadleaf herbicide Tordon® at 1.24 l/ha. Centaurea maculosa is very sensitive to this herbicide, allowing its effective removal with low dosages that minimize impacts on nontarget native plants (Rice and Toney 1998). As obligate parasites of C. maculosa, Urophora food resources are removed by the treatment along with their host plant.

Centaurea maculosa and Urophora sampling

Percent cover of C. maculosa was visually estimated over a 5-m radius circular plot centered on each sampling station (after Pearson et al. 2000, 2001). This was done at the peak of the growing season during the first week in July each year from 1999 to 2003. Urophora larvae were quantified in 1999 and 2000 as the number of larvae per C. maculosa seedhead by haphazardly collecting 10 seedheads from within 1 m of each sampling station in the fall and dissecting the seedheads to count the larvae within. In 2001 and 2002, Urophora were quantified in 0.5 m² quadrats placed 0.5 m uphill from each sampling station. Within each frame, percent cover of C. maculosa was estimated and the number of C. maculosa stems and seedheads were
counted. A random subset of 20 seedheads were selected from each station and dissected to quantify the larvae within. These data were used to calculate the density of larvae per seedhead and the density of larvae per 0.5 m$^2$ in 2001 and 2002 and also to determine the relationship between *C. maculosa* cover, *C. maculosa* seedheads, and *Urophora* larvae for extrapolating larval densities in 1999 and 2000 (see analyses). *Urophora* were not quantified in 2003 since they would be consumed in the winter after the study was terminated.

*Invertebrate sampling*

To quantify the potential effects of herbicide removal on other food resources, I conducted pitfall sampling for invertebrates, which are the dominant food for *P. maniculatus* in arid grasslands in this region (Johnson 1969, Halfold 1981, Pearson et al. 2000, D. E. Pearson unpubl. data). I constructed invertebrate pitfall traps from 2-l clear plastic soft drink bottles. The tops were cut off at the base of the neck, and the bottles were set into the ground so the rim was at or below ground level. Plastic 455 ml cups were placed in the bottom of each pitfall and filled with approximately 100 ml of 10% formalin as a preservative. The tops of the bottles were inverted and set in the body of bottles so they acted as funnels that drained into the cups. The mouth of this funnel (approximately 21 mm) was large enough to accommodate all invertebrates in this system, but small enough to minimize the risk of capturing mice in pitfalls. I placed pitfalls at the center of every 3$^{rd}$ sampling station (30 m intervals) starting 30 m from the treatment boundary so that there were 6 pitfalls on each transect, with 3 on each side of the treatment boundary. I conducted pitfall sampling over 3 3-week periods in spring, summer, and fall, 2000-2003. Pitfall sampling overlapped with trapping (see Deer mouse sampling below) such that pitfall sampling
began 1 week before and ended 1 week after each trapping session. I collected pitfall contents at the end of each 3-week sampling period by straining, packaging, and freezing the contents. Invertebrates were quantified and identified to order in the laboratory.

**Deer mouse sampling**

I sampled *P. maniculatus* populations using Sherman folding live traps (7.6 x 8.9 x 22.9 cm) spaced at 10-m intervals along the 3 transects on each replicate plot (Pearson and Ruggiero 2003). This resulted in 22 trap stations per transect with 11 stations on each side of the treatment boundary, beginning 10 m from the boundary. I placed one trap at each sampling station and ran them for 4 days. I baited traps with peanut butter and whole oats, and I covered traps with closed cell foam and placed polypropylene batting inside to protect mice from cold and rainy weather. Trapping was conducted in spring (last week in April), summer (first week in July), and fall (first week in October). I checked traps each day before 1100 hrs, and captured animals were identified to species and tagged with uniquely numbered 1005-1 monel ear tags (National Band and Tag Company, Newport, Kentucky 41072-0430). In addition, I determined the sex, weight and reproductive status of each individual prior to release at the trap station. *Peromyscus maniculatus* were weighed by the tail to the nearest 0.5 g using a 50-g Pesola scale and age was assigned based on pelage characteristics as juvenile (all gray), subadult (mottled gray-brown), or adult (all brown or beginning the adult molt as indicated by brown near the base of the tail). Females were deemed reproductively active if mamma were visibly swollen or if mice were visibly pregnant. Males were deemed reproductively active if testes were sufficiently swollen to be palpable or fully descended. In addition to live trapping, I
also snap trapped mice to obtain a minimal sample of mice for diet analysis during each sampling period. Snap trap lines made up of 6 standard snap traps were set out at 40 m intervals along 2 transects centered between the 3 live trap transects. I baited snap traps with peanut butter on the first day only and checked them along with live traps during each 4-day sampling period. All plots and treatments were sampled simultaneously during each 4-day trapping period.

**Analyses**

I compared *Urophora* density and relative abundance of invertebrates collected from pitfalls by treatment and over time in separate analyses using mixed linear models (PROC MIXED; SAS Institute 1999), where replicate plot was treated as a random factor and treatment and year were entered as fixed factors with year treated as a repeated measure. I estimated *Urophora* densities per 0.5 m$^2$ for this analysis using the linear regression equation for the relationship between *C. maculosa* percent cover and seedhead densities quantified in 2001 and 2002 ($R^2 = 0.426$, $F_{1,231} = 172.95$, $P < 0.001$). *Urophora* densities were calculated from this equation as $y = (mx+b)u$; where $y = Urophora$ density, $m = 1.992$, $x = C. maculosa$ percent cover, $b = 6.511$, and $u =$ mean *Urophora* density per seedhead (m is the coefficient and b is the constant from the regression equation). Estimates for *C. maculosa* percent cover and mean *Urophora* density per seedhead were based on sampling each station from 1999 to 2002. Analysis of invertebrate populations focused on Orthoptera, Lepidoptera, Coleoptera, and Arachnida, since these 4 orders make up >90% of the *P. maniculatus* diet at this study site (D. E. Pearson unpubl. data). All orders were pooled for analyses because patterns were similar among orders.
I estimated *P. maniculatus* abundance and associated variance for each 4-day trapping interval for each control and treatment plot by considering the population closed within each season (Otis et al. 1978) using Program MARK (White and Burnham 1999). Population abundance was estimated using the jackknife estimator (Model Mh; Otis et al. 1978), which incorporates individual heterogeneity into the probability of capture. Estimates were then analyzed using mixed models in PROC MIXED where treatment, season, and year were fixed factors.

I used a multi-state mark-recapture approach (Schwarz et al. 1993) to estimate the influence of *Urophora* food subsidy removal on the survival and movement of *P. maniculatus*. Each 4-day trapping period was collapsed to a single capture event so that there were 15 total capture events (spring, summer, and fall in each year from 1999 through 2003). Capture histories were then tallied across the 15 capture intervals. Survival \((S)\) was estimated by partitioning apparent survival \((\phi)\) from the probability of movement \((\psi)\) between treatment and control areas. For example, survival during time period \(i\) in treatment areas \((t)\) can be described as \(S_t^i = \phi_t^i / \psi_t^i c\), where \(c\) is the control. Note that while multi-state models do adjust apparent survival for local movement between treatments, survival is still confounded with permanent emigration from plots. I assigned animals initially to control or treatment based on their residence status during their first capture interval. An animal captured in only one treatment was assigned to that treatment, an animal captured in both treatment and control was assigned to the treatment where it was captured most, and any animals captured an equal number of times on both sides of the treatment boundary were removed from the analysis \(n = 27; 2%\) of the total number of individuals captured). Snap trapping data and live trapping mortalities were incorporated into the
population modeling to account for animals removed through live and snap trap mortality (White and Burnham 1999).

This experiment was designed to test for two *a priori* explanatory variables that could influence both survival and movement of *Peromyscus*: 1) treatment, and 2) season (winter versus non-winter). Because there were no estimable differences between *Urophora* food subsidies on control and treatment plots prior to treatment (see results), pretreatment plots were pooled with control plots. Treatment was considered to first potentially influence survival and movement during winter 2000-2001, because *Urophora* produced in the summer provide food for mice beginning in fall (Pearson et al. 2000). Season was considered to be important because prior work documented seasonal changes in *P. maniculatus* predation on *Urophora* (Pearson et al. 2000). I also considered the effect of a drought that began in spring 2000 and eventually reduced the *Urophora* food subsidy starting in fall 2001 (see results; Fig. 1). To estimate this potential effect, I considered the drought to influence survival beginning winter 2001-2002; therefore, “pre-drought” covered 1999-2000, and “post-drought” covered 2001-2003.

Based on these considerations, I developed a candidate model set (Table 1) that reflects the models necessary to evaluate the specific hypotheses being tested (Table 2). The most complex model I considered (the global model) included an interactive effect of treatment (trt), season (seas), and drought (drt) on survival and movement, and season and year interaction on capture probability \(S(\text{trt} \times \text{seas} \times \text{drt}) \times (\text{seas} \times \text{yr})\). To estimate the nuisance capture probability, \(p\), I compared the global model to a series of reduced models that only changed \(p\) estimation \((p(\text{seas} \times \text{yr}) \text{ vs } p(\text{seas}) \text{ vs } p(.))\) to determine the most parsimonious parameterization (based on model-selection criteria; see below). For the remaining
candidate models, I subsequently used the most parsimonious approach identified for estimating $p$, which was estimation of $p$ as (seas×yr). I tested the fit of the global model by estimating the overdispersion parameter, $\hat{c}$, using the median $\hat{c}$ procedure in program MARK. This approach is valuable for estimating goodness-of-fit when global models are not the most complex models testable with the data and allows for model selection to be adjusted for overdispersion, whereas other approaches do not (e.g., Pradel et al. 2003). Hypotheses were tested by hierarchically comparing paired models using likelihood ratio tests, $\text{AIC}_c$ (Akaike's Information Criterion, adjusted for sample size), and Akaike ($\text{AIC}_c$) model weights (relative likelihood of a model given the data; Burnham and Anderson 1998:124) in program MARK (Burnham and Anderson 1998, White and Burnham 1999). The results of the hypothesis tests were evaluated in the context of the overall model comparison for the entire set of candidate models.

I evaluated the response of other demographic and individual fitness parameters to food subsidy removal such as sex ratios, reproductive activity, juvenile recruitment, and body mass separately using mixed linear models in PROC MIXED that compared indices of each population parameter over time with replicate plot entered as a random factor and treatment, year, and season entered as fixed factors in a repeated measures framework (SAS Institute 1999). I combined adults and subadults for sex ratios and reproduction indices to distinguish potential breeders from non-breeding juveniles. I calculated sex ratios as the proportion of adult and subadult males to adult and subadult males and females. Reproductive activity was derived separately for males and females. I defined reproductive activity for males as the ratio of reproductively active adult and subadult males to the total number of adult and subadult males. For females, I defined reproductive activity as the ratio of
reproductively active adult and subadult females to the total number of adult and subadult females. I calculated pregnancy as the ratio of visibly pregnant adult and subadult females to all adult and subadult females. I defined juvenile recruitment as the ratio of juveniles to adult and subadult females. Body mass was indexed using the mass at first capture for adult and subadult males. I excluded females from body mass analysis due to difficulty in identifying pregnant females, and I used only body mass measurements taken on the first capture because repeated captures can cause mass declines over time (Pearson et al. 2003).

RESULTS

Centaurea maculosa and Urophora abundance

Centaurea maculosa declined immediately on herbicide-treated plots by 99%, from 57.3 to 0.4 percent cover (Fig. 1). Most of the decline occurred in the 2000 growing season immediately following herbicide application, but the decline continued into 2001 due to delayed mortality of a small percentage of plants. Unfortunately, C. maculosa on control plots also experienced a dramatic decline, concurrent with the herbicide treatment. Centaurea maculosa cover dropped by approximately 64% by 2001 (from 57.4% cover in 1999 to 20.5% cover by 2001), and it remained at this level for the duration of the study (Fig. 1). The decline in C. maculosa cover on control plots was significantly correlated with prior June precipitation ($R^2 = 0.761, F_{1,3} = 9.576, P = 0.054$) suggesting that acute spring drought conditions during the study caused this decline. This is corroborated by other studies showing that the spring drought conditions killed both young and adult C. maculosa plants and dramatically reduced flowering across western Montana particularly in 2000 and 2001 (Ortega et al. 2004, Stanley 2005, Chapter 4).
Urophora densities closely followed patterns of C. maculosa density, with one notable exception (Fig. 2). In 2000, the general decline in Urophora density was buffered by a 28% increase in seedhead densities of Urophora larvae on the control plots that helped to compensate for the drought-induced decline in its host plant. The increased Urophora seedhead density on the control plots appeared to result from increased competition for seedheads among adult Urophora evicted from the removal plots by C. maculosa elimination. By 2001, the Urophora decline on control plots had leveled off 73% below the pre-drought densities. Urophora densities estimated from the linear regression compared well with the data collected in 2001 and 2002 (Fig. 2). Despite the undermining effects of the drought, the herbicide treatment still reduced Urophora densities 40 to 60 fold on the removal plots relative to control plots by 2001 and 2002. This difference was highly significant between treatments ($F_{1,149} = 60.94, P < 0.001$), and there was a significant treatment by year interaction ($F_{3,254} = 14.69, P < 0.001$).

Invertebrate abundance

Pitfall results indicated that the relative abundance of invertebrates available for consumption by P. maniculatus fluctuated over time in a similar manner on the control and removal plots (Fig. 3). The one exception to this was a brief spike in invertebrate abundance on the removal plots in the spring and summer of 2001. This unusual spike was the result of 2 other C. maculosa biological control agents, the knapweed flower weevils (Larinus spp.), appearing in high numbers in removal plot pitfalls as they emerged from the litter in spring and summer to find no host plants. Excluding the weevils, invertebrate abundance differed by year ($F_{3,215} = 2.54, P = 0.057$), but there was no significant difference in invertebrate abundance between
treatments ($F_{1,155} = 0.02, P = 0.899$) or between treatments by year ($F_{3,215} = 0.64, P = 0.593$).

Deer mouse abundance

*Peromyscus maniculatus* dominated the small mammal community at Calf Creek, comprising 98% of the total live and snap trap captures (2852 captures). The next most abundant small mammals were yellow-pine chipmunks (*Tamias amoenus*) and montane voles (*Microtus montanus*) with 1% of the captures each (36 and 34 captures, respectively). Preble’s shrews (*Sorex preblei*) were occasionally captured in invertebrate pitfalls. Thus, there was little likelihood of interspecific interactions within the small mammal community confounding *P. maniculatus* response to treatment.

Despite fluctuations among seasons ($F_{2,17} = 5.96, P = 0.011$) and years ($F_{4,17} = 15.57, P < 0.001$), *P. maniculatus* populations were significantly more abundant on control plots with abundant *Urophora* food resources than on the biocontrol removal plots ($F_{1,17} = 4.48, P = 0.010$; Fig. 4). At the onset of trapping in spring 1999, *P. maniculatus* were in a decline phase that ended in the spring of 2000. This decline was virtually uninterrupted by the 1999 breeding season. Following a trough in abundance that lasted from spring 2000-2001, *P. maniculatus* initiated a long increase phase in spring 2001 that culminated in the summer and fall of 2002 followed by an overwinter crash. Thus, the removal treatment was initiated at the bottom of the trough in the population cycles. Prior to treatment, there was no difference in *P. maniculatus* populations, though mice tended to be somewhat more numerous on the removal plots (Fig. 4). As populations started the increase phase following removal in May 2000, *P. maniculatus* populations on control and removal plots began to
diverge. Populations on the subsidized control plots increased much more dramatically than on unsubsidized removal plots and remained higher through the following population crash and into the next spring. However, the subsidy did not prevent the crash. In general, subsidized control populations were 2 times more abundant during this period than the unsubsidized treatments.

**Survival and movement**

Estimation of the overdispersion parameter indicated the global model fit the data relatively well ($\hat{c} = 1.133 \pm 0.052$). Overall, Models 1 and 2 were strongly supported relative to the other models, based on AIC$_c$ and model weights (Table 1), but the best model (1) was not substantially better than the second best model (Table 1; $\Delta$AIC$_c = 0.24$). Both of these models were over 5-6 times more likely than the next best model considered (Model 3); the remaining models had very little support (weights < 0.004). The top 2 models estimated survival using treatment, season, and drought, and estimated capture probabilities using season and year, but neither model included parameter estimates for movement. The only difference between these two models was that Model 1 treated drought as an additive effect on survival and Model 2 treated drought as a multiplicative or interactive effect. The similar AIC$_c$'s and lack of a significant difference between these models (Table 2; $H_0$: 2c) suggest that drought may have had both an additive and interactive effect with treatment and season on survival. The fact that the third ranked model differed from the first two only in its parameterization of movement, suggests that movement offered some unique contribution to the overall understanding of the system. However, this contribution was relatively minor given the number of additional parameters required based on a comparison of this model to Model 1 (Table 2; $H_0$: 5). Thus, survival
appeared to be important whereas movement was not important for causing the observed differences in *P. maniculatus* populations resulting from removal of the biocontrol food resource.

