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LETTER

Biological control agents elevate hantavirus by subsidizing deer mouse populations

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Abstract

Biological control of exotic invasive plants using exotic insects is practiced under the assumption that biological control agents are safe if they do not directly attack non-target species. We tested this assumption by evaluating the potential for two host-specific biological control agents (*Urophora* spp.), widely established in North America for spotted knapweed (*Centaurea maculosa*) control, to indirectly elevate Sin Nombre hantavirus by providing food subsidies to populations of deer mice (*Peromyscus maniculatus*), the primary reservoir for the virus. We show that seropositive deer mice (mice testing positive for hantavirus) were over three times more abundant in the presence of the biocontrol food subsidy. Elevating densities of seropositive mice may increase risk of hantavirus infection in humans and significantly alter hantavirus ecology. Host specificity alone does not ensure safe biological control. To minimize indirect risks to non-target species, biological control agents must suppress pest populations enough to reduce their own numbers.

Keywords

Biological control, *Centaurea maculosa*, disease ecology, food subsidies, hantavirus, indirect effects, invasive species, non-target effects, *Peromyscus maniculatus*, *Urophora*.

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INTRODUCTION

Exotic plant invasions threaten the biological diversity of natural ecosystems around the world (Wilcove *et al.* 1998, Mack *et al.* 2000). 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; Gurr & Wratten 2000). However, the introduction of exotic organisms for biological control entails risks to non-target species (Simberloff & Stiling 1996; Louda *et al.* 1997; McEvoy & Coombs 2000; Strong & Pemberton 2000). For example, control agents with broad host ranges sometimes attack native species, causing deleterious non-target effects (Simberloff & Stiling 1996; Louda *et al.* 1997; Stiling 2002). To reduce this threat, rigorous screening for host-specificity is conducted before introduction of biological control agents for weed control (McEvoy 1996). However, this does not prevent agents from indirectly impacting non-target organisms through food web interactions (Holt & Hochberg 2001; Pearson & Callaway 2003). Here we provide evidence that two host-specific biological control agents (*Urophora* spp.) widely established across western North America to control spotted knapweed (*Centaurea maculosa* Lam.), indirectly increase the Sin Nombre hantavirus by providing food subsidies to populations of its native rodent reservoir.

The gall flies *Urophora affinis* (Frauenfeld) and *Urophora quadrifaciata* (Meigen) were first introduced into North America in the early 1970s as biological control agents for spotted (*C. maculosa*) and diffuse knapweed (*Centaurea diffusa* Lam.) (Harris 1980a), exotic forbs that aggressively invade arid habitats of western North America (Sheley *et al.* 1998). Adult *Urophora* lay eggs within immature flowerheads of *Centaurea* where the resulting larvae induce gall formation (Harris 1980a). The larvae overwinter within the seedheads from September to June, then emerge as adults and repeat the cycle (Story *et al.* 1992). *Urophora* have remained host specific since their introduction over 30 years ago and have been shown to substantially reduce seed production in the two *Centaurea* species (Harris 1980b). However, seed reductions have not effectively controlled these weeds (Maddox 1982; Stanley 2005) which continue to spread to new locations 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 & Harris 1980).

The abundance and availability of *Urophora* larvae during fall, winter and spring make them a valuable food resource for many native consumers. Deer mice (*Peromyscus maniculatus* Wagner), in particular, readily exploit this novel food source (Pearson *et al.* 2000). Within *C. maculosa*-invaded grasslands, deer mice switch microhabitats and shift their diet to exploit the seasonally available larvae (Pearson *et al.* 2000). As a result, in areas with abundant *C. maculosa*, *Urophora* larvae make up ≥ 50% of the deer mouse diet during most months of the year and 85% of their diet during key winter months when these mice typically experience a population decline associated with scarce native food resources (Pearson *et al.* 2000). This exploitation of *Urophora* larvae has increased survival of mice and doubled deer mouse populations in habitats invaded by *C. maculosa* (Ortega *et al.* 2004, D. Pearson and R. Fletcher, unpublished results).

