Understanding invasions: the rise and fall of diffuse knapweed (*Centaurea diffusa*) in North America

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Introduction

Diffuse knapweed (*Centaurea diffusa* Lam.) is an aster of Eurasian origin that has colonized 1.4 million hectares of semi-arid grasslands in western North America over the last century [1, 2]. The species can be found from Washington to Michigan and from British Columbia to New Mexico. This species is a short-lived perennial that often produces a rosette in the first year and in the second year will flower, set seed, and die. The senesced adult plant can function as a tumbleweed to disperse seeds. The weed reduces grassland forage and has the potential to exhibit negative effects on other ecosystem services of these rangelands [3–5].

Midway through the last century *C. diffusa* was sufficiently widespread to implement biological control efforts. The first species of biological control insects (gall flies, *Urophora* spp) were released in North America in 1970, followed by the release of about 10 additional species of insects over the next two decades [6]. By 2000, 13 insects had been released [7]. In spite of these efforts, diffuse knapweed continued to spread, and through the 1990s, biological control efforts appeared unsuccessful. As summarized by Carpenter and Murray [8] "At least nine biological control agents that attack *Centaurea diffusa* are established in parts of the United States. Unfortunately, it appears that none of these agents, alone or in combination, effectively controls diffuse knapweed populations."

While evidence was accumulating indicating that top-down controls were not affecting dominance for this and related species of *Centaurea*, the importance of bottom-up controls (competitive and resource acquisition mechanisms) were being documented. Gerlach and Rice [9] indicated that a relative of diffuse knapweed, *C. solstitialis*, was successful as an invader due to its abilities to persist within a community and exploit resource opportunities. Research on diffuse knapweed at our site [5] indicated that the characteristics
of persistence and opportunism also described the invasiveness and dominance of *C. diffusa* as well. Suding et al. [10] demonstrated that rosettes of diffuse knapweed were strong competitors under ambient nutrient conditions, but were less competitive under lower nutrient conditions that may have characterized North American grasslands until recently. Elsewhere, the role of soil biota – collectively the benefits provided by mycorrhizae and the absence of soil pathogens – has also been indicated in the success of invasive species of *Centauraea*. Mycorrhizal fungi provide a competitive advantage to invasive spotted knapweed *C. maculosa* [11, 12]. Callaway et al. [13] demonstrated that mycorrhizal interactions allowed *C. melitensis* to exhibit compensation to grazing damage. Those findings added to the work showing that overcompensation to root herbivory occurred in *C. maculosa* [14].

Allelopathy was also identified as a competitive mechanism used by *Centauraea* species. Spotted knapweed was found to possess an allelopathic agent, (−)-catechin [15]. Another allelopathic chemical, 8-hydroxyquinoline, was subsequently identified for diffuse knapweed [16], supporting the contention of Hierro and Callaway [17] that *C. diffusa* used allelopathy to achieve a competitive advantage. This particular chemical contains nitrogen, an element that is generally more available in many areas of North America due to increased atmospheric nitrogen deposition and chronic fire suppression [18]. Callaway and Ridenour [19] suggested that the relatively high production of allelopathic compounds could explain the dominance of invasive *Centauraea* species.

While specific findings were being reported for *Centauraea* spp, Klironomos [20] demonstrated that invasive plant species with strong dominance (high abundance in their respective communities) often exhibit positive feedbacks with soil biota. Mitchell and Power [21] found that those invasive species that exhibited dominance and were identified as noxious and invasive tended to be those that had escaped their native fungal pathogens and viruses. These same species had yet to accumulate an equivalent number of pathogens in their introduced environments. Not surprisingly, Callaway et al. [22] subsequently showed that *C. diffusa* growth exhibited positive feedbacks in soils of invaded communities. Collectively, these results argue that the combination of traits – persistence and opportunism, allelopathy, and potential positive feedbacks from soil biota – allow for *Centauraea* species, including diffuse knapweed, to function as something we might call “super-invaders” [23]. Such plants appear to be superior competitors and capable of dominance across a broad range of ecological conditions.