In testing specific hypotheses regarding the role of the treatment, season, and drought on survival and movement, it becomes clear that drought was a very important factor in the observed outcomes. Treatment alone did not significantly improve the survival parameter (Table 2; H₀: 1a), nor did treatment by season (Table 2; H₀: 1b). However, incorporating drought into the season by treatment interaction as either an additive (Table 2; H₀: 2a) or an interactive (Table 2; H₀: 2b) effect greatly improved the model fit to the data (Table 1). The movement parameter was not improved by adding treatment (Table 2; H₀: 3a), or a treatment by season interaction (Table 2; H₀: 3b), and incorporating drought as an additive (Table 2; H₀: 4a) or interactive (Table 2; H₀: 4b) factor did not improve the model enough to justify incorporating the movement parameter.

Survival probabilities estimated by averaging estimates from models 1 and 2 (Burnham and Anderson 1998) began to increase following removal on both control and removal plots as populations began to increase at this time (Fig. 5). However, this increase appeared to be subdued on the removal plots compared to the control plots, especially during summer. The overall effect was that estimated survival probabilities were consistently higher on the control plots following treatment. The estimated movement probabilities from model 3 showed that movement from the subsidized control to the unsubsidized removal plots began to steadily decline immediately following treatment while the probability of moving from the removal to the control plots began to increase immediately after treatment (Fig. 6). However, the increased movement toward the subsidy faltered when the drought hit.
Reproduction and body mass

All demographic and fitness measurements differed significantly by season ($F > 3.00, P < 0.05$) and year ($F > 3.00, P < 0.05$) except for sex ratios and body mass, which both differed among seasons ($F > 3.65, P < 0.05$) but not among years ($F < 1.50, P > 0.20$). These patterns reflected seasonal and annual variation expected for a seasonally breeding temperate zone small mammal (Fig. 7). There was no evidence that removing biocontrol agents affected sex ratios or any measure of reproductive allocation or reproductive output, including the proportion of reproductively active males and females, the proportion of pregnant females, or juvenile recruitment (Table 3). Body mass of animals also did not differ between treatments (Table 3).

**DISCUSSION**

Research on food limitation in animal populations suggests that food subsidies, whether from allochthonous or autochthonous sources, can significantly influence individual fitness, population size, community composition, and even ecosystem function (Boutin 1990, Spencer et al. 1991, Polis and Hurd 1996, Jefferies 2000, Roemer et al. 2001, Polis et al. 2004, Croll et al. 2005). Food subsidies can directly affect the populations using the subsidies and indirectly affect other populations, communities, and ecosystem functions when subsidized consumers have strong interactions within the system. Thus, the establishment of exotic organisms has the potential to reassemble communities by altering consumer populations, trophic interactions, and food web complexity. However, little work has been done to examine the effects of exotic organisms on consumer populations and consumer interactions in native systems (Roemer et al. 2001, Richman and Lovvorn 2004).
I found that exotic *Urophora* gall flies introduced for the biological control of *C. maculosa* substantially subsidize *P. maniculatus* populations. Within a year following removal of these agents, *P. maniculatus* significantly declined on removal plots relative to controls. Populations remained lower on these plots for 2 years, until the removal effect began to dissipate. Although I used herbicide to remove *Urophora* by eliminating its host plant, there was no significant effect of the treatment on other invertebrate prey of *P. maniculatus*. These invertebrates make up >90% of the *P. maniculatus* diet on the study area in the absence of *Urophora* (D. E. Pearson unpublished data). Furthermore, *P. maniculatus* do not eat *C. maculosa* seeds (Pearson et al. 2000), nor do they appear to utilize the invader in any other way. Thus, the primary effect of the *C. maculosa* removal was the elimination of *Urophora* as a supplemental food source on removal plots. In evaluating the mechanism for the observed differences in *P. maniculatus* populations, I found evidence for higher survival in biocontrol-subsidized populations, but little indication that movement or recruitment played any significant role. Thus, decreased survival appeared to be the key factor driving reductions in *P. maniculatus* populations following removal of the *Urophora* food subsidy.

Food supplementation studies have a number of well-recognized limitations that constrain inferences regarding how food subsidies in natural systems are likely to actually affect population and community dynamics (e.g., Boutin 1990, Galindo-Leal and Krebs 1998). However, food supplementation studies provide >98% of the information on the effects of food limitation (Boutin 1990), because experiments involving removal of natural foods within systems are logistically difficult to accomplish. Thus, there is a need to evaluate the applicability of current understandings of food limitations in the context of more natural ecological settings.
In a seminal review on food limitation in animal populations, Boutin (1990) formulated 2 key hypotheses about the effects of food limitation based on food supplementation studies. These hypotheses were 1) animal populations should increase 1.5 – 2.5 fold in response to food subsidies (originally posed by Gilbert and Krebs 1981), and 2) subsidies generally will increase but not prevent the dynamics of subsidized populations. To my knowledge, these predictions have never been evaluated in the context of long-term removal of natural resources representative of natural temporal and spatial distributions and concentrations of resources within actual communities. The changes in abundance of *P. maniculatus* that I observed in response to removal of the *Urophora* food subsidy fit the first hypothesis well. The difference in *P. maniculatus* populations during the affected period from spring 2001 to spring 2003 (discounting summer 2001 when mice did not appear to differ) ranged from 1.6 to 2.6 fold ($\bar{x} = 1.98$). I also found strong support for the second hypothesis. The relatively long-term nature of this experiment allowed me to observe *P. maniculatus* populations over a period of essentially 2 population cycles that included 2 decline phases, 2 troughs, and 1 increase phase. As a result, I was able to observe the effect of the subsidy on 1 full population cycle. Aside from the approximately 2-fold higher *P. maniculatus* populations on the subsidized control plots, both populations cycled in remarkable synchrony (Fig. 4). Both populations initiated their increase in spring 2001, peaked in summer 2002, and crashed in spring 2003. The key differences were that the increase and decline phases were much steeper and the peak much higher in the presence of the subsidy. These data provide strong support for both of the 2 key hypotheses regarding the effects of food limitation in animal populations.
However, my conclusions regarding the demographic mechanisms driving the changes I observed in *P. maniculatus* populations in response to food removal differ from those drawn from food supplementation studies. From a mechanistic standpoint, the most consistent finding from food supplementation studies has been that immigration is very important in determining the observed population responses (Gilbert and Krebs 1981, Taitt 1981, Boutin 1984, 1990, Law 1995, Lofgren et al. 1996, Prevot-Julliard et al. 1999, Banks and Dickman 2000). However, food supplementation experiments create islands of concentrated resources that can draw in consumers from surrounding areas, and this problem is rarely experimentally controlled (but see Desy and Batzli 1989). Thus, immigration in response to food supplementation may reflect an experimental artifact rather than a treatment effect. This situation is exacerbated by the fact that most food supplementation studies are too short-term (Boutin 1990) to sort out the relative importance of immigration versus survival and recruitment. For example, using radio telemetry, Boutin (1984) showed that snowshoe hares (*Lepus americanus*) immigrating onto treatment grids in response to supplemental feeding eventually returned to their own territories, possibly in response to territoriality exhibited by hares on the feeding areas. To address the problem of immigration, I designed this study specifically to look at issues of movement across treatment boundaries. By estimating movement probabilities across these boundaries in a mark-recapture framework before and after removal treatment, I was able to evaluate the relative contribution of movement to the observed response to treatment. Although there was evidence for greater immigration versus emigration on the subsidized control relative to unsubsidized removal plots (Fig. 6), movement parameters were not included in the two models that best fit the data, indicating that movement was not an important factor determining the differences in *P. maniculatus* populations.
populations in response to subsidy removal. More work is needed to better understand the role of movement in response to food subsidies, but my data suggest that the strong immigration response observed on so many food supplementation studies may be an experimental artifact.

Regarding reproduction, food supplementation studies provide more variable results, but commonly show an increase in reproduction or an increase in allocation of resources toward reproduction (Guyer 1988, Desy and Batzli 1989, Boutin 1990, Cittadino et al. 1994, Schweiger and Boutin 1995, Galindo-Leal and Krebs 1998, Banks and Dickman 2000, Diaz and Alonso 2003). I found no evidence that removing the subsidy changed reproduction or allocation of energy toward reproductive output. Although the *Urophora* food subsidy disappears annually during the peak of the breeding season from June through August, the biocontrol food subsidy carries well into the breeding season, which begins in this area in February for male and March for females (Pearson et al. 2000). Moreover, others have shown through winter supplemental feeding that energy from winter food additions can be allocated to increased reproductive output (e.g., Schweiger and Boutin 1995, Diaz and Alonso 2003). Nonetheless, I saw no indication of changes in body mass, sex ratios, proportions of reproductively active males or females, proportions of pregnant females, or juvenile recruitment rates in response to removal of the food subsidy despite examining this response over several years (Fig. 7).

Survival was the only demographic parameter that differed in response to treatment. Survival was an important parameter in the top 2 mark-recapture models and was also important in the third ranked model (Table 1). Collectively these 3 models garnered 99% of the support from the pool of models tested. Although I anticipated that overwinter survival would be elevated by the food subsidy based on
prior work (Ortega et al. 2004), survival differences between treatments appeared strongest in the breeding season (Fig. 5). This suggests that the availability of the *Urophora* subsidy in spring and fall increases survival of *P. maniculatus* during the breeding season, and the subsidy may be particularly important for increasing survival of young animals in the fall before the onset of winter. This could account for the significantly higher numbers of *P. maniculatus* observed by Ortega et al. (2004) in the fall on *C. maculosa*-invaded versus uninvaded sites. However, the specific role of survival in driving population differences between treatments is difficult to discern, because the drought weakened the treatment effect by 73% almost simultaneously with the removal treatment (Fig. 2). As a result, the drought drew much of the action away from the removal to the point where treatment alone did not significantly affect survival as shown from the comparison of models 5 and 6 (Table 2; H₀ 1a). In contrast, adding drought to the model with treatment and season either as an additive or as an interactive effect resulted in the 2 best-fitting models (Table 2; H₀ 2a, 2b). Thus, drought appeared to reduce the effect of the treatment (the additive or in this case subtractive drought effect; Table 1, model 1), and this effect appeared to differ by treatment (the interactive drought effect; Table 1, model 2) given that the additive drought and interactive drought models, models 1 and 2, could not be differentiated (Table 2; H₀ 2c). This makes intuitive sense, because the drought decreased *Urophora* food subsidies (the additive or subtractive effect), but this only happened on the control plots (interactive or drought by treatment effect), because there were virtually no *Urophora* left on the removal plots. Thus, the drought had a huge impact on this system by reducing moisture inputs and weakening the effect of the *Urophora* food subsidy on mice to such an extent that it nearly overwhelmed the treatment effect. Drought effects altering interactions in long-term ecological studies are well
documented in the western United States (Brown et al. 2001). Despite this undermining effect of the drought, survival clearly differed by treatment when season and drought were factored into the model as indicated by the overall model comparisons that showed treatment was important in both dominant models (Table 1). In fact, survival was the only demographic parameter that explained the observed differences in treatment populations.

Prior and ongoing research in this system suggests that when exotic organisms establish strong interactions with important consumers they can have widespread effects throughout the community by way of trophic interactions (Pearson and Callaway 2003). At this same study site, I found that *P. maniculatus* predation on native plant seeds can reduce seedling emergence and survival, thereby affecting recruitment in native plant populations (Chapter 3). I also showed that *P. maniculatus* density is an important factor determining seed predation impacts, and that *Urophora* food subsidies can increase *P. maniculatus* predation on native seeds by increasing *P. maniculatus* populations. Research elsewhere in western Montana provides strong evidence that *Urophora* food subsidies to *P. maniculatus* populations elevate the prevalence of the deadly Sin Nombre hantavirus, with important implications for human health (Chapter 4). Consumer interactions are an important aspect of the ecology of exotic species that requires further attention.

**Conclusions**

As a large-scale, long-term experimental removal of a natural food resource, this research provides a test of food limitation understandings derived from supplemental feeding studies in the context of understanding autochthonous inputs of exotic species. My experimental results support prior observational studies that
suggest *P. maniculatus* populations double in response to the *Urophora* subsidy (Ortega et al. 2004, Chapter 4). The inability of food subsidies to prevent population cycles suggests that intrinsic density-dependent factors and extrinsic factors such as predation or parasitism (Korpimaki and Krebs 1996, Krebs 1996, Korpimaki and Norrdahl 1998, Hudson et al. 1998) may be more important in driving population fluctuations than food resources. Nonetheless, as I show here (Fig. 4), food subsidies may amplify population highs and buffer population lows, with potentially important implications for population dynamics of the subsidized consumer and its interactions with other organisms in the system. The fact that herbicide treatment of *C. maculosa* can reduce *Urophora* and *P. maniculatus* populations has important implications for managing the undesirable nontarget effects of this biological control agent (Chapters 3 and 4). In particular, the risk of hantavirus infection may be reduced through herbicide treatment of the host plant in cases where *C. maculosa* provides abundant *Urophora* food subsidies to *P. maniculatus* near homes and outbuildings.

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Table 1. Candidate model set. AICc is Akaike information criterion corrected for number of parameters used to test the fit of the model. Delta AICc progressively compares each model to the best fit model with the lowest AICc (model #1). AICc weight indicates the relative likelihood of the model for the given data. K indicates the number of parameters in the model.

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<td>2087.48</td>
<td>14.28</td>
<td>0.0004</td>
<td>14</td>
</tr>
<tr>
<td>12</td>
<td>s(trt<em>seas+drt) ψ(trt</em>seas+drt) p(seas)</td>
<td>2105.30</td>
<td>32.09</td>
<td>0.0000</td>
<td>18</td>
</tr>
<tr>
<td>13</td>
<td>s(trt<em>seas+drt) ψ(trt</em>seas+drt) p(.)</td>
<td>2109.73</td>
<td>36.52</td>
<td>0.0000</td>
<td>17</td>
</tr>
</tbody>
</table>

Notes: s = survival, ψ = movement, p = capture probability, trt = treatment, seas = season, drt = drought, yr = year.
Table 2. Model comparison results. This table provides a verbal statement of each question, shows which models are compared to evaluate each hypothesis, provides sample-size corrected AIC (AICc), the number of parameters in the model (N), and the $\chi^2$, degrees of freedom, and P-values from the likelihood ratio tests for model comparisons.