The significant direct effects of *Urophora* food subsidies on deer mouse populations may translate into significant indirect effects on other organisms through food web interactions (Pearson & Callaway 2003). For example, 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 subsidies that elevate deer mouse populations may increase the prevalence of SNV and thereby elevate the risk of humans contracting HPS (Mills *et al.* 1999; Yates *et al.* 2002). To test the hypothesis that *Urophora* indirectly increase SNV incidence through food subsidies to deer mice (food subsidy hypothesis), we compared deer mouse abundance and SNV incidence between deer mouse populations in areas with high and low abundance of *C. maculosa* and *Urophora*.

METHODS

Study sites and overall design

We compared deer mouse abundance and SNV incidence between locations with high and low *C. maculosa* infestations, representing correspondingly high and low *Urophora* densities, for 3 years (2001–2003) at eight replicate sites in bluebunch wheatgrass (*Pseudoroegneria spicata*) and rough fescue (*Festuca scabrella*) grassland types across western Montana. Replicate sites represented distinct mountains or valleys and were separated by major drainages and spread over > 5000 km^2. We placed three 1-ha grids at each replicate site with one grid located in an area of low *C. maculosa* abundance (mean cover < 2%) and two grids in areas with high *C. maculosa* abundance (mean cover > 20%). The two high *C. maculosa* grids at each site were originally selected to represent high and medium infestations of *C. maculosa*. However, comparisons of *C. maculosa* cover estimates showed no difference between the high and medium grids, so these were pooled to form the high *C. maculosa* category. Each grid was comprised of 100 sampling stations at 10 m spacing in a 10 by 10 array. At each site, grids were placed in areas with similar topography and vegetation composition and separated by $\bar{x}$ = 3500 m (SD = 5400) to ensure independence among grids. Each site was trapped at the same time each year in spring (April to May) 2001–2003. Spring was the focus because *Urophora* food subsidies are most likely to affect SNV through increased overwinter survival of mice (Ortega *et al.* 2004, D. Pearson and R. Fletcher, unpublished results). Increased overwinter survival should reduce the risk that mouse populations and SNV will be locally extirpated (e.g. Boone *et al.* 1998). It may also facilitate the horizontal transmission of the virus through higher overwinter mouse densities and winter aggregations of mice in *C. maculosa* stands (Pearson *et al.* 2000; Ortega *et al.* 2004, D. Pearson and R. Fletcher, unpublished results). Spring is also significant for SNV transmission since SNV seasonally peaks in spring (Bennett *et al.* 1999; Calisher *et al.* 1999; Douglass *et al.* 2001) and HPS cases increase at this time (Mills *et al.* 2002).

Urophora density and host abundance

Overwintered mice captured in spring are affected by *Urophora* produced the previous summer, because *Urophora* produced during the growing season are only available to mice as larvae during the period from the fall after eggs are laid until the following spring when the mature flies leave the flowerheads (Pearson *et al.* 2000). Therefore, to determine the amount of *Urophora* available as a food resource to overwintering mice sampled during the springs of 2001–2003, we sampled *Urophora* produced during the summers of 2000–2002. In 2001 and 2002, we estimated *Urophora* densities at the end of the growing season in 0.5-m^2 quadrat frames systematically located at 33 sampling stations across each grid. At each station, we counted all *C. maculosa* stems, determined the number of seedheads per stem for 10 randomly selected stems and estimated the number of *Urophora* larvae per seedhead by dissecting 20 seedheads randomly selected from the 10 stems. This allowed us to estimate the density of *Urophora* per 0.5 m^2 in 2001 and 2002 and to back-estimate *Urophora* densities in 2000. The extrapolation of *Urophora* densities in 2000 was necessary because we initiated mouse sampling in 2001 but the *Urophora* food source for these mice was produced in 2000. Additionally, to address what appeared to be a significant