By 2003, however, evidence suggested that certain insects were having a strong influence on diffuse knapweed densities in Colorado [24]. Subsequent reports from Montana [25] and British Columbia [26] indicated that this response was widespread. The common factor in this reduction was the addition, to the existing suite of herbivores, of a weevil that consumed both seeds and maturing plants. Differences in nutrient availability and plant competition have the potential to mediate weed responses to herbivory (e.g., [27, 28]).
Accordingly, tests at multiple sites and under multiple climatic regimes are warranted to establish the generality of top-down controls on diffuse knapweed.

Here, we use results from studies of insect herbivory on diffuse knapweed populations in the Colorado Front Range to make inferences about factors controlling invasiveness of diffuse knapweed and its relatives in North America. We show that top-down controls negate the characteristics that made knapweed successful as an invader in Colorado. We also hypothesize that these controls, as represented by the current list of biological control insect species released in North America, may not be uniformly effective for all of the invasive Centaurea species.

**Monitoring effects of herbivores on knapweed populations and seed production**

A monitoring site dominated by diffuse knapweed (25–30% of plant cover) was established in Boulder County, Colorado, USA in 1997. In that year small numbers of *Sphenoptera jugoslavica* Obenb., (Coleoptera: Buprestidae), *Cypholeonaxis achatas* Fahlæus (Coleoptera: Curculionidae), both root feeders of rosettes, and *Larinus minutus* Gyllenhall (Coleoptera: Curculionidae), the lesser knapweed flower weevil, were released at the site. In addition, there were existing populations of *Urophora quadrijasciata* Meigen, (the knapweed seed head fly, Diptera: Tephritidae). A second species of fly, *Urophora affinis* Frauenfeld, the banded gall fly, invaded the site from unknown sources by 1999.

Knapweed abundance and reproduction was obtained by counting rosettes, flowering stems, seed heads per plant, and seeds per seed head. The abundance of *Larinus minutus*, which reproduces by placing eggs in flowers of the knapweed, was also obtained by during the inventory of seed heads. Details on data collection procedures at the Boulder site are reported in Seastedt et al. [24].

In addition to quantifying seed production and weevil abundance at the above site, additional areas containing large densities of knapweed were sampled for seeds and weevils. Sites included three mountain meadow sites and eight additional grasslands at distances up to 100 km from the original study site. Insects were released at these sites in the late 1990s or insects from other sites eventually colonized the areas. Data on seed and weevil densities in seed heads reported here were collected during the 2001–2004 interval. With one exception, counts were based on inspection of 180 seed heads from 30 different plants at each site collected in the mid August to mid September interval. At one site this analysis was limited to 108 seed heads from 18 plants.

The patterns observed for knapweed stem densities at our 1997 release site show that about four years were required for insect populations to build up to levels where knapweed densities were significantly reduced (Fig. 1). In contrast to the sharp decline in densities observed at this site, other sites not experiencing these levels of insect herbivory continued to maintain high densities
and cover of knapweed [24]. By 2004, the seed head weevils had expanded to almost all remnant grasslands in the area, and two plots that had substantial knapweed when first censused in 2001 had no flowering stems or rosettes of this species in 2004.

Summer precipitation during the study interval was variable and typical of semiarid grassland found in a continental climate. During the last five years rainfall was average or above average in 2001, 2003, and 2004. The year 2000 was moderately dry and the autumn and winter of 2001 and all of 2002 were extremely dry. Knapweed rosettes may have refrained from flowering in 2002 but persisted through the drought. Seeds germinated by substantial rains in 2003 produced a modest increase in knapweed in 2004, but at levels well below those observed prior to 2001. Rosette densities of knapweed were about 50 plants m⁻² in 1997 [24], but ranged from 1–3 plants m⁻² at the release site in 2004 (data not shown). Given the potential persistence of a seed bank for this species [29] the decline in rosettes likely reflected both the reduction in seed production as well as substantial mortality of seedlings.