<table>
<thead>
<tr>
<th>H</th>
<th>Question</th>
<th>Model comparison</th>
<th>AICc</th>
<th>N</th>
<th>$\chi^2$</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>Does treatment alone affect survival?</td>
<td>$s(.) \psi(.) p(seas<em>yr)$ vs $s(trt) \psi(.) p(seas</em>yr)$</td>
<td>2084.20</td>
<td>11</td>
<td>1.86</td>
<td>1</td>
<td>0.173</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$s(trt) \psi(.) p(seas*yr)$</td>
<td>2084.37</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>Does treatment affect survival by season?</td>
<td>$s(trt) \psi(.) p(seas<em>yr)$ vs $s(trt</em>seas) \psi(.) p(seas*yr)$</td>
<td>2084.37</td>
<td>12</td>
<td>0.97</td>
<td>2</td>
<td>0.614</td>
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<tr>
<td></td>
<td></td>
<td>$s(trt<em>seas) \psi(.) p(seas</em>yr)$</td>
<td>2087.48</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2a</td>
<td>Does drought have an additive effect on treatment?</td>
<td>$s(trt<em>seas) \psi(.) p(seas</em>yr)$ vs $s(trt<em>seas+drt) \psi(.) p(seas</em>yr)$</td>
<td>2087.48</td>
<td>14</td>
<td>16.32</td>
<td>1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$s(trt<em>seas+drt) \psi(.) p(seas</em>yr)$</td>
<td>2073.21</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>Does drought interact with the treatment effect?</td>
<td>$s(trt<em>seas) \psi(.) p(seas</em>yr)$ vs $s(trt<em>seas+drt) \psi(.) p(seas</em>yr)$</td>
<td>2087.48</td>
<td>14</td>
<td>22.24</td>
<td>4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$s(trt<em>seas+drt) \psi(.) p(seas</em>yr)$</td>
<td>2073.45</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>Is drought best described as additive or an interaction?</td>
<td>$s(trt<em>seas+drt) \psi(.) p(seas</em>yr)$ vs $s(trt<em>seas+drt) \psi(.) p(seas</em>yr)$</td>
<td>2073.21</td>
<td>15</td>
<td>5.92</td>
<td>3</td>
<td>0.116</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$s(trt<em>seas+drt) \psi(.) p(seas</em>yr)$</td>
<td>2073.45</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3a</td>
<td>Does movement alone affect mouse populations?</td>
<td>$s(.) \psi(.) p(seas<em>yr)$ vs $s(.) \psi(trt) p(seas</em>yr)$</td>
<td>2084.20</td>
<td>11</td>
<td>2.93</td>
<td>1</td>
<td>0.087</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$s(.) \psi(trt) p(seas*yr)$</td>
<td>2083.30</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>Does movement by season affect mouse populations?</td>
<td>$s(.) \psi(trt) p(seas<em>yr)$ vs $s(.) \psi(trt</em>seas) p(seas*yr)$</td>
<td>2083.30</td>
<td>12</td>
<td>0.14</td>
<td>2</td>
<td>0.935</td>
</tr>
<tr>
<td></td>
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<td>$s(.) \psi(trt<em>seas) p(seas</em>yr)$</td>
<td>2087.25</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4a</td>
<td>Does drought have an additive effect on movement?</td>
<td>$s(.) \psi(trt<em>seas) p(seas</em>yr)$ vs $s(.) \psi(trt<em>seas+drt) p(seas</em>yr)$</td>
<td>2087.25</td>
<td>14</td>
<td>2.12</td>
<td>1</td>
<td>0.146</td>
</tr>
<tr>
<td></td>
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<td>$s(.) \psi(trt<em>seas+drt) p(seas</em>yr)$</td>
<td>2087.18</td>
<td>15</td>
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<td></td>
</tr>
<tr>
<td>b</td>
<td>Does drought have an interaction with movement?</td>
<td>$s(.) \psi(trt<em>seas) p(seas</em>yr)$ vs $s(.) \psi(trt<em>seas+drt) p(seas</em>yr)$</td>
<td>2087.25</td>
<td>14</td>
<td>9.04</td>
<td>4</td>
<td>0.060</td>
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<tr>
<td></td>
<td></td>
<td>$s(.) \psi(trt<em>seas+drt) p(seas</em>yr)$</td>
<td>2086.42</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Does movement improve the overall survival model for treatment effects on mice?</td>
<td>$s(trt<em>seas+drt) \psi(.) p(seas</em>yr)$ vs $s(trt<em>seas+drt) \psi(trt</em>seas+drt)$</td>
<td>2073.45</td>
<td>18</td>
<td>2.65</td>
<td>7</td>
<td>0.916</td>
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<tr>
<td></td>
<td></td>
<td>$p(seas*yr)$</td>
<td>2085.28</td>
<td>25</td>
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Notes: $s =$ survival, $\psi =$ movement, $p =$ capture probability, $trt =$ treatment, $seas =$ season, $drt =$ drought.
Table 3. Results from PROC MIXED analysis of sex ratio, reproductive measures, and body mass by treatment (treat) and treatment interactions with year (yr) and season (seas). Least square means are provided with SEs only for the treatment effect.

<table>
<thead>
<tr>
<th>Demographic/fitness measure</th>
<th>Factor</th>
<th>Control</th>
<th>Treatment</th>
<th>F</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex ratio (male/total)</td>
<td>treat</td>
<td>0.49 ±0.03</td>
<td>0.46 ±0.03</td>
<td>1.23</td>
<td>1,106</td>
<td>0.269</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>treat*yr</td>
<td>1.30</td>
<td>4,169</td>
<td>0.274</td>
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<tr>
<td></td>
<td>treat*seas</td>
<td>0.10</td>
<td>2,215</td>
<td>0.905</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion reproductive males</td>
<td>treat</td>
<td>0.70 ±0.02</td>
<td>0.74 ±0.03</td>
<td>2.34</td>
<td>1,93.4</td>
<td>0.129</td>
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<td></td>
<td>treat*yr</td>
<td>1.26</td>
<td>4,128</td>
<td>0.288</td>
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<tr>
<td></td>
<td>treat*seas</td>
<td>0.18</td>
<td>2,192</td>
<td>0.834</td>
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<tr>
<td>Proportion reproductive females</td>
<td>treat</td>
<td>0.51 ±0.03</td>
<td>0.49 ±0.03</td>
<td>0.32</td>
<td>1,111</td>
<td>0.575</td>
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<tr>
<td></td>
<td>treat*yr</td>
<td>1.01</td>
<td>4,171</td>
<td>0.406</td>
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<tr>
<td></td>
<td>treat*seas</td>
<td>0.80</td>
<td>2,200</td>
<td>0.449</td>
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<tr>
<td>Proportion pregnant females</td>
<td>treat</td>
<td>0.21 ±0.03</td>
<td>0.22 ±0.03</td>
<td>0.88</td>
<td>1,123</td>
<td>0.876</td>
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<tr>
<td></td>
<td>treat*yr</td>
<td>0.16</td>
<td>4,174</td>
<td>0.957</td>
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<td></td>
<td>treat*seas</td>
<td>0.35</td>
<td>2,216</td>
<td>0.702</td>
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<tr>
<td>Recruitment</td>
<td>treat</td>
<td>0.24 ±0.04</td>
<td>0.22 ±0.04</td>
<td>0.15</td>
<td>1,106</td>
<td>0.703</td>
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<tr>
<td></td>
<td>treat*yr</td>
<td>1.68</td>
<td>4,156</td>
<td>0.157</td>
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<tr>
<td></td>
<td>treat*seas</td>
<td>0.69</td>
<td>2,202</td>
<td>0.501</td>
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<tr>
<td>Body mass</td>
<td>treat</td>
<td>20.79 ±0.35</td>
<td>20.78 ±0.35</td>
<td>0.00</td>
<td>1,98.8</td>
<td>0.968</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>treat*yr</td>
<td>0.87</td>
<td>4,145</td>
<td>0.484</td>
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<tr>
<td></td>
<td>treat*seas</td>
<td>0.34</td>
<td>2,171</td>
<td>0.711</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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FIG. 1. *Centaurea maculosa* response (± SE) to aerial application of the broadleaf herbicide Tordon®. Herbicide was applied 5 May 2000. The decline in *C. maculosa* on the controls was driven by spring drought conditions. Precipitation inputs from the previous June explained >76% of the variance in *C. maculosa* cover on the controls (R² = 0.761, P = 0.05).

FIG. 2. Change in *Urophora* larvae densities (± SE) in response to herbicide treatment (sprayed treatments) and spring drought (unsprayed controls) from 1999 to 2002 in western Montana. Intensive sampling conducted in 2001 and 2002 provided estimates of actual densities of *Urophora* larvae and percent cover of *C. maculosa* per 0.5 m² that were used to extrapolate *Urophora* larvae densities in 1999 and 2000 based on linear regression. In 2001 and 2002, both the actual density estimates (closed symbols) of *Urophora* and the extrapolated estimates (open symbols) based on linear regression for data pooled over 2001 and 2002 are given for comparison. *Urophora* abundance was not estimated in 2003, because *Urophora* produced in 2003 provide food for mice beginning in fall 2003, which was the end of the study.

FIG. 3. Mean (± SE) abundance of *P. maniculatus* invertebrate food sources from 2000 to 2003 on controls with *Urophora* winter food subsidies and on treatments where food subsidies were removed by herbicide treatment of its host plant. Data represent the 4 most abundance invertebrate orders (Orthoptera, Coleoptera, Lepidoptera, and Arachnida) in the diet of *P. maniculatus* on the study area (D. E. Pearson unpublished data).
Fig. 4. Population estimates for *P. maniculatus* from spring 1999 through fall 2003 on control plots with *Urophora* winter food subsidies present and treatment plots where the food subsidies had been removed by herbicide treatment of their host plant. Treatment was initiated in May 2000.

Fig. 5. Estimates (± SE) of *P. maniculatus* survival probabilities over time on control plots where the *Urophora* food subsidy is present and on treatment plots where the food subsidy has been removed. Survival probabilities are estimated over winter (w) and summer (s) periods. Estimates come from model averaging between the two competing best-fit models, models 1 and 2 (Table 1).

Fig. 6. Estimates (± SE) of *P. maniculatus* movement probabilities over time on control plots where the *Urophora* food subsidy is present and on treatment plots where the food subsidy has been removed. Movement probabilities are estimated within winter (w) and summer (s) periods. Estimates come from the best-fit model for movement, model 3 (Table 1).

Fig. 7. Changes in *P. maniculatus* demographic variables over time on control plots where the *Urophora* food subsidy is present and on treatment plots where the food subsidy has been removed.
**C. maculosa**

---

**Unsprayed**

---

**Sprayed**

---

**Precipitation**

---

**Fig. 1**

Prior June precipitation (cm) vs. C. maculosa cover (%) over the years 1999 to 2003. The graph shows the impact of spraying and precipitation on the cover of C. maculosa.
Fig. 64

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Fig. 3

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Subsidy present

Subsidy removed

P. maniculatus population estimates

- Subsidy present
- Subsidy removed

FIG. 4

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From subsidy to removal
From removal to subsidy

Movement probability

1999 2000 2001 2002 2003

s w s w s w s w

Treatment

Fig. 6

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CHAPTER 3
DOES DEER MOUSE SEED PREDATION INFLUENCE SPOTTED Knapweed
INVASION?

Abstract. Small mammal consumers play important roles in structuring plant communities that may significantly affect the outcome of plant invasions. I examined deer mouse (Peromyscus maniculatus) seed predation on seedling emergence and establishment of a dominant grass, Pseudoroegneria spicata, and a forb, Balsamorhiza sagittata, in the context of Centaurea maculosa (spotted knapweed) invasion in grasslands of the intermountain West. I also studied how herbicide removal of C. maculosa and its gall fly (Urophora spp.) biological control agents, which subsidize P. maniculatus populations, affected P. maniculatus predation on native plant seeds. Peromyscus maniculatus readily took seed of both native plants, but removed significantly more B. sagittata than P. spicata seeds. This seed predation reduced emergence and establishment of both species, but had greater impacts on B. sagittata than P. spicata. Seed predation correlated with P. maniculatus abundance, suggesting that the abundance of this predator largely determines its impacts on native plants. Accordingly, removal of C. maculosa and Urophora reduced P. maniculatus abundance, resulting in reduced seed removal rates. However the strength of the effect of P. maniculatus on seed predation attenuated at the levels of emergence and establishment. Because P. maniculatus avoids consuming C. maculosa seeds, these results suggest that P. maniculatus is an important seed predator that can influence invasion by selectively preying on native plant seeds while avoiding seeds of exotic plants. Most importantly, Urophora
biological control agents may indirectly increase predation on native seeds through a complex form of apparent competition.

Key words: Balsamorhiza sagittata; biological control agents; Centaurea maculosa; deer mice; exotic plants; food subsidies; food-web interactions; indirect effects; nontarget effects; Peromyscus maniculatus; plant-herbivore interactions; Pseudoroegneria spicata; seed predation; Urophora

INTRODUCTION

Exotic plant invasions are a well-recognized threat to native ecosystems around the world (Wilcove et al. 1998, Mack et al. 2000), but management of this problem has been hindered by limited understanding of the ecological processes that drive invasion. The dominant hypothesis proposed to explain successful exotic plant invasions is the enemy release hypothesis (Maron and Vila 2001, Keane and Crawley 2002, Colautti et al 2004), which states that exotic species become invasive by escaping the controlling influence of natural enemies in their native range (Williams 1954). However, enemy release depends not only on the invader escaping natural enemies in its native range; release also depends on the natural enemies encountered by the invader, the biotic resistance, in the introduced range (Elton 1958). Enemy release and biotic resistance remain controversial explanations for the success or failure of invaders (e.g., Loreau et al. 2001, Colautti et al. 2004), but consumer interactions clearly play an important role in invasion (Mitchell and Power 2003, DeWalt et al. 2004, Colautti et al 2004). Yet, despite the prevalence of consumer effects, little work has been done to understand how direct and indirect effects of
consumer interactions in the recipient community may affect the outcome of invasion (Duffy 2002).

Small mammals play important roles in structuring many native plant communities through a variety of interactions (Huntly and Inouye 1988, Brown and Heske 1990, Hulme 1994a, 1994b, 1996, Gutierrez et al. 1997, Ostfeld et al. 1997, Manson et al. 2001, Seabloom and Richards 2003). In particular, selective seed predation and herbivory by small mammals can significantly alter the composition and structure of native plant communities (Brown and Heske 1990, Hulme 1996, Ostfeld et al. 1997, Edwards and Crawley 1999). Given their importance as native consumers, small mammals have substantial potential to influence the invasion of exotic plants into native systems. For example, small mammal consumers that incorporate exotic plants into their diet could serve as a strong form of biotic resistance, whereas the same species may facilitate invasion by rejecting the invader as a novel food source (Manson and Stiles 1998), by consuming less of the invader relative to natives (Vilà and Gimeno 2003), or by dispersing seeds of invaders after consuming but not destroying seeds (Vilà and D’Antonio 1998). Consumer interactions associated with small mammals in invaded communities may play important roles in the process of invasion that need to be considered in order to advance invasion ecology.

In western North America, spotted knapweed (Centaurea maculosa) is an exotic Eurasian forb that aggressively invades grasslands and savannas (Sheley et al. 1998) and dramatically reduces the abundance of many native plant species (Ridenour and Callaway 2001, Ortega and Pearson 2005). In the intermountain grasslands most severely impacted by C. maculosa invasions, deer mice (Peromyscus maniculatus) are the dominant small mammal consumers (Pearson et al. 2000, 2001), and these mice
are aggressive seed predators capable of reducing plant populations (Maron and Simms 1997, 2001). Moreover, *P. maniculatus* readily consume native plant seeds (D. E. Pearson personal observations) but avoid consuming *C. maculosa* seeds (Pearson et al. 2000). Therefore, *P. maniculatus* has the potential to facilitate invasion of *C. maculosa*. Additionally, *P. maniculatus* successfully exploits the larvae of two *C. maculosa* biological control agents *Urophora* spp. that overwinter in galls within its seedheads (Pearson et al. 2000), and this winter food resource serves as a subsidy that can double or triple *P. maniculatus* populations (Ortega et al. 2004, Chapters 2 and 4). Thus, as *C. maculosa* invades, it significantly elevates *P. maniculatus* populations through the indirect effect of the biocontrol agents. This in-turn provides the potential for a form of consumer-mediated apparent competition (Holt 1977) where *C. maculosa* invasion may elevate seed predation on native plants by indirectly increasing mouse populations (Pearson and Callaway 2003).

I examined *P. maniculatus* seed predation and its effects on seedling emergence and seedling establishment for two native plants in intermountain grasslands invaded by *C. maculosa*. My objectives were to: 1) quantify the effect of *P. maniculatus* seed predation on seedling emergence and establishment of two dominant native plant species representing different functional groups (grasses and forbs), 2) evaluate the implications of *P. maniculatus* seed predation on the invasion ecology of *C. maculosa*, and 3) determine whether experimental removal of *C. maculosa* and its *Urophora* parasite alters these interactions.
METHODS

Study area

The study was conducted at the Calf Creek Wildlife Management Area approximately 10 km northeast of Hamilton in the foothills of the Sapphire Mountains in western Montana. The study site is dominated by Palouse-type grasslands (Lynche 1955, Mueggler and Stewart 1980) on rolling hills that are separated by conifer-lined drainages. Average annual precipitation is approximately 32 cm coming mostly as snow during the winter and rain in May and June. Study plots were located in the grasslands and were dominated by *Pseudoroegneria spicata* (bluebunch wheatgrass) and *Koeleria cristata* (June grass), with scattered *Artemisia tridentata* (Great Basin sage). *Balsamorhiza sagittata* (arrowleaf balsamroot) and *Lupinus* species are dominant native forbs. *Centaurea maculosa* now dominates the community averaging >50% cover across the study area.