negative effect of a drought in 2000 on *C. maculosa* flowering and *Urophora* densities at the onset of the study, we back-estimated densities of *Urophora* to 1999, the pre-drought period. This allowed evaluation of the drought effect in 2000 relative to both pre- and post-drought years. To estimate *Urophora* densities in 1999 and 2000, we estimated densities of knapweed stems (stems were used because they are more persistent than seedheads) 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. Stems rarely persist more than 2 years. Linear regressions from each site were used to estimate the density of *Urophora* larvae by site as a function of *C. maculosa* stem density based on regression relationships in 2001 and 2002. Data from 2001 and 2002 were pooled to incorporate interyear variation into the regression models, and stem counts in 1999 and 2000 were representative of the range of stem counts in 2001 and 2002 used to construct the regressions. Although this process involves estimating *Urophora* densities in 1999 and 2000 from relationships measured in 2001 and 2002, we feel the extrapolation provides a reasonable approximation of *Urophora* density since the purpose is not to precisely estimate *Urophora* densities, but rather to provide a qualitative index for comparing *Urophora* between high and low *C. maculosa* plots and between drought and normal years. To evaluate abundance of *Urophora*'s host plant, we estimated per cent cover of *C. maculosa* within a 3-m radius of each of the 100 stations on each grid at the eight study sites in June 2001–2003 when *C. maculosa* had reached peak biomass following bolting.

Deer mouse abundance

On each trapping grid, one Sherman live trap was placed at each of the 100 sampling stations and checked in the morning each day for 4 days. Trapping was conducted once each year in the spring and all grids (treatments) at a site were trapped simultaneously. Sites were trapped sequentially, with those at low elevations sampled first so that sites were sampled at similar phenological stages. Trapped small mammals were identified, ear tagged, and their sex, mass, reproductive condition and age were determined prior to release at the trap station. To evaluate whether food subsidies altered agonistic behaviours associated with hantavirus transmission (Glass *et al.* 1988; Douglass *et al.* 2001), animals were checked for signs of agonistic behaviour indicated by scars and wounds found primarily on the ears and tail (Douglass *et al.* 2001). Only adult animals were used in the analyses to focus on the effects of the *Urophora* food subsidy on overwintered mice. Overwintered adults were defined as animals ≥ 16.5 g based on the split in the bimodal distribution of masses between juveniles and adults observed at the onset of breeding in spring.

Hantavirus incidence

Blood samples were taken from each mouse upon first capture during each trapping period (Mills *et al.* 1995). Blood samples were tested for hantavirus antibodies at the Montana Public Health Laboratory, Helena, MT using the enzyme-linked immunosorbent assay method (Feldmann *et al.* 1993). Mice with titre counts $\geq 1 : 400$ were classified as seropositive for hantavirus, indicating they had been exposed to or were currently infected with hantavirus. The blood results allowed us to examine densities of seropositive mice and proportions of seropositive mice (seroprevalence). Abundance or density of seropositive mice was defined as the number of seropositive individuals captured per grid. Seroprevalence was defined as the total number of mice testing positive for hantavirus antibodies divided by the total number of mice successfully tested, i.e. mice with insufficient samples or equivocal results were excluded. 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.

Analyses

We employed mixed linear models (PROC MIXED; SAS Institute 1999) to evaluate the effect of year and *C. maculosa* abundance (high vs. low) on the number of mice captured, the number of seropositive mice captured, seroprevalence, sex ratio and per cent of wounded mice. Year and *C. maculosa* abundance class were entered as fixed factors in a repeated measures design and site was treated as a random blocking factor. We compared sex ratio and wounding between mouse populations to understand potential mechanisms for differences in SNV prevalence between high and low *C. maculosa* grids, because sex and agonistic behaviour can influence SNV prevalence (e.g. Glass *et al.* 1988; Douglass *et al.* 2001). Analyses were based on 694 overwintered mice. We also used PROC MIXED to evaluate the effect of a spring drought on *C. maculosa* cover and stem densities among years on high *C. maculosa* grids. In these models, year was a repeated measure and site was a random factor. Contrasts were used following the overall tests to determine which years differed significantly using $\alpha = 0.05$. Data for these analyses included 2001–2003 for cover estimates and 2000–2002 for stem densities using data from all eight sites. The different time periods correspond to how these variables relate to mice. Cover of *C. maculosa* relates directly to mice within the same year as this represents the

habitat aspect of the plant for mice, but stem densities relate to mice in the following year as stem densities link directly to flower production and represent the food aspect of the plant for mice, i.e. *Urophora* production.