A single Larinus weevil larva will consume all of the seeds found in a diffuse knapweed seed head. Thus, the average seed production of knapweed impacted by this insect is determined by the number of seed heads with weevils (all contributing zero seeds) as well as the seeds produced in those seed heads not containing weevils. The relationship is 'triangular', i.e., when weevil abundance is high, seed production is uniformly low, but when weevil
Figure 2. Relationship between seed and weevil production in seed heads of diffuse knapweed. Each point represents the mean value of seeds per seed head and weevils per seed head for 12 sites in the Colorado Front Range area collected during the 2001–2004 interval. (n = 38; not all sites were sampled in all years.)

abundance is moderate-to-low, seed production can be low, moderate or high (Fig. 2). The difference in seed production likely relates to the amount of defoliation experienced by the flowering plants. *Larinus minutus* over-winters as adults beneath the knapweed, and they tend to defoliate plants in the spring following their emergence from the soil. If sufficient defoliation occurs, the vigor of flowering is suppressed, and weevils apparently lay few eggs on stressed plants. Such plants produce few seeds and contain few weevils, providing the low seed–low weevil results seen in Figure 2. The weevils that defoliate flowering plants disperse to healthy plants that do produce seeds in those seed heads not fed upon by the weevil larvae. Those plants produce the moderate seed-low weevil points seen in Figure 2.

Knapweed not subjected to significant weevil herbivory generally produced 4–8 seeds per seed head (Fig. 3). Once weevil populations become established, seed production is greatly reduced, both by the direct consumption of seeds and the reduction in plant vigor caused by the feeding activities of the adults. While the summer of 2004 was spectacular in terms of rainfall and plant growth, weevil damage on a seed head basis was about the same as that seen in previous years (Fig. 3). Seed rain, the amount of seed produced per m², was initially above 4,000 seeds per m² in 1997, declined to about 700 seeds per m² in 2000 (the year before the large population decline shown in Fig. 1), and was estimated below 200 seeds per m² in 2004 (Fig. 3). The failure for knapweed to maintain its flowering stem densities, given these seed inputs, argues
for substantial mortality of seeds, seedlings, and rosettes. We do not know the extent to which that mortality results from plant competition versus direct mortality from herbivory and pathogens, but we believe this mortality is likely important in the sustained low densities of the knapweed. Interestingly, 'weevil rain' (number of adult weevils emerging from seed heads) has been as high as 2,000 weevils per m². Unless an effective predator or parasite for this species appears, knapweed experiencing these densities of herbivores is doomed to either an early death or very low reproduction.

Harris [30] suggested that the objective of knapweed control should be to achieve less than 5% cover by the weed on rangelands. This has been accomplished in Colorado, and similar results are underway in other regions (e.g., [25, 26] and unpublished results). Myers and Bazely [31] make the strong case that this decline is likely due to the combination of effects that the insects have on multiple stages of the knapweed lifecycle.

The reduction in knapweed densities in Colorado is attributed largely to activities of the lesser knapweed flower weevil, *Larinus minutus*. The other insects present in this study have not been able to control the weed [31], or, as in the case of *Cyphocleonus achates*, were not particularly abundant during intervals of knapweed decline (Seastedt, unpublished results). However, these observations do not exclude the possibility that the addition of the other species collectively have more impact on the rate and extent of knapweed decline than *L. minutus* operating alone. Seedling mortality appears to be a significant
part of the reduction in plant densities, because densities decline when seed production is still moderately high [24]. Since root feeders have been observed to cause mortality in immature plants [31], these species in particular likely do assist in the speed of the decline in knapweed densities. Myers and Bazely [31] indicate that seed predators should only be effective when host plants are poor competitors and have low rosette survival. In Colorado, seedling survival appears to be a vulnerable stage for this plant, and soil nutrient availability and plant competition influence survival ([5, 10] and unpublished results). Thus, similar to findings of McEvoy et al. [27] and research summarized by Muller-Scharer and Schroeder [6], we believe that resource competition, generated by low soil resource availability or through plant competition, mediates the exotic plant response to herbivorous insects. Accordingly, we predict that in spite of large reductions of knapweed in grassland areas, knapweed will remain common in areas of soil disturbance with high nutrients and little plant competition. To date, our observations match this prediction.