Overall sampling design

Sampling was conducted at four replicate plots. Plots were selected for homogeneous vegetation conditions and were located 500 to > 1000 m apart. Each plot consisted of three primary transects 220 m long and parallel to each other and to the slope and separated by 50 m (Fig. 1). Herbicide treatment was randomly assigned to half of each plot splitting transects in half, and treatments included large buffer strips 50 to > 500 m on three sides. On 5 May 2000, *C. maculosa* was removed from half of each plot by helicopter spraying of the broadleaf herbicide Tordon® at 1.24 l/ha. *Centaurea maculosa* exhibits a high degree of sensitivity to this herbicide allowing effective removal of this plant with low dosages that minimize impacts on
nontarget native forbs (Rice and Toney 1998). *Urophora* are also removed in this process as they are obligate parasites of *C. maculosa*.

To evaluate seed predation on native species, I chose the forb, *B. sagittata*, and the grass, *P. spicata*, because they are community dominants that produce some of the largest seeds within their corresponding functional groups (seed weight is 0.0024 g for *P. spicata* and 0.0080 g for *B. sagittata*) and therefore should be especially susceptible to small mammal seed predation (e.g., Brown and Heske 1990, Hulme 1994a, Garb et al. 2000). Additionally, these species are two of the natives most significantly negatively impacted by *C. maculosa* invasion (Ortega and Pearson 2005). Seed removal and seedling emergence experiments were conducted only in the post treatment years from 2001 to 2004. However, the current study was part of a larger experiment examining *P. maniculatus* population response to the removal of the *Urophora* food subsidy (Chapter 2). In that study, *C. maculosa* and *P. maniculatus* sampling were conducted beginning in 1999 prior to herbicide treatment, and pretreatment sampling showed no differences between control and treated areas for *C. maculosa* or *P. maniculatus* (Chapter 2). Results from that study are described and referenced in the text where they apply.

**Seed removal**

To quantify relative rates of seed removal of the two plant species in the two herbicide treatments, I offered *B. sagittata* and *P. spicata* seeds in wire cages designed to allow predation by *P. maniculatus*, but exclude all other potential predators. I placed seeds in 57 ml plastic cups set within similar cups that were glued to plywood surfaces (45 x 45 x 0.6 cm) and covered by wire mesh cages (25 cm on sides, 15 cm tall; mesh size 0.5 cm). Cages had 2 small entrances 5 cm wide by 3.5 cm tall.
cm tall cut in all 4 sides to provide mice access, but prevent birds, larger rodents, and other mammals from reaching the offerings. The only other small mammals captured on the study areas during seed experiments were rare Preble’s shrews (*Sorex preblei*), which are insectivores, and montane voles (*Microtus montanus*), which are herbivores that primarily eat plant leaves and shoots and comprised 1% of the captures (Chapter 2). A sticky layer of Tanglefoot® was painted around the bases of the fastened plastic cups to prevent insects from removing seeds. However, neither species produces elaiosomes on their seeds, reducing the potential for myrmecochory. The seed removal cages were located every 30 m along the primary transects with the innermost stations starting 10 m from the treatment boundary (Fig. 1). This resulted in four stations per transect on each side of the treatment boundary and 24 stations per replicate plot.

I conducted seed removal experiments in the spring, summer, and fall of 2001 and 2002. Seed offerings were split into two periods (first and second weeks) with each species of seed randomly assigned to either the first or second period on each plot in each season. Seed offerings were comprised of 10.00 ± 0.01 g dry weight of seeds per station. Dry weight was measured on an electronic triple-beam scale after air drying seeds at approximately 27°C and reweighing seeds daily until seed weights stabilized. Seed offerings were replaced after two days in the cages and offerings and cages were removed on the fourth day. A total of 20.00 g of seed were offered at each station over a four-day period. Pilot studies indicated that such offerings would generally exceed *P. maniculatus* removal rates even at relatively high mouse densities so that residual seeds would remain for collection after each two-day interval. Residual seeds collected from the field were air dried as described above and subtracted from starting weights to determine removal rates. Seed removal rate was
quantified as the percent of seeds removed after summing the two individual samples set out during each sampling period. Data were not included if cages showed signs of disturbance other than mice.

Seedling emergence and establishment

To examine the effect of granivory on *B. sagittata* and *P. spicata* establishment, I set out seeds of each species and compared seedling emergence and establishment rates in treatments with no predation (all small mammals, birds, and insects excluded; seeds added) and only *P. maniculatus* predation (birds, insects, and small mammals larger than *P. maniculatus* excluded; seeds added) while controlling for cage effects and the effects of natural seed rain and seed reserves in the soil (*P. maniculatus* allowed access, but birds, insects, and small mammals larger than *P. maniculatus* excluded, no seeds added). Cages were made of wood frames forming blocks of three cells 45 x 45 x 9 cm covered with a 1-cm mesh screen. Each cell in a cage was randomly assigned to control, no predation, or *P. maniculatus* predation treatments. Cells assigned to *P. maniculatus* predation and control cells were drilled with six 3-cm diam. holes located approximately 4.0 cm from the bottom and evenly spaced on two opposite sides to provide mouse access. Cages were dug into the ground approximately 2 cm. Cages were secured by setting wooden stakes into the ground at the four corners and connecting the stakes with wire across the top of the cage. Tanglefoot® was applied to the outer bottom edge of each cage near ground level to prevent granivorous insects from entering. Cages were set out in June when *B. sagittata* and *P. spicata* naturally disperse seeds, and cages were located ≥1 m from mature plants of either of these species to avoid natural seed rain. One hundred seeds of each species obtained from a commercial distributor within the region (Sunmark
Seeds International, Inc.) were scattered in each predation and no predation cell to provide a known quantity of seeds sufficient to ensure seedling emergence. No seeds were added to the control cell, which allowed me to account for natural seed rain, seed bank, and cage effects. Seedling emergence and establishment cages were set out along four secondary transects running parallel to and 10 m from the primary transects. Six cages were set out 40 m apart along these transects so that three cages were on each side of the herbicide treatment boundary (Fig. 1). The innermost cages were 20 m from the treatment boundary. This resulted in 24 cages per replicate plot.

Cages were checked periodically for seedling emergence starting in March and continuing until seedling emergence ended in April or May. This experiment was first initiated in spring 2001. In spring 2002, all seedlings were quantified and removed and new seeds were added to repeat the experiment. In spring 2003, seedlings were counted, but then left to grow in order to examine establishment into the population in 2004. Establishment was quantified in the spring of 2004 by counting all surviving seedlings at that time. All seedlings were removed at the end of the experiment. Data were excluded for cages that were not fully secure when checked in spring.

Analyses

Seed removal rates were compared using mixed ANOVA models in PROC MIXED (SAS Institute 1999) where replicate plot was treated as a random factor and herbicide treatment, seed type, and season, were treated as fixed factors within a repeated measures framework. In this design, the cage defined the sample unit that was repeated across seasons. Each year was analyzed separately. Seedling emergence rates were not normally distributed, so these data were analyzed with
GENMOD using a Poisson distribution scaled for over-dispersed data (SAS Institute 1999). *Peromyscus maniculatus* treatment (cells with seeds added and *P. maniculatus* access or no access), herbicide treatment, and seed type were treated as fixed factors, replicate plot was treated as a random factor, and the cage control (cage with *P. maniculatus* access but no seeds added to evaluate cage and background seed effects) was included as a covariate. Each year was analyzed separately.

**RESULTS**

*Seed removal*

In 2001, *P. maniculatus* populations were relatively low and did not begin to respond to *C. maculosa* removal until the fall (Chapter 2). During this period, seed removal rates were also relatively low (Fig. 2) and did not differ between the herbicide treatment and the control (*F*<sub>1,91</sub> = 0.00, *P* = 0.981). However, seed removal rates were significantly higher for *B. sagittata* than for *P. spicata* (*F*<sub>1,94</sub> = 318.56, *P* < 0.001) and removal rates showed a strong seasonal trend of increasing seed removal as the season progressed from spring to fall (*F*<sub>2,188</sub> = 122.78, *P* < 0.001; Fig. 2). This seasonal trend of increasing seed removal rates applied to both plant species (Fig. 2), but was much stronger for *B. sagittata* than for *P. spicata* as indicated by the significant seed type x season interaction (*F*<sub>1,176</sub> = 64.98, *P* < 0.001). These patterns were not altered by the herbicide treatment; there was no significant interaction for herbicide treatment x seed type (*F*<sub>1,94</sub> = 1.59, *P* = 0.211), herbicide treatment x season (*F*<sub>2,188</sub> = 2.07, *P* = 0.130), or herbicide treatment x seed type x season (*F*<sub>2,176</sub> = 0.63, *P* = 0.536).

In 2002, when *P. maniculatus* populations were higher and mice declined in response to *C. maculosa* removal (Chapter 1), seed removal rates were significantly
lower on the *C. maculosa* removal plots ($F_{1,91} = 15.11, P < 0.001$; Fig. 2). As in the previous year, there were greater removal rates of *B. sagittata* seeds than *P. spicata* seeds ($F_{1,94} = 436.54, P < 0.001$), and seed removal increased from spring to fall for both species ($F_{2,188} = 19.97, P < 0.001$). However, the seasonal increase in removal rates was weaker for *B. sagittata* than *P. spicata* as reflected by the significant seed type x season interaction ($F_{2,187} = 7.62, P < 0.001$). Relative to *P. spicata*, *B. sagittata* removal started very high and leveled off very quickly. This leveling off of *B. sagittata* seed removal was partly because *P. maniculatus* predation on *B. sagittata* was so intense by summer and fall that mice were emptying seed dishes. I expect that even stronger differences would have been found had mice been offered more *B. sagittata* seeds. As in 2001, none of the observed patterns in seed removal were altered by herbicide treatment as indicated by the lack of significant interactions for herbicide treatment x season ($F_{2,188} = 0.67, P = 0.512$), herbicide treatment x seed type ($F_{1,94} = 0.38, P = 0.539$), and herbicide treatment x seed type x season ($F_{2,187} = 0.41, P = 0.667$).

**Seedling emergence**

*Peromyscus maniculatus* populations were relatively low and had only begun to respond to herbicide treatments at the end of the period when mice had access to the seeds set out in 2001 and emerging in 2002 (Chapter 1). Seedling emergence results in 2002 indicated that *P. maniculatus* access to seeds significantly reduced seedling emergence of both species ($\chi^2 = 9.20, df = 1, P = 0.002$; Fig. 3), but mice had a stronger effect on the larger seeded *B. sagittata* as indicated by the *P. maniculatus* treatment x seed type interaction ($\chi^2 = 7.93, df = 1, P = 0.005$). These patterns arose despite the fact that *P. spicata* seedling emergence was significantly higher than *B.
sagittata in this year ($\chi^2 = 16.18, \text{df} = 1, P < 0.001$; Fig. 3). Herbicide treatment had no effect on seedling emergence rates ($\chi^2 = 1.19, \text{df} = 1, P = 0.276$), and herbicide treatment did not alter P. maniculatus effects on seedling emergence rates; there was no significant P. maniculatus x herbicide treatment interaction ($\chi^2 = 0.76, \text{df} = 1, P = 0.384$) and no significant P. maniculatus treatment x herbicide treatment x seed type interaction ($\chi^2 = 0.78, \text{df} = 2, P = 0.678$). The cage control covariate was significant ($\chi^2 = 6.85, \text{df} = 1, P = 0.009$), but little seedling emergence was observed in the control relative to seed additions.

During the period when P. maniculatus had access to the seeds that ultimately germinated in spring 2003, mouse populations were substantially higher than in the previous year, and P. maniculatus were significantly less abundant on the C. maculosa-removal plots (Chapter 2). As a result, P. maniculatus impacts on seedling emergence in 2003 were much stronger than in 2002 (Fig. 3). Peromyscus maniculatus access to seeds significantly reduced seedling emergence in both species ($\chi^2 = 7.76, \text{df} = 1, P = 0.005$; Fig. 3) with stronger effects on B. sagittata as indicated by the P. maniculatus treatment x seed type interaction ($\chi^2 = 5.91, \text{df} = 1, P = 0.015$). In this year, B. sagittata seedling emergence tended to be higher than P. spicata seedling emergence (Fig. 3) though these differences were not statistically significant ($\chi^2 = 2.10, \text{df} = 1, P = 0.147$), presumably because P. maniculatus reduced B. sagittata seedling emergence and suppressed this effect (Fig. 4). As in 2002, herbicide treatment had no effect on seedling emergence rates ($\chi^2 = 1.41, \text{df} = 1, P = 0.235$), and herbicide treatment did not alter P. maniculatus effects on seedling emergence rates; the interaction between P. maniculatus and herbicide treatment was only marginally significant ($\chi^2 = 3.24, \text{df} = 1, P = 0.072$). Neither was there a significant P. maniculatus treatment x herbicide treatment x seed type interaction ($\chi^2$.
Seedling establishment

Establishment of seedlings from 2003 to 2004 generally followed patterns of seedling emergence in 2003. *Peromyscus maniculatus* access to seeds significantly reduced seedling establishment ($\chi^2 = 12.00$, $df = 1$, $P < 0.001$) with a stronger effect on *B. sagittata* than on *P. spicata* (Fig. 3) as indicated by a *P. maniculatus* treatment x seed type interaction ($\chi^2 = 12.50$, $df = 1$, $P < 0.001$). Seedling establishment did not differ between species ($\chi^2 = 0.87$, $df = 1$, $P = 0.352$), but there was an herbicide treatment effect on establishment ($\chi^2 = 4.23$, $df = 1$, $P = 0.040$). *Centaurea maculosa* removal by herbicide did not alter *P. maniculatus* effects on seedling establishment as indicated by the non-significant *P. maniculatus* x herbicide treatment interaction ($\chi^2 = 0.47$, $df = 1$, $P = 0.491$). The interaction between *P. maniculatus* treatment x seed type x herbicide treatment was not significant ($\chi^2 = 5.2$, $df = 2$, $P = 0.074$). The cage control covariate was not significant ($\chi^2 = 1.87$, $df = 1$, $P = 0.171$), and very little establishment was observed in the control relative to seed additions.

**DISCUSSION**

Small mammal consumers play important roles in structuring native plant communities (Brown and Heske 1990, Ostfeld et al. 1997, Maron and Simms 1997, 2001) that have significant but generally overlooked implications for invasion ecology and management. My results indicate that *P. maniculatus* can have strong effects on native plant establishment through seed predation, and that increased *P. maniculatus*
density in response to food subsidies from biological control agents may have significant indirect effects on native plants. However, the degree to which these indirect effects carry through to the level of plant recruitment may depend on inputs like precipitation that limit productivity within the system.

Seed removal

Seed removal experiments established that \textit{P. maniculatus} are aggressive, but selective, predators of \textit{B. sagittata} and \textit{P. spicata} seeds. Seed removal rates were approximately 2 to 20 times higher for the larger seeded \textit{B. sagittata} than the smaller seeded \textit{P. spicata} (Fig. 2). This selection for larger seeds is consistent with size-dependent seed selection documented for other small mammal seed predators (Mittlebach and Gross 1984, Brown and Heske 1990, Hulme 1994a, Garb et al. 2000) and holds significant implications for the role of \textit{P. maniculatus} in influencing plant community composition in this system (e.g., Brown and Heske 1990).

Seed removal was variable, as reported by other authors (Hulme 1994b, Maron and Simms 1997, Manson and Stiles 1998), but patterns of seed removal tended to correlate with patterns of \textit{P. maniculatus} abundance. Seed removal rates increased across seasons from spring to fall and between years from the first year to the second in accordance with seasonal and annual increases in \textit{P. maniculatus} populations on the study site (Chapter 2). The seasonal increase in seed removal may partly reflect a behavioral shift in foraging as seeds naturally increase in \textit{P. maniculatus} diets from spring to fall concurrent with the seasonal increase in availability of this resource (Johnson 1961, Pearson et al. 2000). However, the strong seasonal increase in \textit{P. maniculatus} abundance (Chapter 2) is likely an important factor driving this trend given the seasonal increase in populations from spring to fall.
in both years. The few studies that have effectively quantified small mammal abundance in conjunction with seed predation experiments have generally shown positive correlations between seed removal rates and small mammal abundance that are consistent with the results presented here (Ostfeld et al. 1997, Kelt et al. 2004, but see Morris 1997). These studies and my results suggest that the intensity of small mammal seed predation is largely a density driven process, indicating that the factors determining *P. maniculatus* density will also determine the intensity of seed predation. True to this expectation, I found that experimental removal of *C. maculosa*, which reduced *P. maniculatus* populations in 2002 (Chapter 2), was associated with significantly lower rates of seed removal. In 2001, when there was no difference in *P. maniculatus* between the herbicide treatment and control, there was also no difference in seed removal (Fig. 2).