RESULTS

Urophora density and host abundance

In most years *Urophora* densities were > 200 larvae per 0.5 m^2 on high *C. maculosa* grids vs. < 25 larvae per 0.5 m^2 on low *C. maculosa* grids (Fig. 1). However, in 2000 *Urophora* densities on high *C. maculosa* grids were 70% lower than in other years. This reduction in *Urophora* in 2000 was associated with a 53% reduction in *C. maculosa* stem densities on high *C. maculosa* grids in 2000 relative to other years ($F = 19.01$, d.f. = 2, 30, $P < 0.001$; mean stem density ± 1 SE = 8.1 ± 2.2, 16.5 ± 2.2 and 17.6 ± 2.2 in 2000–2002 respectively). This suggests that many *C. maculosa* plants did not bolt and flower in 2000 as in normal years, resulting in the dramatic decline in *Urophora* observed in 2000. This reduction in *C. maculosa* flowering was observed across western Montana and has been attributed to unusually dry spring conditions (Ortega *et al.* 2004; Stanley 2005, D. Pearson and R. Fletcher, unpublished results). Despite this decline in stem densities, *C. maculosa* cover estimates did not change significantly from 2001 to 2003 ($F = 1.36$, d.f. = 2, 27, $P = 0.274$; mean per cent cover ± 1 SE = 21.9 ± 2.3, 18.9 ± 2.3 and 21.9 ± 2.3 in 2001–2003). These results establish that during the 3 years that mice and hantavirus were sampled, 2001–2003, overall cover of *C. maculosa* did not change, but abundance of the *Urophora* food source available to mice was much lower in 2001 than 2002–2003 on high *C. maculosa* grids due to reduced *C. maculosa* flower production in 2000.

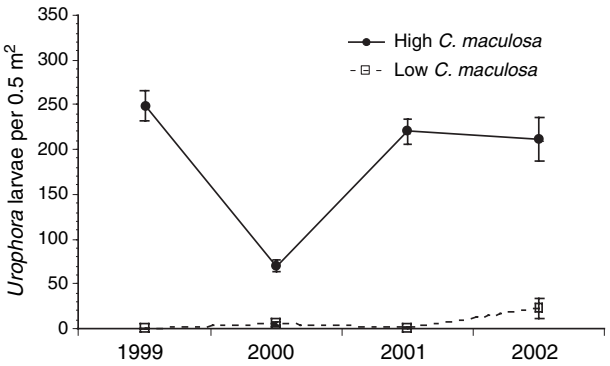

Figure 1 Estimated mean density (±SE) of *Urophora* larvae per 0.5-m^2 from 2000 to 2002 for two western Montana sites with high and low *Centaurea maculosa* density. Error bars do not show for some points. Note that *Urophora* produced in 1 year subsidize deer mice in the next year, because the food is consumed overwinter.

Deer mouse abundance

Deer mice were significantly more abundant on high vs. low *C. maculosa* grids ($F = 6.67$, d.f. = 1, 16, $P = 0.020$; Fig. 2a), despite significant fluctuations in mouse populations among years ($F = 13.69$, d.f. = 2, 45, $P < 0.001$). However, differences between high and low *C. maculosa* grids were only apparent in 2002 and 2003 and not in 2001, as indicated by the significant interaction between *C. maculosa* level and year ($F = 6.68$, d.f. = 2, 45, $P = 0.003$). Thus, *C. maculosa* and *Urophora* appeared to double mouse populations in 2002 and 2003 under conditions of