The relationship between biotic controls and ‘super invaders’

Our findings do not negate the studies that show Centaurea species to possess allelopathic chemicals, to maximize mutualistic associations with mycorrhizae, or to benefit from positive feedbacks with soil biota. Further, Centaurea species may have escaped pathogens found in soils of their native habitat, thereby conferring additional advantage. These mechanisms, however, operate most strongly at high population densities. These factors are relatively ineffective at maintaining dominance if propagule pressure (seed production) and survivorship of juvenile plants are insufficient to maintain high densities of the invader. Our data suggest that the combination of herbivore stress to flowering plants and direct seed consumption by weevil larvae in undisturbed vegetation can reduce the abundance of this species to relatively low densities. Therefore, top-down controls (i.e., the components of classical biological control) are capable of negating or overriding ‘novel weapons’ or other mechanisms that confer competitive advantage to this species. Demonstration of top-down controls in North America does not prove that these insects controlled knapweed in their native lands (e.g., [32, 33]), but these results do show that this negative feedback is sufficient to deter dominance in the invaded areas studied here.

The Centaurea species that have invaded North America possess numerous traits that have interacted with the invaded communities in ways that have enhanced the dominance of these species. However, allelopathy may be the equivalent of “bringing a knife to a gun fight” as a mechanism for maintaining dominance. The ability to acquire and preempt limiting resources is a requirement for plant dominance. When that ability is precluded by the absence of propagules or the absence of tissues necessary to obtain those resources in an efficient manner, then dominance is unobtainable. Knapweed’s ability to grow
and produce seeds is often limited by nitrogen availability [5]. Since the allelopathic agent of diffuse knapweed requires nitrogen, we suspect that a trade-off between allocation of nitrogen for root exudates and allocation of nitrogen to seed production occurs in this species. Our data suggest that knapweed in grasslands containing sufficient competitors becomes seed limited once the herbivore *L. minitius* becomes abundant. Thus, allocation to allelopathic materials in the face of significant top-down controls is not going to contribute to the success of the species. We note that, in the context of either the enemy release hypothesis or the evolution of increased competitive ability hypothesis (see Inderjit et al., this volume), escape from herbivory and root pathogens may have allowed *Centaurea* species to produce sufficient root exudates to generate allelopathic chemicals that is described by the novel weapons hypothesis. Hence, the existence of novel weapons, should it be proven to be a fairly common trait of invaders could be a consequence of the absence of biological controls in the invaded communities and, if this is the case, should be regarded as a consequence of factors identified in the enemy release hypothesis.

Missing from our knowledge is the extent to which *Centaurea diffusa* and soil biota of the Colorado Front Range are similar to other communities of *C. diffusa* in other regions of North America. Certain populations of *C. diffusa* may differ in their abundances of allelopathic agents, and certain populations may have more soil pathogens and fewer soil symbionts in other regions (e.g., [22, 33]). If so, then our results on top-down controls in these areas might not have generality. We strongly believe, however, that we have described the general case. Top-down controls have been reported in Montana and British Columbia [25, 26], areas with different climates, different soils, and, presumably, very different soil biota. Second, *C. diffusa* was able to invade and dominate in our region regardless of its specific chemistry or the specific composition of soil biota. Thus, for now at least, top-down controls in some and perhaps most regions appear to negate whatever combination of invasive traits *C. diffusa* has mustered. Not all of our study areas have responded as quickly as the site shown in Figure 1, but sites that appear slower to increase in seed head weevil abundance and decrease seed production have all started with much larger densities and seed sources of knapweed.