**Seedling emergence**

Seed predation may not always translate into population-level effects on plants, because seed predation may be largely compensatory if plants are safe-site limited rather than seed-limited (Crawley 1992, Harper 1977, Maron and Gardner 2000). Therefore, evaluating whether seed removal translates into reductions in plant recruitment is crucial (e.g., Louda 1983, Maron and Simms 2001). By examining seedling emergence and establishment rates under conditions where *P. maniculatus* were permitted or excluded from access to a known number of seeds, I evaluated the effect of seed predation on establishment of *B. sagittata* and *P. spicata* at the level of seedling emergence and seedling establishment.

Seed addition experiments indicated that *P. maniculatus* had significant effects on seedling emergence of both species as indicated by lower emergence rates when
mice were allowed access to seeds (Fig. 3). Additionally, the preference exhibited by
*P. maniculatus* for *B. sagittata* seeds in the seed offering experiments was also
reflected in seedling emergence experiments. *Peromyscus maniculatus* reduced
seedling emergence of *B. sagittata* much more than *P. spicata*. These patterns held
for both years, despite substantial differences in seedling emergence rates for both
species between years. The *P. maniculatus* abundance effect was also reflected in the
seedling emergence results. The effect of *P. maniculatus* was much stronger in the
second year when mouse populations were higher, particularly for *B. sagittata* (Fig.
3). However, the effect of removing *C. maculosa* on *P. maniculatus* seed predation
was attenuated at the level of seedling emergence. The effect of *C. maculosa* removal
on seedling emergence was only marginally significant despite significant differences
in *P. maniculatus* abundance and seed removal between treatments.

*Seedling establishment*

Seedling establishment corresponded with seedling emergence results for the
2002 seed cohort except that for *B. sagittata* there was a shift from higher seedling
emergence on the treatment where *C. maculosa* was removed to higher establishment
on the control (Fig. 3). This suggests that another factor was affecting establishment
of the *B. sagittata* seedlings that escaped mouse predation. Since *C. maculosa*
removal significantly increased plant establishment, it is possible that the shift from
higher seedling emergence on the herbicide treatment to higher establishment on the
herbicide control was due to a direct effect of residual herbicide on seedling
establishment (Fig. 3). However, this seems unlikely given that herbicide did not
significantly affect seedling emergence in either of the 2 previous years. It is possible
that some other unmeasured factor affecting seedling establishment differently by treatment.

*Peromyscus maniculatus in the invasion ecology of Centaurea maculosa*

My experimental results indicate that *P. maniculatus* predation on the seeds of two dominant native plants in this system reduced seedling emergence and seedling establishment for both species. Additionally, predation differed enough between plant species for *P. maniculatus* to influence the relative abundance of these community dominants. These results establish that *P. maniculatus* has the potential to be an important factor in structuring native plant communities in this system, with the capacity to significantly influence plant invasion. Although, I did not evaluate *P. maniculatus* predation on *C. maculosa* seeds in this study, prior work examining stomach contents of *P. maniculatus* in *C. maculosa*-invaded habitats indicates that *P. maniculatus* actually avoids consuming *C. maculosa* seeds; these mice rarely ingest the seeds even when they forage on *Urophora* larvae within *C. maculosa* seedheads (Pearson 1999, Pearson et al. 2000). Thus, *P. maniculatus* may facilitate *C. maculosa* invasion through differential predation on native versus exotic seeds. This situation may be further exacerbated by the fact that as *C. maculosa* invades it also elevates *P. maniculatus* populations by providing food subsidies to mice in the form of biological control agents (Pearson et al. 2000, Ortega et al. 2004, Chapter 4). Given these interaction pathways, *C. maculosa* may indirectly impact native plants through *Urophora* food subsidies to *P. maniculatus* at the same time that it directly impacts them through competition.

The result is a form of tri-trophic or second-order apparent competition (Fig. 4). This interaction pathway, postulated by Pearson and Callaway (2003), can now be
qualitatively parameterized using results from this and other studies. The direct negative effect of *C. maculosa* on most native plants is quite strong (Ridenour and Callaway 2003, Ortega and Pearson 2005), but is reciprocated by a weak negative effect of native plants on *C. maculosa* (e.g., Ridenour and Callaway 2003).

*Centaurea maculosa* has a very strong positive effect on *Urophora* species (Myers and Harris 1980), which in turn have a very weak negative effect on *C. maculosa* (Maddox 1982, Stanley 2005). *Urophora* have a strong positive effect on *P. maniculatus* (Ortega et al. 2004, Chapters 2 and 4), but *P. maniculatus* reciprocate with a weak negative effect on *Urophora* (Stanley 2005). Finally, this study shows that *P. maniculatus* can have strong negative effects on native plants, which presumably provide some positive effect on *P. maniculatus*. Based on the general strengths and directions of these interactions, I hypothesized that removal of *C. maculosa* would reduce *P. maniculatus* seed predation on native plants by reducing *Urophora* food subsidies to *P. maniculatus*.

Removal of *C. maculosa* in 2000 reduced *P. maniculatus* population by fall 2001 (Chapter 2) and this translated to reduced seed removal by *P. maniculatus*, providing support for the hypothesized interaction chain from *C. maculosa* to native plants (Fig. 4). At the level of seedling emergence, this pattern also largely held, but was much weaker. *P. maniculatus* effects on seedling emergence were higher in the second year when mouse populations were higher and there was a trend toward larger mouse effects on the controls where *Urophora* food subsidies maintained higher mouse populations, but this was only marginally significant. At this point other factors appeared to take over as establishment of seedlings from seeds that escaped *P. maniculatus* predation could not be attributed to mice. Thus, the overall effect of *P. maniculatus* on plants attenuated as seed cohorts moved from seeds to seedlings to
first year recruits. Maron and Simms (2001) showed a similar attenuation of seed predation effects for young plants due to compensatory invertebrate mortality, but they showed that despite this compensatory mortality, *P. maniculatus* had large effects on adult populations of *Lupinus arboreus*. Although I did not evaluate the effects of seed predation beyond the first year seedling stage, the observed impacts of seed predation were certainly sufficient to affect adult plant populations despite compensatory survivorship after seedling emergence. Additionally, given that *P. maniculatus* seed predation appeared to be driven by *P. maniculatus* abundance, and given the importance of *Urophora* subsidies to *P. maniculatus* populations (Ortega et al. 2004, Chapters 2 and 4), it seems likely that stronger inputs into the system could increase the strength of the overall interaction chain and compensate for some of the attenuation of the indirect effects on plant establishment. Evidence for this can be seen by examining the drought effects.

*Resource inputs: the drought effect*

Beginning in 2000 an exceedingly dry spring caused a severe reduction in *C. maculosa* and *Urophora* populations across western Montana (Ortega et al. 2004, Stanley 2005, Chapters 2 and 4). These conditions reduced the *Urophora* resource for deer mice to such low levels on *C. maculosa*-invaded sites that *P. maniculatus* populations across western Montana declined to densities equivalent to those on uninvaded, unsubsidized sites (Ortega et al. 2004, Chapter 4). A similar phenomenon was observed at Calf Creek. In 2000 and 2001 *C. maculosa* decreased in the controls (no *C. maculosa* removal) within the study area by approximately 64% resulting in a 73% reduction in *Urophora* (Chapter 2). At many study areas in western Montana, *C. maculosa* began to recover as early as 2001 (Ortega et al. 2004, Chapter 4), but this
was not the case at Calf Creek where dry spring conditions continued to suppress *C. maculosa* into 2003 (Chapter 2). Demographic modeling indicated that the drought was very important in determining the population response of *P. maniculatus* to the treatment on these study sites (Chapter 2). The drought greatly weakened the population response of *P. maniculatus* by reducing the effect of the treatment relative to the controls by 73%. Thus, the drought appears to have functioned as a natural experiment that reduced precipitation inputs into the system. This reduction in precipitation then weakened the interaction chain by reducing *C. maculosa* populations. Reductions in *C. maculosa* populations in turn reduced *Urophora* populations, which reduced *P. maniculatus* populations, which presumably reduced the effects of *P. maniculatus* on seed predation, seedling emergence and seedling establishment given the relationship between *P. maniculatus* abundance and seed predation effects observed in this study. Under normal precipitation inputs, the interaction chain likely would have been much stronger with less attenuation of the effect of mice on seedling emergence and seedling establishment given that the food subsidy was 73% higher prior to the drought. Drought has been reported to alter the nature and strength of community interactions elsewhere in the western United States as well (Brown et al. 2001).

**Conclusions**

This study establishes that *P. maniculatus* are capable of reducing establishment of dominant native plants in this system through seed predation. Moreover, through selectivity in seed predation, *P. maniculatus* are capable of influencing the relative abundance of these species. These results reinforce prior conclusions that small mammal consumers play important roles in structuring plant
communities (Brown and Heske 1990, Gutierrez et al. 1997, Edwards and Crawley 1999, Hulme and Hunt 1999, Ostfeld et al. 1997, Manson et al. 2001). They also suggest that *P. maniculatus* may play an important role as a consumer in the invasion ecology of this system. Not only are these mice capable of suppressing establishment in native species, they also avoid consuming seeds of *C. maculosa*. This sets up a situation where *P. maniculatus* may facilitate *C. maculosa* invasion by preying more on native than exotic seeds. Additionally, because *C. maculosa* indirectly increases *P. maniculatus* populations through food subsidies from its biological control agents (Ortega et al. 2004, Chapters 2 and 4), *C. maculosa* likely benefits indirectly from a form of second-order apparent competition by increasing *P. maniculatus* predation on native seeds. This second-order apparent competition has important implications for biological control (Pearson and Callaway 2003, 2005, Chapter 4). Host-specificity screening in weed biological control is intended to prevent nontarget effects associated with apparent competition by preventing the biological control agent from directly attacking nontarget species (McEvoy 1996, Hajek 2004). However, as this study shows, even if a biological control agent remains host-specific, if it is eaten by another organism that in turn attacks potential plant competitors, the control agent can still impact nontarget plants through this more indirect form of apparent competition. Other management strategies for invasive plants can also affect biotic interactions important to invasion in the recipient community. Herbicide treatment of *C. maculosa* did not change the nature of the plant consumer interactions, but it did affect the strength of those interactions. Herbicide reduced the rate of *P. maniculatus* removal of native plant seeds and tended toward reducing seedling emergence rates, but the effect on seedling emergence was not significant, possibly due to a drought which decreased the general strength of this interaction chain in the system. Better
understandings of the biotic and abiotic interactions important to invasion and the effects of management efforts in altering these interactions is critical to addressing the threat of exotic plant invasions.

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Fig. 1. General study design. Vertical line indicates treatment boundary. Crosshatching indicates herbicide treatment of study plot and variable buffer zone on three sides of treatment (buffers range from 50 to >500 m). Treatment sides of plots were randomly assigned. Seed removal cages begin 10 m from the treatment boundary and are separated by 30 m thereafter. Seedling emergence and recruitment cages start approximately 20 m from the treatment boundary and are separated by 40 m. Seed removal cages are located on the primary transects that are spaced 50 m apart and seedling emergence and recruitment transects are on secondary transects that are 10 m from the primary transects. Symbols for cages are oversized relative to plot scaling.

Fig. 2. Mean percentage (±SE) of *P. spicata* and *B. sagittata* seeds removed from cups by *P. maniculatus* in spring, summer, and fall of 2001 and 2002 in the presence and absence of *C. maculosa* and its parasitic *Urophora* gall flies that provide food subsidies to *P. maniculatus*. Herbicide application on treatments in 2000 removed *C. maculosa* and *Urophora*. *Peromyscus maniculatus* populations began to decline significantly on the removal treatments in the fall of 2001, and they were significantly lower on unsubsidized treatments all through 2003 (Chapter 2).

Fig. 3. Mean number (±SE) of *P. spicata* and *B. sagittata* seedlings that germinated in 2002 and 2003 and recruited to first year seedlings in 2004 in the presence and absence of *P. maniculatus* predation and in the presence and absence of *C. maculosa* and its parasitic *Urophora* gall flies that provide food subsidies to *P. maniculatus*. Herbicide application on treatments in 2000 removed *C. maculosa* and *Urophora*. *Peromyscus maniculatus* populations were not significantly lower on *C. maculosa*...
removal treatments during the period that seeds germinating in 2002 were out, but they were significantly lower on *C. maculosa* removal treatments during the period when seeds germinating in 2003 were out. The scales differ between seedling emergence (2002 and 2003) and seedling recruitment (2004). Data presented are not transformed.

**Fig. 4.** Community interaction diagram showing direct and indirect interactions between spotted knapweed, gall flies, deer mice, and native plants. Arrows indicate direction of interactions and arrow weight indicates the relative strength of the interactions. Signs indicate whether interaction is positive or negative. Interactions were parameterized as described in the text.
Seed offering cage

Seed germination and recruitment cage

FIG. 1
Seed removal (%)

P. spicata

B. sagittata

Season

FIG. 2

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C. maculosa removal

Fig. 3
Deer mice

Gall flies

Knapweed

Native plants

Fig. 4
CHAPTER 4

BIOLOGICAL CONTROL AGENTS ELEVATE DEADLY HANTA VIRUS

BY FEEDING MICE

Abstract. Exotic plant invasions threaten the biological diversity of natural ecosystems around the world (Wilcove et al. 1998). Classical biological control, the introduction of exotic organisms to control exotic invasive species, is a promising strategy that has proven effective at controlling exotic pests once they become widely established in natural ecosystems (McFadyen 1998). However, the introduction of exotic organisms for biological control entails risks to nontarget species (Harris 1988, Simberloff and Stiling 1996, Louda et al. 1997, Strong and Pemberton 2000, Henneman and Memmott 2001, McEvoy and Coombs 2001). For example, control agents with broad host ranges sometimes attack native species causing deleterious nontarget effects (Simberloff and Stiling 1996, Louda et al. 1997, Stiling 2002). To reduce this threat, rigorous screening for host-specificity is conducted before introduction of weed biological control agents (McEvoy 1996, Pemberton 2000). However, this does not prevent control agents from indirectly impacting nontarget organisms through food web interactions (Holt and Hochberg 2001, Pearson and Callaway 2003). I demonstrate that two host-specific biological control agents (Urophora spp.), widely established across western North America to control spotted knapweed (Centaurea maculosa), indirectly increase the incidence of a deadly hantavirus by providing food subsidies to native rodent populations. Host specificity alone does not ensure safe biological control. Biological control agents must suppress
pest populations enough to reduce their own numbers in order to minimize indirect risks to nontarget species.

INTRODUCTION

The gall flies *Urophora affinis* and *U. quadrifaciata* were first introduced into North America in the early 1970s as biological control agents for spotted (*Centaurea maculosa*) and diffuse knapweed (*C. diffusa*) (Harris 1980a), exotic forbs that invade arid habitats of western North America and displace native species (Shelely et al. 1998, Ortega and Pearson 2005). Adult *Urophora* lay eggs within immature flowerheads of *Centaurea* where the larvae induce gall formation that reduces seed production (Harris 1980a). The larvae over winter within the seedheads from September to June, then emerge as adults and repeat the cycle. *Urophora* have remained host specific since their introduction over 30 years ago and have been shown to substantially reduce seed production in the two *Centaureas* (Harris 1980b). However, seed reductions have not effectively controlled these weeds (Maddox 1982), which continue to spread and increase in abundance. As a result, *Urophora* now infest *C. maculosa* and *C. diffusa* populations across western North America and have become as superabundant as their prolific hosts, occurring at densities many times greater than in their native Europe (Myers and Harris 1980).

The abundance and availability of *Urophora* larvae during fall, winter, and spring make them a valuable food resource for native consumers. Deer mice (*Peromyscus maniculatus*) readily exploit this novel food source within *C. maculosa*-invaded grasslands by switching microhabitats and shifting their diet to utilize the seasonally available larvae (Pearson et al. 2000). *Urophora* larvae now make up 85% of the deer mouse diet during key winter months when these mice typically
experience a population decline associated with scarce native food resources (Pearson et al. 2000). This food subsidy has increased over-winter survival of mice and doubled deer mouse populations in *C. maculosa*-invaded habitats (Ortega et al. 2004).