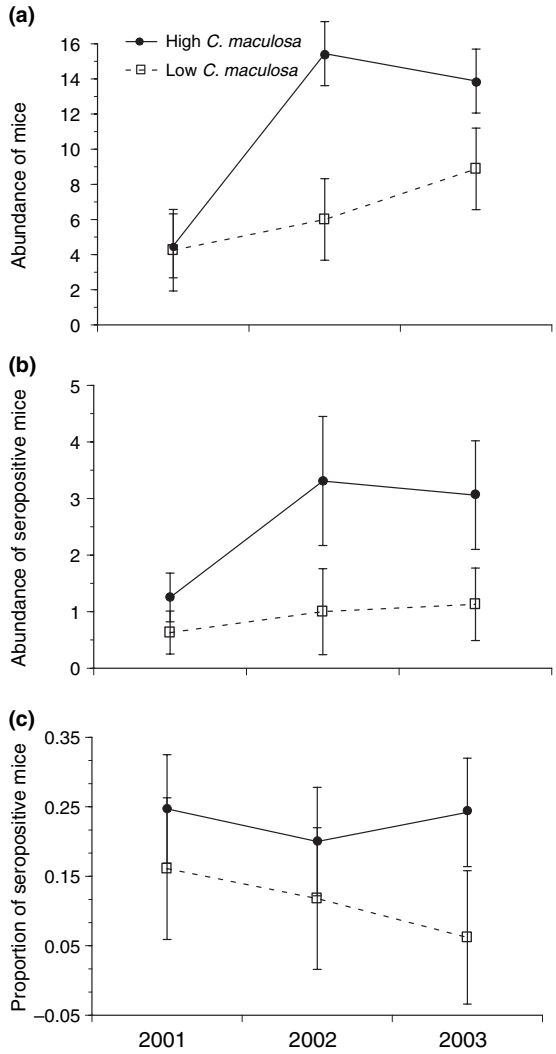

Figure 2 Mean (±SE) for (a) abundance of deer mice (*Peromyscus maniculatus*), (b) abundance of seropositive deer mice and (c) proportion of seropositive deer mice captured on grids from 2001 to 2003 in western Montana grasslands with high vs. low levels of *Centaurea maculosa*.

good precipitation that favoured *Urophora* production, but this effect was negated in the spring of 2001 following reductions in *Urophora* production in 2000.

Hantavirus incidence

The abundance of seropositive mice was significantly greater on high vs. low *C. maculosa* grids ($F = 4.40$, d.f. $= 1, 20$, $P = 0.049$; Fig. 2b). Year ($F = 1.60$, d.f. $= 2, 46$, $P = 0.214$) and the year by *C. maculosa* interaction ($F = 0.70$, d.f. $= 2, 46$, $P = 0.502$) were not significant. The *proportion* of seropositive mice tended to be greater at sites with high vs. low *C. maculosa* (Fig. 2c; $F = 3.88$, d.f. $= 1, 17$, $P = 0.065$), while year ($F = 0.41$, d.f. $= 2, 38$, $P = 0.666$) and the year by *C. maculosa* interaction ($F = 0.40$, d.f. $= 2, 38$, $P = 0.674$) were not significant. Mean proportion of male deer mice did not differ between high and low *C. maculosa* grids ($F = 0.01$, d.f. $= 1, 23$, $P = 0.929$), among years ($F = 0.30$, d.f. $= 2, 37$, $P = 0.742$) or among years by *C. maculosa* ($F = 0.21$, d.f. $= 2, 37$, $P = 0.808$). The mean proportion of wounded deer mice did not differ between high and low *C. maculosa* grids ($F = 0.03$, d.f. $= 1, 28$, $P = 0.856$), among years ($F = 0.65$, d.f. $= 2, 37$, $P = 0.529$) or among years by *C. maculosa* ($F = 1.09$, d.f. $= 2, 37$, $P = 0.348$).

DISCUSSION

Host specificity is the key screening criterion used to reduce non-target risks associated with the biological control agents introduced for weed control (McEvoy 1996). However, this focus on host specificity ignores the potential for biocontrol agents to interact with non-target organisms through means other than direct consumption (Holt & Hochberg 2001; Pearson & Callaway 2003, 2005). Here, we show that food subsidies from the host-specific *Urophora* biocontrol agents which increase overwinter survival in deer mice (Ortega *et al.* 2004, D. Pearson and R. Fletcher, unpublished results), are associated with more than three-fold higher spring densities of deer mice that test positive for SNV, the aetiological agent for HPS in humans.