Will the top-down effects we observed for *C. diffusa* be repeated for other species of invasive *Centaurea* in North America? The demise of a dominant species requires greatly reducing propagule pressure, plant survivorship, or significant reductions in both of these variables. We suspect that short-lived species that by necessity rely heavily on seeds as a mechanism for success will likely be most susceptible to the top-down controls observed here. Longer-lived, iteroparous plants such as *C. maculosa* may be less vulnerable to insects like the seed head weevil for several reasons. First, annual seed production per plant appears relatively low, implying that maximum seed head weevil numbers, which are limited by the number of seed heads produced, will also be relatively low. Densities of adult weevils on a per-plant basis therefore may never match those numbers occurring on *C. diffusa*. This would diminish tissue dam-
age caused by the adult weevils. Second, perennial plants may be able to persist over multiple years by producing few or even no flowers. Seed head weevils could not persist in such areas and therefore the densities of weevils required to control perennial *Centaurea* species may never be obtained. Finally, the allelopathic agent identified for spotted knapweed is a carbon-based compound. Hence, nitrogen allocation conflicts are not likely to be as important. This does not preclude the possibility of other insect species with different life history strategies to function as potential biological control agents. For example, there exists some hope that the root-feeding weevil, *Cyphocleonius achates*, may eventually function in this fashion at least in some regions for *C. maculosa* [34].

A subset of exotic plants exhibit a combination of traits such as enhanced rates of allelopathic chemical production and beneficial plant-microbial interactions formed when exotic plants enter new communities. These ‘bottom-up’ effects appear to give this group the properties of ‘super invaders’. Exotic control agents, some which may also exhibit more effective control characteristics due to release from their own predators and pathogens, can greatly diminish the dominance of these invaders. While high competitive ability can confer high survivorship of mature plants, seeds, seedlings and juvenile plants appear more vulnerable to top-down controls and also appear more susceptible to competition-mediated resource limitations. The net outcome between the relative strengths of ‘bottom-up’ and ‘top-down’ benefits and constraints produces the patterns we observe for invasiveness and dominance. While *Centaurea diffusa* appears to no longer be an unmanageable invasive threat, we do not know if the control mechanisms that reduce the dominance of diffuse knapweed will work for other invasive *Centaurea* species.

**Summary**

The relative importance of mechanisms explaining the invasiveness and dominance of alien plant species remains a subject of active debate. Diffuse knapweed has been identified as a strong competitor capable of using allelopathic chemicals to achieve dominance in plant communities that have not co-evolved with this species. Positive feedbacks with soil biota may further enhance the competitive abilities of *Centaurea* species. The failure of classical biological control after 30 years of effort was seen as negative evidence for the enemy escape hypothesis as a mechanism explaining dominance. However, control of this invasive species by insect herbivory now appears to have been achieved in widely separated ecosystems in North America. While we do not know if these same insects exerted this regulatory function in the native habitat of diffuse knapweed, we do see top-down controls operating effectively in the invaded ecosystems. Traits conferring strong competition such as enhanced rates of allelopathic chemical production or those produced by new plant-microbial interactions formed when exotic plants enter new communities can be negated by biological control mechanisms.
Acknowledgments

This work was funded in part by a USDA-NRCSGP and EPA Region VIII PESP grants to the authors. We thank Marybeth Gamo, Colin Maun, Emily Mathis, and Cindy Shand for assistance in the most recent field and laboratory measurements. Research sites were provided by Boulder County Open Space, City of Boulder Open Space and Mountain Parks, and by private landowners in Boulder, Larimer, and Douglas Counties.

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Nature 427: 731–733