The direct effects of *Urophora* food subsidies on deer mouse populations may translate into indirect effects on other organisms through food-web interactions (Pearson and Callaway 2003). Deer mice are the primary reservoir for the Sin Nombre virus (SNV; Childs et al. 1994), which causes the deadly hantavirus pulmonary syndrome (HPS) in humans. Thus, food subsides that elevate deer mouse populations may increase the prevalence of SNV and elevate the risk of contracting HPS.

To test the hypothesis that *Urophora* indirectly increase SNV prevalence through food subsidies to deer mice (food subsidy hypothesis), I compared deer mouse abundance and SNV seroprevalence between deer mouse populations in grasslands with high and low *C. maculosa* abundance for three years at eight replicate sites across western Montana, USA. At each site, I sampled mice in plots with high (> 20% cover) or low (< 5% cover) *C. maculosa* abundance that were similar in topography and composition of native vegetation. Increases in *C. maculosa* in the low abundance plots during the study indicate that the original differences in *C. maculosa* abundance were due to the timing of invasion rather than underlying abiotic or biotic factors. *Centaurea maculosa* abundance is a good surrogate for *Urophora* abundance because *Urophora* are obligate parasites significantly correlated with *C. maculosa* (Fig. 1). Deer mice are linked to *C. maculosa* only by feeding on *Urophora* larvae. Mice do not forage on *C. maculosa* tissues or seeds, and they avoid *C. maculosa* when *Urophora* are not in the seedheads (Pearson et al. 2000, Ortega et al. 2004).
To quantify the direct effects of *Urophora* on deer mouse populations, I live-trapped and marked mice in the springs of 2001, 2002 and 2003 on plots with high and low *C. maculosa* abundance at each of the eight study sites. By combining these results with those of a spatially independent but temporally overlapping study previously conducted in western Montana (Ortega et al. 2004), I obtained a five-year record that shows that deer mouse populations closely tracked the abundance of *Urophora* and *C. maculosa* as predicted by the food subsidy hypothesis. Deer mice were two times more abundant in stands with high versus low *C. maculosa* density in 1999, 2000, 2002, and 2003 (Fig. 2), years preceded by normal precipitation that produced abundant reproductive *C. maculosa* and *Urophora* (Fig. 1). In contrast, deer mouse abundance did not differ between stands with low versus high *C. maculosa* density in 2001 (Fig. 2), a year preceded by exceptional spring drought that reduced the density of *C. maculosa* flowering stalks, and by extrapolation *Urophora* larvae, by 69% compared with other years (Fig. 1). Because *Urophora* increase over-winter survival of mice (Ortega et al. 2004), this reduction in mouse populations in 2001 in response to reduced *Urophora* production in 2000 is consistent with the food subsidy hypothesis. This pattern in 2001 is corroborated by both studies despite their spatial independence and inherent differences in deer mouse densities. Because site effects were not experimentally controlled in these studies, I conducted another 6-year study in west-central Montana that compared deer mouse response to experimental removal of *C. maculosa* and *Urophora* using herbicide treatments targeting *C. maculosa*. Deer mice declined in response to *C. maculosa* and *Urophora* removal as predicted by the food subsidy hypothesis (Fig. 3).

To determine how *Urophora* food subsidies to deer mice might influence the incidence of SNV in mouse populations, I drew blood from mice captured in the
springs of 2001, 2002, and 2003 and tested it for hantavirus antibodies (Feldmann et al. 1993). Abundance of SNV-positive mice closely tracked deer mouse abundance (compare Fig. 4a with 2b), indicating that *Urophora* food subsidies indirectly increased SNV by increasing host populations. In 2002 and 2003, years following normal precipitation that produced abundant *Centaurea* and *Urophora*, there were approximately three times more deer mice that tested positive for hantavirus antibodies at sites with high versus low *C. maculosa* abundance (Fig. 4a). In 2001, following the spring drought that reduced *Centaurea* and *Urophora*, these differences were greatly reduced.

The greater abundance of seropositive mice in heavily-invaded grasslands is primarily attributable to higher mouse numbers. However, the difference in the relative abundance of mice between grasslands with high versus low *C. maculosa* (two-fold difference; Fig. 2b) does not fully account for the difference in the relative abundance of seropositive mice (three-fold difference; Fig. 4a). This suggests that the rate of hantavirus transmission among deer mice is also higher in *Urophora*-subsidized mouse populations. This observation is supported by the fact that the proportion of seropositive mice is consistently higher at sites with high versus low *C. maculosa* densities (Fig. 4b). Although these differences are not statistically significant (*P* = 0.065; Fig. 4b), the results are conservatively biased because no mice were captured on 13-25% of the grids with low *C. maculosa* abundance, a result consistent with the food subsidy hypothesis, but one that conservatively biased these data, because seroprevalence is undefined when no mice are present (see Notes).

Thus, my results suggest that *Urophora* food subsidies may increase the incidence of SNV in mouse populations not only by increasing deer mouse populations directly but also by increasing transmission rates among mice within elevated populations.
In North America, SNV is the primary etiological agent of HPS, a deadly zoonotic disease that infects humans annually with a 37% fatality rate (Mills et al. 2002). Current understanding of the epidemiology of HPS in the southwestern USA where the disease first emerged is based on the hypothesis that increased moisture from El Niño Southern Oscillation events releases deer mice and other rodent populations from food limitations (Yates et al. 2002). This results in increased deer mouse populations followed by elevated SNV prevalence and ultimately outbreaks of HPS in humans (Yates et al. 2002). Thus, the current understanding of HPS epidemiology is based on the hypothesis that food-limited deer mouse populations, when released by increased food resources, can lead to elevated SNV and additional cases of HPS. My results support this hypothesis by showing that food subsidies from biological control agents can augment food-limited deer mouse populations and elevate SNV prevalence. The drought effects emphasize this by showing that precipitation inputs control the food resources that drive this system. The fact that *C. maculosa* is not common in the southwestern U.S. indicates that *Urophora* species were not associated with the initial emergence of HPS in 1993. Nonetheless, the widespread and overlapping distributions of *C. maculosa*, *Urophora*, deer mice, and SNV (Sheley et al. 1998, Mills et al. 2002) suggest that *Urophora* food subsidies have the potential to increase SNV over a large region of northwestern USA and southwestern Canada where *C. maculosa* is abundant.

Destabilization of the equilibrium state of a disease’s ecology can lead to new emerging infectious diseases (Daszak et al. 2000). Lyme disease in the northeastern USA is a serious disease associated with a *Peromyscus* rodent that emerged from human disruption of the disease’s natural ecology (Allen et al. 2003). Widespread increases in populations of rodents like deer mice, which are reservoirs for HPS and
other zoonoses such as plague (Gage et al. 1995), holds potentially serious implications for human health with regard not only to the current state of HPS, but also as it relates to the potential for a new emergence of this and other diseases.

Our results suggest that although host-specificity of biocontrol agents is necessary, it is not sufficient to ensure the safety of exotic organisms introduced for biological control. Biological control agents that establish, but fail to control their target species can become superabundant, thereby increasing the risk to nontarget species (Holt and Hochberg 2001, Pearson and Callaway 2003). If a biological control agent effectively controls its target species and remains host-specific, it will minimize risks to nontarget species by reducing its own populations (Holt and Hochberg 2001, Pearson and Callaway 2003). Efficacy may be as important as host-specificity for safe and effective biological control.

METHODS

Mouse sampling

Eight study sites were located in western Montana within grassland habitats dominated by *Pseudoroegneria spicata* and *Festuca scabrella* and spanning a region 170 by 80 km. Three 90 by 90 m grids were set out at each replicate site with one grid placed in an area with very low *C. maculosa* abundance (mean cover < 5%) and two grids placed in areas with high *C. maculosa* abundance (mean cover > 20%). Grids were placed in areas similar in topography and vegetation composition. Each grid on a site was separated from the others by ≥200 m and sampled simultaneously during each four-day trapping period. Different sites were trapped sequentially, with the sites at low elevations sampled first so sites were sampled at similar phenological
stages. Each site was sampled at the same time each year in spring (April to May). Spring was the focus because *Urophora* food subsidies are most likely to affect SNV through increased over-winter survival of mice (Ortega et al. 2004) that reduces the risk that mouse populations and SNV will be locally extirpated (Abramson et al. 2003) and facilitates the horizontal transmission of the virus through higher over-winter mouse densities and aggregations of mice in *C. maculosa* stands (Pearson et al. 2000, Ortega et al. 2004). Spring is also significant for SNV transmission since, SNV seasonally peaks in spring (Douglass et al. 2001) and HPS cases seasonally increase at this time (Mills et al. 2002). One Sherman live trap was placed at each of 100 sampling stations at 10-m spacing and checked in the morning before 1100 hr each day for four days. Trapped small mammals were identified, ear tagged, and their sex, mass, reproductive condition and age was determined prior to release at the trap station. Only adult animals were used in the analyses to focus on over-wintered mice and control for the fact that sites trapped last in the rotation began to show young-of-the-year animals, whereas sites trapped early were made up only of over-wintered adults. Over-wintered adults were defined as animals \( \geq 16.5 \) g based on the split in the bimodal distribution of masses between juveniles and adults. Analyses are based on 694 over-wintered mice, 541 on high *C. maculosa* grids and 153 on low *C. maculosa* grids.

**Hantavirus sampling**

Blood samples were taken from each mouse upon first capture during each trapping period (Douglass et al. 2001). Blood samples were tested for hantavirus antibodies at the Montana Public Health Laboratory, Helena, Montana using the enzyme-linked immunosorbent assay method (Feldmann et al. 1993). Titer counts
≥1:400 were classified as seropositive for hantavirus. Testing positive for antibodies indicates that mice have been exposed to or are currently infected with hantavirus. Seroprevalence, was defined as the total number of mice testing positive for hantavirus antibodies divided by the total number of mice testing positive or negative. All surfaces and handling equipment were disinfected after each mouse was handled and traps were disinfected between captures to ensure that hantavirus was not transmitted among mice or study sites.

*Centaurea and Urophora removal experiment*

This study was located in *P. spicata*- and *Artemisia tridentata*-dominated grasslands at Calf Creek Wildlife Management Area in west-central Montana. Trapping protocols followed those described above, except that 22 Sherman live traps were set at 10 m intervals along three transects spaced 50 m apart. Sampling was replicated on four plots, and trapping was conducted each spring in late April from 1999 to 2004. On 5 May 2000, the broadleaf herbicide, Tordon® was applied by helicopter spraying at 1.24 l/ha to remove *C. maculosa* and its *Urophora* parasites. This treatment split each plot in half with three transects of 11 traps on each side.

*Centaurea stem and Urophora larval density estimation*

To address what appeared to be a significant negative effect of the 2000 drought on *C. maculosa* and therefore *Urophora* densities, I estimated densities of knapweed stems back to 1999 (the pre-drought period) on two study sites by counting old stems from 1999 and 2000 in the spring of 2001. This was done by assigning fresh stems from the previous fall, notable by their light tan colour, to the 2000
growing season and assigning older stems, notable by their dark grey colour, to the 1999 growing season. In 2001 and 2002, stems were sampled at the end of the growing season in the fall each year. Additionally, in 2001 and 2002, the number of stems and seedheads per stem were counted in 0.5-m quadrat frames systematically located at 33 trap stations across each grid, and the number of *Urophora* larvae were estimated per seedhead by dissecting 20 random seedheads from each station. Linear regression was used to estimate the density of *Urophora* larvae as a function of *C. maculosa* stem density using the equation $y = 3.44x - 6.48$ based on a significant linear correlation between *Urophora* larvae and *C. maculosa* stems counted within these 0.5 m$^2$ quadrats ($R^2 = 0.47$, $F = 144.86$, df = 1, 163 $P < 0.001$).

**Notes**

In 2001 and 2002 no mice were captured on 25% of the low *C. maculosa* invasion grids, and in 2003 no mice were captured on 13% of the low *C. maculosa* invasion grids. Low mouse populations are common early in the spring and these can approach or reach zero on sites with poor conditions for over-winter survival. The lack of mice captured in the spring of these years on only the low *C. maculosa* abundance grids is consistent with expectations based on the food subsidy hypothesis, but this situation precludes estimation of seroprevalence because seroprevalence is defined as the number of seropositive mice divided by the number of mice tested. Since no mice are tested when none are captured, this results in a division by zero, which is undefined. However, evidence suggests that there was in fact no SNV on these sites and seroprevalence would have been estimated as zero if any mice had been captured, rendering this test highly significant. On one low *C. maculosa*-abundance site, mice were captured in other years, but never tested positive for SNV, suggesting SNV would also be zero in this particular year. On another site, no mice
were ever captured over the three year period on the low *C. maculosa*-abundance site and no mice captured on the high *C. maculosa*-abundance sites at this replicate location ever tested positive for SNV, suggesting that SNV prevalence was zero on the low invasion site at this replicate in all years.

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LITERATURE CITED


Fig. 1. Mean (± SE) density of *C. maculosa* stems from 1999 through 2002 for two sites in western Montana, USA with high and low *C. maculosa* density. Corresponding *Urophora* densities were estimated on the right axis using linear regression for the relationship between larvae and stems (see Methods). Not all error bars show.

Fig. 2. Mean (± SE) numbers of deer mice captured on plots with high and low *C. maculosa* abundances for two spatially independent but temporally overlapping studies in western Montana. (a) is from Ortega et al. (2004). (b) is from this study. Analyses for this study indicate *C. maculosa* abundance ($F_{1,16.4} = 6.67, P = 0.020$), year ($F_{2,44.9} = 13.69, P < 0.001$), and year by *C. maculosa* abundance interactions ($F_{2,44.9} = 6.68, P = 0.003$) are significant. Scales on left and right axes reflect differences in site productivity and sampling methodologies between studies.

Fig. 3. Mean (± SE) abundance of deer mice on four plots in west-central Montana before and after herbicide treatment removed *C. maculosa* and *Urophora* larvae. Before treatment, *C. maculosa* and *Urophora* were equally abundant, and deer mouse populations did not differ between treatments ($F_{1,11} = 0.02, P = 0.898$) or among years by treatment ($F_{1,22} = 0.78, P = 0.781$), though relative abundance of mice differed between years ($F_{1,22} = 66.88, P < 0.001$). After treatment, mice declined 50% on the treatments, but not on untreated controls ($F_{1,19.5} = 9.51, P = 0.006$) despite differences across years ($F_{3,61.2} = 15.86, P < 0.001$). Strength of the treatment effect differed across years ($F_{3,61.2} = 2.89, P = 0.043$) as mice fluctuated. (a) shows overall effects presented as least square means (±SE) pooled across years, and (b) shows least square means (±SE) by year.
Fig. 4. Mean (± SE) for (a) abundance and (b) proportion of seropositive deer mice captured from 2001 to 2003 on grids with high versus low *C. maculosa* abundance. Abundance of seropositive mice was greater on high versus low *C. maculosa* sites ($F_{1,20.4} = 4.40, P = 0.049$), but year ($F_{2,45.9} = 1.60, P = 0.214$) and year by *C. maculosa* interaction ($F_{2,45.9} = 0.70, P = 0.502$) were not significant. Proportion of seropositive mice was generally greater on high versus low *C. maculosa* sites, but not significantly ($F_{1,17} = 3.88, P = 0.065$). Year ($F_{2,37.9} = 0.41, P = 0.666$) and year by *C. maculosa* interactions ($F_{2,37.9} = 0.40, P = 0.674$) were not significant.
FIG 1

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Deer mouse abundance

Fig 2
Figure 3

(a) Deer mouse abundance comparison between sprayed and unsprayed areas pre-treatment and post-treatment.