We found that deer mice were two times more abundant in *C. maculosa*-dominated grasslands than in grasslands where *C. maculosa* was rare in 2002 and 2003 (Fig. 2a), years preceded by normal precipitation that produced abundant *Urophora* (Fig. 1). In contrast, deer mouse abundance did not differ between stands with low vs. high *C. maculosa* in 2001 (Fig. 2a), a year preceded by an exceptional spring drought that dramatically reduced the abundance of *Urophora* larvae by reducing *C. maculosa* flowering (Fig. 1). Thus, the drought served to partition the indirect effect of *C. maculosa* on mice in terms of the *Urophora* food resource from the direct effect of *C. maculosa* in terms of cover by reducing *Urophora* production but not *C. maculosa* cover in 2001. Moreover, since deer mice do not consume *C. maculosa* tissue and only rarely incidentally consume *C. maculosa* seeds (Pearson *et al.* 2000, D. E. Pearson, unpublished results), and since mice avoid *C. maculosa* cover when *Urophora* are not available within the seedheads (Pearson *et al.* 2000, Ortega *et al.* 2004), the greater abundance of deer mice associated with high *C. maculosa* appears to be due to the *Urophora* food resource. These findings are corroborated by a spatially independent but temporally overlapping study conducted in west-central Montana from 1999–2001 showing that deer mice were two times more numerous on sites with high vs. low *C. maculosa* abundance in 1999 and 2000, years preceded by normal precipitation, but not in 2001, following the 2000 drought (Ortega *et al.* 2004). Combined, these studies represent a sampling area > 8500 km^2 and a time period covering 5 years, documenting that deer mice closely tracked the abundance of *Urophora* in a manner consistent with the food subsidy hypothesis. Although site effects could not be experimentally controlled in these observational studies, another 5-year study in west-central Montana that compared deer mouse response to experimental removal of *C. maculosa* and *Urophora* showed that deer mice declined in response to *C. maculosa* and *Urophora* removal in a manner also consistent with the food subsidy hypothesis (D. Pearson and R. Fletcher, unpublished results). Thus, there is extensive evidence that *Urophora* directly subsidize deer mouse populations, but the potential for these consumer subsidies to affect other organisms has not been demonstrated until now.

In this study, *Urophora* food subsidies to deer mouse populations appeared to indirectly increase the incidence of SNV. Blood results from deer mice captured in the springs of 2001–2003 indicated that the relative abundance of seropositive mice was significantly greater on grids with high vs. low *C. maculosa* and *Urophora* abundance. In 2002 and 2003, years following normal precipitation that produced abundant *Centaurea* and *Urophora*, there were over three times more deer mice that tested positive for hantavirus antibodies at high *C. maculosa* sites. In 2001, following the spring drought that reduced *Urophora*, but not *C. maculosa*, differences in the abundance of seropositive mice between sites with high and low *C. maculosa* abundance were greatly reduced. This pattern of higher densities of seropositive mice on high *C. maculosa* grids only when *Urophora* are abundant is consistent with expectations of the food subsidy hypothesis.

The greater abundance of seropositive mice in heavily invaded grasslands is primarily attributable to the increase in the total number of mice. However, the difference in abundance of mice between grasslands with high vs. low *C. maculosa* density (two-fold difference) does not appear to fully account for the difference in the relative abundance of