(b) Deer mouse abundance over years 1999 to 2004.
a

![Graph showing the abundance of seropositive mice in High and Low Centaurea over years 2001 to 2003.](image)

- High Centaurea
- Low Centaurea

b

![Graph showing the proportion of seropositive mice over years 2001 to 2003.](image)

- 2001
- 2002
- 2003

**FIG 4**

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CHAPTER 5

INDIRECT NONTARGET EFFECTS OF HOST-SPECIFIC BIOLOGICAL CONTROL AGENTS: IMPLICATIONS FOR BIOLOGICAL CONTROL

Abstract. Classical biological control of weeds currently operates under the assumption that biological control agents are safe (i.e., low risk) if they do not directly attack nontarget species. However, recent studies indicate that even highly host-specific biological control agents can impact nontarget species through indirect effects. This finding has profound implications for biological control. To better understand the causes of these interactions and their implications, I evaluate recent case studies of indirect nontarget effects of biological control agents in the context of theoretical work in community ecology. I find that although particular indirect nontarget effects are extremely difficult to predict, all indirect nontarget effects of host-specific biological control agents derive from the nature and strength of the interaction between the biological control agent and the pest. Additionally, recent theoretical work suggests that the degree of impact of a biological control agent on nontarget species is proportional to the agent’s abundance, which will be highest for moderately successful control agents. Therefore, the key to safeguarding against indirect nontarget effects of host-specific biological control agents is to ensure the biological control agents are not only host-specific, but also efficacious. Biological control agents that greatly reduce their target species while remaining host-specific will reduce their own populations through density-dependent feedbacks that minimize risks to nontarget species.
**Keywords:** Biological control; Nontarget effects; Host-specificity; Indirect effects; Efficacy; Natural enemies; Multiple release approach; Lottery approach; *Peromyscus maniculatus; Centaurea maculosa; Agapeta zoegana*

**INTRODUCTION**

Classical biological control is based on the enemy release hypothesis. This hypothesis states that exotic species become pests in new environments by escaping the influence of those natural enemies that suppressed their populations in their native range (Crawley, 1997; Keane and Crawley, 2002). Thus, the strategy behind classical biological control is to reestablish top-down control by reintroducing the natural enemies of the pest into its new range. This has been the conceptual underpinning of classical biological control for over a hundred years and it continues to be today (Hajek, 2004; Van Driesche and Bellows, 1996). Although a variety of natural enemies may help control a pest in its native range, not all potentially effective natural enemies will serve as safe biological control agents in a pest’s new environment. In particular, natural enemies with broad host ranges are unlikely to provide the surgical precision we desire in biological control, because they may attack important nontarget organisms in the new environment and become exotic pests in their own right (Follett and Duan, 2000; Harris, 1990; Howarth, 1991; Louda et al., 1997; Simberloff and Stiling, 1996; Wajnberg et al., 2001). As a result, biological control programs emphasize host specificity in selecting agents for introduction to avoid these undesirable nontarget effects. The outcome has been that biological control operates under the assumption that nontarget effects arise only when biological control agents directly attack nontarget species, or conversely that host-specific biological control agents are safe (I define safe as low risk or safe enough for introduction).
Although the importance of host specificity for the safety of biological control should not be understated (e.g., Louda et al., 1997), perhaps it has been overstated (e.g., Hoddle, 2004a). The emphasis on host specificity has diverted attention from other potential sources of risk to nontarget species that has contributed, at least in part, to certain biocontrol strategies like the “lottery approach” (Myers, 1985) which may unnecessarily elevate nontarget risk, especially indirect nontarget risk. The lottery approach is a multiple release strategy in classical biological control that promotes the deployment of multiple host-specific biological control agents for each target pest (Hokkanen and Pimentel, 1984; McEvoy and Coombs, 2000; Myers, 1985). This approach places great emphasis on host specificity of individual agents, but does not weigh efficacy as heavily in this process (McEvoy and Coombs, 2000; Sheppard, 2003). This lack of emphasis on efficacy derives from the assumption that the most effective agent or combination of agents will emerge from the milieu of introductions. The biological control of spotted knapweed (*Centaurea maculosa* Lam.) provides a classic example of the lottery approach. Thirteen species of biological control agents have been introduced for the control of spotted knapweed (Lang et al., 2000), and the pool of agents that are sufficiently host specific to warrant introduction may be exhausted (Müller-Shärer and Schroeder, 1993). Thus, the entire suite of host-specific biological control agents may have been introduced for this weed. Although there is currently little indication of successful control of spotted knapweed (Maddox, 1982; Müller-Shärer and Schroeder, 1993), in other cases where the lottery approach has been successful, it is often only one or two of several released agents that end up ultimately effecting control (Denoth et al., 2002; Forno and Julien, 2000; McFadyen, 2003; Myers, 1985). For example, in the classical success story of klamath weed
(Hypericum perforatum L.), three agents were introduced, but success was attributed to only one of these (Huffaker and Kennett, 1959).

The lottery approach is only one of several multiple-release strategies in biological control (Harris, 1991; Sheppard, 2003), but it is the one that has been most criticized because relative to other multiple-release approaches it depends the most on chance and the least on explicit knowledge of community interactions in the introduction of multiple biological control agents for each target weed (McEvoy and Coombs, 1999, 2000; Myers, 1985; Myers et al., 1989; Sheppard 2003; Strong and Pemberton, 2000). The result of multiple-release strategies in general and the lottery approach in particular is that exotic organisms intentionally introduced for classical biological control exceed the number of exotic pests targeted for control (Hokkanen and Pimentel, 1984; McEvoy and Coombs, 1999; Myers, 1985). Although the introduction of any individual agent, will present some risk to nontarget species, the degree of risk will increase with increasing numbers of agents. If host specificity does not sufficiently ensure the safety of biological control agents, multiple-release strategies like the lottery approach that emphasize numbers of agents over agent efficacy may present undue risks toward nontarget species. Here, I apply recent advances in community ecology theory to two recent case studies of community interactions in biological control to evaluate the implications of indirect nontarget effects of host-specific biological control agents for the practice of biological control.

THEORY ADDRESSING NONTARGET EFFECTS OF BIOLOGICAL CONTROL AGENTS

Application of community ecology theory to biological control suggests that there are many ways in which biological control agents can indirectly impact nontarget organisms. For example, Holt and Hochberg (2001) identified five general
scenarios based on community modules (sets of interactions described by three to six strongly interacting organisms) through which biological control agents could indirectly affect nontarget species (Fig. 1). Four of these scenarios involve an indirect effect that is mediated through a direct attack by the biological control agent on a nontarget species, i.e., these scenarios depend on some aspect of host infidelity by the biological control agent. This is reassuring because, in theory, contemporary biological control strategies that ensure a high degree of host specificity should safeguard against most of these indirect nontarget effects (this assumes screening is effective at predicting host range, but see Louda et al., 2003). However, one scenario (Fig. 1e), referred to as “enrichment” by Holt and Hochberg (2001), only requires the presence of a generalist natural enemy capable of exploiting the biological control agent. In this case, the biological control agent can be an extreme specialist on the target weed and still profoundly impact other organisms in the systems where they have been introduced. If the biological control agent becomes sufficiently abundant, this interaction can be strong enough to subsidize populations of generalist natural enemies and indirectly affect other organisms attacked by that natural enemy. I believe that such indirect nontarget effects are of particular concern because they are not currently guarded against. This is primarily because indirect nontarget effects that arise from biological control agents with broad host ranges are well documented (Follett and Duan, 2000; Wajnberg et al., 2001), but only a handful of studies have recently begun to evaluate the potential viability and significance of indirect nontarget effects arising from host-specific biological control agents (Pearson and Callaway, 2003). Though these studies are currently few, they help to illustrate the nature and extent of the problems associated with indirect nontarget effects of host-specific biological control agents.
EMPIRICAL EVIDENCE FOR NONTARGET EFFECTS OF HOST-SPECIFIC BIOCONTROL AGENTS

I recently examined empirical evidence for indirect nontarget effects of host-specific biological control agents and identified three categories of indirect nontarget effects that can arise from highly host-specific control agents (Pearson and Callaway, 2003). These categories include 1) ecological replacement, 2) compensatory responses, and 3) food-web interactions (Fig. 2). This last category equates with the enrichment scenario described by Holt and Hochberg (2001; Fig. 1e), but the other two categories are not yet recognized by their framework. I briefly introduce these concepts here (Fig. 2) and provide examples of compensatory responses and food-web interactions in order to illustrate the implications of these indirect nontarget effects for the practice of biological control.

Ecological replacement

Ecological replacement occurs when an established invader replaces displaced native species in such a way that other native species become dependent on the invader. Nontarget effects occur when successful control of the invader deleteriously impacts the nontarget native species that have come to depend on it (Fig. 2a). Biological control under conditions of ecological replacement can result in undesirable indirect nontarget effects, but this is because the targeted pest has become important or desirable with regard to some aspect of its ecology, not because a biological control agent has misbehaved or otherwise failed. For example, saltcedar (Tamarix spp.) is a serious invasive pest in the southwestern United States which has replaced native trees and shrubs in many riparian areas (DeLoach et al., 2000). The
southwestern willow flycatcher (*Epidonax traillii extimus*), which is an endangered subspecies of the willow flycatcher, normally nests in willows (*Salix* spp.), but in some areas where willows have been replaced by saltcedar, the flycatcher now nests in the saltcedar (Sogge 2000). The proposed biological control program for saltcedar was initially held up due to concerns that successful control of the invader would leave the flycatcher without nesting habitat in some areas (DeLoach et al. 2000). However, this program has resumed after careful examination of the risks and assessment of potential mitigation on behalf of the flycatcher. Avoiding the unintended indirect nontarget effects associated with ecological replacement involves careful assessment of the target weed and its community interactions before introductions are made. I see the issue of ecological replacement as it relates to biological control as more of a policy issue than a problem with the ecological understandings of biological control. I am more concerned here with the ecological aspects of deploying biological control.

**Compensatory responses**

Compensatory responses can cause deleterious indirect nontarget effects by host-specific biological control agents when an agent’s attack elicits a response from the target species that actually increases its negative impact on nontarget species or shifts its impact to other nontargets (Fig. 2b). Compensatory effects may occur when a damaged plant increases relative growth rates and competitive effects (Ramsell et al., 1993), induces the production of chemicals that might harm neighbors (Siemans et al., 2002), or stimulates the release of root exudates (Hamilton and Frank, 2001). Plant compensatory responses to herbivory are quite common (Crawley, 1989; Trumble et al., 1993), and there are numerous examples of compensatory responses of
exotic plants to mechanical clipping (Callaway et al., 2001, unpublished; Gerlach and Rice, 2003) and to insects used as biological control agents (Islam and Crawley, 1983; Julien et al., 1987; Katovich et al., 1999; Müller, 1989; Steinger and Müller-Shärer, 1992), but it is not clear how often compensation results in negative effects on neighbors. Ramsell et al. (1993) showed that Tipula paludosa Meigen feeding on Lolium perenne L. actually increased its negative impacts on Rumex obtusifolius L. due to a compensatory response to root grazing. Over compensation to clipping was reported for the invasive Centaurea solstitialis by Gerlach and Rice (2003), suggesting the potential for this weed to increase its negative effects under herbivory, and Callaway et al. (unpublished) showed that clipping C. solstitialis did increase its negative impacts on native and naturalized California grasses, but acknowledged that clipping differs from herbivory in many regards. Callaway et al. (1999) and Ridenour and Callaway (2003) found that application of the biological control agent Agapeta zoegana L. (Lepidoptera: Tortricidae) to its host plant spotted knapweed did not reduce biomass or fecundity in spotted knapweed, but instead caused significant reductions in reproduction and trends toward reduced biomass in neighboring Idaho fescue (Festuca idahoensis Elmer). Thus, the extent to which compensatory responses might result in indirect nontarget effects of biological control introductions is not yet clear given the limited research. However, given the variability in the nature and strength of compensatory responses of plants to herbivory (Crawley, 1989; Trumble et al., 1993), it is likely that indirect effects of biological control agents that do occur through compensatory responses would be highly variable and difficult to predict.
Food-web interactions

Food-web interactions can arise when generalist consumers or other generalist natural enemies exploit a host-specific biological control agent (Figs. 1e, 2c). If the biological control agent is sufficiently abundant, this interaction can result in a subsidy that significantly elevates the consumer’s populations. Such a subsidy can translate to indirect effects on nontarget species through food-web interactions via the consumer.

For example, the gall flies (*Urophora affinis* (Frauenfeld) and *U. quadrifaciata* (Meigen), Lepidoptera: Tortricidae) introduced to North America to control spotted knapweed (Müller-Shärer and Schroeder, 1993) have become extremely abundant (Harris, 1980) and are now exploited by many native consumers (Story et al., 1995). Earlier studies indicated that exploitation of this resource by the native deer mouse (*Peromyscus maniculatus* Wagner) significantly altered deer mouse diets with potential to elevate mouse populations in knapweed-invaded grasslands (Pearson et al., 2000). This finding spawned a recent debate in *Conservation Biology* about the sufficiency of host specificity as a safeguard against nontarget effects (Hoddle, 2004a, b; Louda and Stiling, 2004). In question, in part, was whether gall flies simply served as an extra food resource for mice or whether gall flies actually functioned as a subsidy that elevated mouse populations and with them the potential for indirect nontarget effects. New research that was in press during this debate establishes that *Urophora* food subsidies actually double or triple mouse populations by increasing overwinter survival of mice in knapweed-invaded habitats (Ortega et al., 2004). Additional studies have since corroborated this result (Pearson and Callaway, unpublished; D. E. Pearson unpublished data). This increase in deer mouse populations is very significant and significantly increases the potential
for gall flies to indirectly affect other nontarget species through food-web interactions (Pearson and Callaway, 2003). In fact, Pearson and Callaway (unpublished) show that gall fly food subsidies to mice have tripled the prevalence of the Sin Nombre virus, a hantavirus that causes hantavirus pulmonary syndrome in humans (Childs et al., 1994). Their study area covered over 1600 km², but the affected area likely includes a much larger region of knapweed-infested habitats in several western states and provinces. Additional research suggests that as spotted knapweed invades native grasslands, gall fly subsidies to deer mice indirectly increase deer mouse seed predation and reduce recruitment in native plants already directly impacted by spotted knapweed (Pearson, unpublished data).

Native species are not the only nontarget organisms susceptible to impacts of biological control food-web interactions. Biological control agents themselves can also be affected. Coleomegilla maculata De Geer is an aggressive predator of Galerucella pusilla Duft. and Galerucella calmariensis L., two biological control agents introduced against purple loosestrife (Lythrum salicaria L.) (Landis et al., 2003). Thus, C. maculata is a shared natural enemy between these two agents that has the potential to affect their relative abundance through apparent competition - a special case of food-web interactions that arises when an organism affects the abundance of a potential competitor by subsidizing a shared enemy (Holt, 1977).

Although host-specificity in weed biological control guards against negative affects of apparent competition that arise from the biological control agent becoming the shared natural enemy between a target weed and nontarget plants, it does not guard against apparent competition occurring through higher trophic interactions involving natural enemies that attack the biological control agent. Recent surveys monitoring introductions of G. calmariensis and G. pusilla indicate that G. calmariensis...
established successfully at 100% of 24 release sites whereas *G. pusilla* failed to establish at any of these release sites (Landis et al., 2003). Although differential establishment of the two conspecifics could be due to intrinsic differences in abiotic interactions or direct competition between the two agents, it is quite possible that apparent competition plays a role. *Coleomegilla maculata* is a strong predator of both species (Sebolt and Landis, 2004). If *G. calmariensis* is better able to suffer this predation, it may indirectly contribute to the demise of *G. pusilla* by subsidizing the *C. maculata* attack on *G. pusilla*.

Given the frequency with which biological control agents are exploited by natural enemies in the introduced range (e.g., Goeden and Louda, 1976; Julien and Griffiths, 1998; Kluge, 1990; Müller and Goeden, 1990; Nuessly and Goeden, 1984; Pearson et al., 2000; Pratt et al., 2003; Reimer, 1988; Sebolt and Landis, 2004; Story et al., 1995), food-web interactions are likely a common outcome of the establishment of host-specific biological control agents. For example, Nuessly and Goeden (1984) documented intensive predation by the house mouse (*Mus musculus* L.) on the stem-boring moth (*Coleophora parthenica* Meyrick) introduced for the biological control of Russian thistle (*Salsola australis* R. Brown) in California. This system is highly reminiscent of the knapweed-gall fly-deer mouse system described above. However, as in virtually all cases of biotic interference with biological control agents, the emphasis of Nuessly and Goeden was on evaluating the effect of the mouse on the control agent not the effect of the control agent on the mouse and other nontarget organisms. Biological control agent-food-web interactions appear to be widespread, but their implications are poorly understood largely because their impacts are virtually unexplored.
SAFEGUARDING AGAINST NONTARGET EFFECTS OF HOST-SPECIFIC BIOCONTROL AGENTS

These examples show that host-specificity alone does not ensure the safety of biological control programs as previously argued (Frank, 1998; Hoddle, 2004a). Moreover, they indicate that the nature of indirect nontarget effects that can arise from even highly host-specific biological control agents are such that they simply cannot be ignored. This conclusion has serious implications for biological control and raises the crucial question of whether or not indirect nontarget effects of host-specific biological control agents can be predicted well enough to screen for them or if a better understanding of the types of interactions that result in indirect nontarget effects will allow us to avoid deleterious outcomes by designing around them.

Predictability has historically been an important element for safeguarding against nontarget effects. In the case of weed biological control, knowledge of the host range of the natural enemy is utilized to develop screening tests to determine the degree of host specificity of biological control agents and identify potentially at-risk nontarget species (Briese, 2003; McEvoy, 1996; Wapshere, 1974). This approach has clearly reduced the risks associated with biological control agents introduced for weed control (Pemberton, 2000), but the key to employing this technique has been the predictability associated with host-range expansion that has allowed testing to focus on a finite number of prospective alternative hosts without having to test all nontarget species present in the new environment (Briese, 2003; Pemberton, 2000).

Examination of the *C. maculosa-Urophora* spp. and *C. maculosa-A. zoegana* examples suggests that specific indirect nontarget effects are highly unpredictable. It is extremely unlikely that one would anticipate at the outset of these introductions that gall flies would elevate the prevalence of hantavirus via subsidies to deer mouse populations or that *A. zoegana* would increase the negative effect of *C. maculosa* on
F. idahoensis. In general, predicting specific indirect nontarget effects seems unlikely. However, understanding the process by which these interactions occur may allow us to more effectively guard against the types of pathways that can lead to these indirect nontarget effects.

Based on the theoretical and empirical evidence presented above, there are only two basic pathways currently recognized by which indirect nontarget effects can arise from host-specific biological control agents, and both are driven by the interaction between the biological control agent and the weed (Fig. 2). Better understanding of the components of this critical interaction may help improve our ability to avoid indirect nontarget effects while simultaneously increasing the success of biological control. Food-web interactions are one route to indirect nontarget effects of host-specific biological control agents that has been identified by both theoretical and empirical research (Fig 2c). As illustrated by the C. maculosa-Urophora spp. case study, food-web subsidies depend on an interaction between the biological control agent and the weed that translates into an overall bottom-up effect (Pearson and Callaway, 2003). That is to say, the effect of the weed on the biological control agent is stronger than the effect of the biological control agent on the weed so that the overall outcome is an increase in the biological control agent instead of a decrease in the weed. This situation creates conditions ripe for subsidies to other food-web elements via generalist natural enemies that are capable of exploiting both the biological control agent and other organisms in the system because the overall interaction is bottom-up rather than top-down as intended. Equally important is the strength of this interaction. For example, in the C. maculosa-Urophora spp. case even though the direction of the interaction is bottom-up, if the interaction between C. maculosa and Urophora spp. were weak (i.e., C. maculosa only very weakly
subsidized *Urophora* spp.) the indirect effects of gall flies would rapidly attenuate. Mice would eat gall flies, but gall flies would not be sufficiently abundant to subsidize mouse populations and indirect effects passing through mice to other species would be negligible.

The second route by which indirect nontarget effects can arise from host-specific biological control agents is through compensatory responses (Fig. 2b). This type of indirect nontarget effect has not yet been recognized by theoretical work in biological control, but is illustrated by the empirical example of *C. maculosa* and *A. zoegana*. In this case, the direction of the interaction appears to be top-down as intended (Müller-Shärer, 1991), but the weed is able to compensate by displacing the negative impact of the biological control agent, thereby increasing the negative effects on the recipient organism. Interaction strength appears to be key here as well. Although *C. maculosa* seems able to displace the negative impacts of *A. zoegana* in the current scenario, if the impact of *A. zoegana* on *C. maculosa* could be increased, it seems likely that eventually *C. maculosa* would no longer be able to compensate and successful control would be achieved. In general, if the biological control agent is strong enough (e.g., it kills or nearly kills the plant outright), it is unlikely that the plant will be able to compensate for the attack.

Thus, disregarding issues of ecological replacement as policy problems, I currently recognize two pathways by which host-specific biological control agents can cause indirect impacts on nontarget species: 1) compensatory responses (Fig. 2b), which are top-down in nature and 2) food-web subsidies (Fig. 2c), which are bottom-up in nature. These examples indicate that the nature of the biological control-weed interaction (top-down versus bottom-up) and the strength of this interaction are both very important aspects determining the potential for indirect nontarget effects of host-
specific biological control agents. This information is valuable for isolating the source of indirect nontarget effects arising from host-specific biological control agents in order to identify the species likely to be at risk, but how do we predict the potential degree of impacts expected?

Theoretical work suggests that indirect effects arising from biological control agents will be proportional to the agent’s abundance (Holt and Hochberg, 2001). This means, indirect nontarget effects will be closely linked to the biological control agent’s success. Unsuccessful biological control agents that are not effective at establishing or exploiting their host in the new environment will not become sufficiently abundant to threaten nontarget species. Highly successful biological control agents will over-exploit the target species with a resultant reduction in their own numbers and associated risks to nontarget species (Holt and Hochberg, 2001). In contrast, biological control agents of intermediate success, that effectively establish and exploit their host without greatly reducing its populations, are the agents most likely to reach high equilibrium densities in the introduced range and present the greatest risks to nontarget species (Holt and Hochberg, 2001). The implication here is that efficacy is the key to understanding and predicting indirect nontarget effects of host-specific biological control agents. Highly effective host-specific biological control agents will present low risk to nontarget species. So long as the agents do not host-switch, they will reduce their own numbers through a density-dependent feedback as they reduce the target species. Even if the biological control agent becomes superabundant in the initial process of establishment, which increases its potential indirect nontarget impacts, as long as the biological control agent is ultimately successful, these indirect nontarget effects should be ephemeral (exceptions could include extirpation of a nontarget species or other permanent impacts during the
abundant phase). Classical biological control successes such as klamath weed in California, USA and prickly pear (*Opuntia* spp.) in Australia and elsewhere very effectively illustrate this phenomenon (DeBach et al., 1976; Huffaker and Kennett, 1959). Efficacy therefore is not only important for biocontrol success, it is also important for ensuring the safety of biological control.

**DELIBERATE COMMUNITY ASSEMBLY**

The ultimate intent of biological control is deliberate community assembly (*sensu* Holt and Hochberg, 2001). Whenever we introduce biological control agents we do so with the intent of achieving a specific outcome in terms of community interactions. Although all multiple release strategies share this common goal, they differ in their routes to achieving it. Multiple release strategies represent a continuum in biological control that ranges from the lottery approach at one extreme to deliberate community assembly at the other, with the cumulative stress model and others somewhere in between (Harris, 1991; Myers, 1985; Sheppard, 2003). Strategies like the lottery and cumulative stress models rely on chance and the assumption that multiple host-specific biological control agents will have additive or synergistic effects with regard to their overall impact on the weed. However, multiple agents are just as likely to increase the chances of antagonistic interactions like competition or intraguild predation among biological control agents (e.g., Ehler and Hall, 1982; Story et al., 1991; Wang and Messing, 2003; Woodburn, 1996) that can undermine effective control while increasing risk to nontarget species. Deliberate community assembly requires an understanding of the ecology and biology of the weed as well as the biological control agent in order to select and introduce the minimal number of agents while maximizing control. The importance of these understandings are being
increasingly recognized in biological control (Briese, 2004; Hinz and Schwarzlaender, 2005; Sheppard, 2003), and recent studies in weed biological control have begun to show how knowledge of the relative sensitivities of a weed’s life-cycle transitions can indicate which natural enemy attacks are most likely to be effective (McEvoy et al., 1993; McEvoy and Coombs, 1999; 2000). These studies have begun to pave the way toward deliberate community assembly as a minimalist multiple release strategy in biological control and recent biological control programs are increasingly moving in this direction (Briese et al., 2002; Briese and Zapater, 2002; Blossey et al., 1996). Recent findings regarding nontarget effects in biological control (Pearson and Callaway 2003) argue now more than ever for shifting multiple release strategies away from lottery-style approaches toward more deliberate community assembly by minimizing agent numbers and reducing redundancy while attempting to maximize efficacy of a few select agents through greater knowledge of the weed and prospective biocontrol agents.

**HOST SPECIFICITY VERSUS EFFICACY**

Given that host-specificity and efficacy are both critical for safe and effective biological control, it is of interest to revisit the question of whether these two goals are biologically at odds with each other. Degree of host-specificity is seen as an indication of highly coevolved relationship between natural enemy and host (Allee et al., 1949) and some have argued that this coevolved process undermines the efficacy of the natural enemy (Hokkanen and Pimentel, 1984; Pimentel, 1963). If this is true, evolution may tend to deny us the best ecological combination for biological control — those organisms that serve as both highly host-specific and highly efficacious agents. Certainly the huge success of myxoma virus in controlling European rabbits illustrates
just how effective new natural enemy-host associations can be (Moore, 1987). However, the risks associated with implementing biological control based on new natural enemy-host associations are deemed too great to accept given that this practice involves introducing natural enemies that are sufficiently generalist that they are willing to establish on new host species (Goeden and Kok, 1986). Moreover, older and more coevolved associations can also be very successful as noted for *Chrysolina* control of klamath weed (Syrett et al., 2000; Huffaker and Kennett, 1959). The question then arises, what conditions cause biological control agents derived from older coevolved associations to at times be so virulent? We need to better understand how and when mechanisms such as conditions in the new environment or escape from natural enemies by the biological control agent are likely to facilitate successful control (Colautti et al., 2004; Hinz and Schwarzlænder, 2005) if we are to use this understanding to engineer more predictable and successful biological control. In particular, better understanding of the potential tradeoffs between host-specificity and efficacy is critical given the need for maximizing both of these factors for safe and effective biological control.

**EFFICACY TESTING**

The notion of elevating efficacy standards for biological control introductions to the level of those standards currently applied to host-specificity testing seems onerous indeed given the current costs, time, and effort required for host-specificity testing (Van Driesche and Bellows, 1996). However, recent theoretical work suggests that by turning this process around, time and costs might actually be saved in the testing process over current approaches. McClay and Balciunas (2005) suggest that because efficacy testing can be much simpler than host-specificity testing (it involves
testing only one natural enemy-plant interaction per natural enemy instead of many), it can actually function as a fast, effective method for reducing the list of control agents being tested for introduction. Even if such a method is only crudely applied, it could provide a more objective means of prescreening for efficacy before host-specificity testing that could be systematically applied and formally evaluated. Under a deliberate community assembly approach, agents that test poorly for efficacy simply would not get evaluated further because they are rejected for release. Evaluating weed life-cycle transitions (McEvoy et al., 1993; McEvoy and Coombs, 1999) can also reduce the list of species that need to be tested for host specificity by screening out organisms unlikely to effect control over the weed. For example, seedhead flies may be inappropriate for species that are not seed limited (Myers and Risley, 2000; Stanley, 2005). Although, efficacy tests in the laboratory and in the field in the native range will never provide a fail-safe predictor for the outcomes of complex community interactions in the new environment, using efficacy testing to drive biological control agent selection is consistent with a deliberate community assembly approach to biological control that focuses on fewer more efficacious control agents that will reduce risk to nontarget species and increase chances for successful biological control.

**DEFINING SUCCESS**

The conclusion that indirect nontarget effects arising from host-specific biological control agents are linked to biocontrol success has important ramifications for how successful control is defined. From a theoretical perspective, successful biological control is defined based on a threshold of economic or ecological impact and therefore is dichotomous (Van den Bosch and Messenger, 1973). However, in practice, the definition of successful biological control has evolved into a rather
continuous concept including different degrees of partial control being variously identified as success (Gurr and Wratten, 2000; McFadyen, 1998). This has resulted in a general lack of agreement on a common definition of successful control that has contributed to the widely divergent estimates of biological control success seen in the literature (e.g., DeLoach, 1991; McFadyen, 1998; Williamson, 1996). However, as pointed out by McEvoy (1996) and Syrett et al. (2000), it is important to appropriately assess costs when evaluating biocontrol success. If one considers that a partially successful control agent that provides marginal financial or ecological returns from a minor reduction in weed populations may simultaneously have disproportionately strong impacts and costs associated with its nontarget effects, then the notion of partial success must be reevaluated in this context. If moderately successful agents hold the greatest potential risk to indirect nontarget species (Holt and Hochberg, 2001), this understanding must be incorporated in the evaluation of success to develop more objective standards for quantifying biological control success.

FUTURE DIRECTIONS

Additional work is needed to advance our understandings of how weed and natural enemy biology and ecology determine not only biological control success, but also community-level outcomes of biological control introductions so that we can begin to more predictably engineer community outcomes resulting from these introductions (e.g., McEvoy and Coombs, 1999; McEvoy et al., 1993). For example, little is known about compensatory responses of weeds or invertebrate pests to biological control agents. More work is needed in the realm of efficacy testing and evaluation of sensitivities of weed life-cycle transitions to determine to what extent such information can serve to better filter out weak agents that offer little chance for
successful control (McClay and Balciunas, 2005). Biological control agents as a whole should be evaluated with regard to efficacy versus potential indirect nontarget risks to determine if certain biological control groups or strategies that have low efficacy also have high potential risks for indirect nontarget effects. If certain categories of biological control agents have low efficacy, high potential risks, or both, these groups should be considered for exclusion from future biological control programs. Finally, we need to expand on our understanding of potential tradeoffs between host-specificity and efficacy if we are to determine how to best maximize both of these factors in the agents we choose.

CONCLUSIONS

The fact that host-specific biological control agents can deleteriously impact nontarget species has profound implications for biological control and multiple release strategies like the lottery approach. The lottery approach has been challenged on the grounds that 1) it is risky to introduce more biological control agents than are necessary to achieve effective control and 2) multiple biological control agents can just as well negatively affect the outcome of biological control as result in additive or synergistic interactions as intended (McEvoy and Coombs, 2000; Myers, 1985; Myers et al., 1989; Pearson and Callaway, 2003; Strong and Pemberton, 2000). Until now, the assumption that host-specific biological control agents are safe has helped to sustain multiple release approaches like the lottery approach despite these attacks. However, recognition of the fact that serious indirect nontarget effects can arise from even the most host-specific biological control agents changes the rules of the game. Host specificity is necessary, but it is not a sufficient criterion for the safe release of biological control agents. The relationship between biocontrol efficacy and risk to
nontarget species suggests that efficacy of biological control agents may be as important as host-specificity for safe and effective biological control. To address the problem of indirect nontarget effects of host specific biological control, multiple release strategies will need to shift further toward a deliberate community assembly approach that minimizes numbers of agents and agent redundancy, while maximizing efficacy through better knowledge of biocontrol agent and weed interactions.

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FIG. 1. Community modules showing pathways for nontarget effects of biological control agents (after Holt and Hochberg, 2001). The first four interactions resulting in nontarget effects (a-d) involve host infidelity on the part of the biological control agent, but the last nontarget effect can occur for even highly host-specific biological control agents. Interactions are named as follows (see Holt and Hochberg 2001): (a) shared predation, (b) mixed predation and competition, (c) exploitative competition, (d) intraguild predation, and (e) enrichment or food-web interaction. Arrows indicate consumption except in (b) where the double-sided arrow indicates competition.

FIG. 2. Community modules depicting pathways for indirect nontarget effects of host-specific biological control agents. (a) Ecological replacement: agent is host specific and strongly suppresses the target weed thereby releasing suppressed natives, but this also weakens dependencies that have developed between the weed and other native species thereby negatively impacting these nontarget species. (b) Compensatory response: agent is host specific and the overall interaction between the biological control agent and the weed is top-down, but the target pest is only weakly impacted, because it displaces the negative impacts onto nontarget species through compensatory responses. (c) Food-web interaction: agent is host-specific, but the overall interaction between the biological control agent and the pest is strongly bottom-up so that the biological control agent becomes superabundant and then serves to subsidize other natural enemies in the system. These natural enemies then translate this subsidy into significant interactions with other nontarget species. Arrow direction indicates direction of the dominant interaction and the weight indicates the strength of the interaction. Lines without arrows in (a) simply indicate some sort of dependency.

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FIG. 2

a

Nontarget

Agent

\[\text{Nontarget} \rightarrow \text{Pest} \rightarrow \text{Nontarget}\]

b

Agent

\[\text{Pest} \rightarrow \text{Nontarget}\]

c

Nontarget

\[\text{Nontarget} \rightarrow \text{Nontarget}\]

Agent

\[\text{Pest} \rightarrow \text{Nontarget}\]