seropositive mice (three-fold difference). This suggests that the *rate* of hantavirus transmission among deer mice may also be higher in *Urophora*-subsidized mouse populations. Our data showed a consistent trend towards higher *proportions* of seropositive mice where *C. maculosa* and *Urophora* were abundant (Fig. 2c), but this difference was only marginally significant. However, the data were also conservatively biased, because in 2001 and 2002 no mice were captured on 25% of the low *C. maculosa* grids, and in 2003 no mice were captured on 13% of the low *C. maculosa* grids. This precluded calculations of seroprevalence for many low *C. maculosa* invasion grids – grids that showed zero seroprevalence in years when mice were present. Thus, there is some evidence that *Urophora* food subsidies may increase the incidence of SNV in mouse populations not only by increasing deer mouse populations directly, but possibly also by increasing transmission rates among mice within elevated populations. Additionally, increased over-winter survival associated with *Urophora* food subsidies (Ortega *et al.* 2004, D. Pearson and R. Fletcher, unpublished results) appears to reduce chances for local extirpation of deer mouse populations, suggesting that *C. maculosa* infestations may provide refugia habitats for both mice and virus (Glass *et al.* 2002; Abramson *et al.* 2003) and so provide source populations crucial for the 'travelling waves' postulated to spread the virus across the landscape during epidemics (Abramson *et al.* 2003).

There are three factors commonly associated with deer mice that test antibody positive for hantavirus: age, sex and wounding (e.g. Bennett *et al.* 1999; Douglass *et al.* 2001). As such, differences in densities or proportions of seropositive mice between populations could arise due to differences in age structure, sex ratios or antagonistic interactions. Age structure was controlled for in this study because only adult animals were analysed and the ratio of males to females was not affected by *C. maculosa* infestation. Agonistic behaviour might increase in dense stands of *C. maculosa* because mouse population densities are higher or because deer mice seasonally aggregate in dense *C. maculosa* patches during the winter months when they forage on *Urophora* larvae (Pearson *et al.* 2000; Ortega *et al.* 2004). However, mean proportion of wounded deer mice did not differ among high and low *C. maculosa* density sites, suggesting the increase in SNV was not due to increased agonistic encounters associated with changes in behaviour. Thus, we found no evidence that the observed increase in numbers of SNV-positive mice or possible increases in seroprevalence due to *Urophora* food subsidies were associated with changes in agonistic behaviour or shifts in demographic structure. We conclude that *Urophora* subsidies to deer mouse populations may increase SNV by simply increasing mouse densities and therefore increasing contact among mice rather than by altering the nature of these contacts through behavioural changes (Mills *et al.* 2002). However, the trends shown toward a proportional increase in SNV suggest this question should be examined further.

In North America, SNV is the primary aetiological agent of HPS, a deadly zoonotic disease that infects humans annually with a 37% case fatality (Mills *et al.* 2002). Current understanding of the epidemiology of HPS in the south-western 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 (Mills *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. Our results support this hypothesis by showing that food subsidies from biological control agents can augment food-limited deer mouse populations and thereby elevate densities of SNV-positive mice. The fact that *C. maculosa* is not common in the southwestern USA 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 suggest that *Urophora* food subsidies have great potential to increase the incidence of SNV over a large region of western USA and Canada where *C. maculosa* is abundant. Moreover, because *C. maculosa* (and therefore *Urophora*) commonly achieves its highest densities in disturbed areas near humans, it may concentrate deer mice near houses and outbuildings. Contraction of HPS occurs most often in enclosed buildings (Armstrong *et al.* 1995) where the virus remains protected from destructive UV radiation and can readily become airborne when disturbed through cleaning activities (Morbidity and Mortality Weekly Report 1993). It is notable that Montana, the epicentre for *C. maculosa* invasion, has recently been ranked as second only to New Mexico in per capita cases of HPS despite the fact that it lies well beyond the Four Corners region, the recognized epicentre for HPS cases (Douglass *et al.* 2005). Destabilization of the equilibrium state of a disease's ecology can lead to new emerging infectious diseases (Daszak *et al.* 2000). For example, 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 (Allan *et al.* 2003). Widespread increases in populations of rodents such as 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.

We show that host-specific biological control agents can have potentially significant non-target effects through food web interactions. Despite strong host specificity, *Urophora* biological control agents have indirectly elevated SNV more than three-fold by providing food subsidies to deer mice. Although host specificity 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 have the potential to become superabundant, thereby increasing their capacity to indirectly impact non-target species (Holt & Hochberg 2001; Pearson & Callaway 2003, 2005). We suggest that efficacy is as important as host specificity for ensuring safe and effective biological control.

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