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How Many and What Kind of Agents for the Biological Control of Weeds: a Case Study with Diffuse Knapweed

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Overview: Following its introduction to North America, diffuse knapweed came to occupy millions of hectares of rangeland. This is the story of biological control efforts done over 25 years before an effective agent was introduced. The moral is that for optimal success of biological control, thorough work should be done to understand the ecology of the target weed and its natural enemies. Effective agents are unlikely to be abundant in the native habitat, but are likely to be able to kill the plants in the exotic habitat.

Introduction

Classical biological control of weeds is the introduction of natural enemies from native areas to exotic sites where their host plants have become invasive and detrimental (overview in Myers and Bazely, 2003). Three important questions that confront biological control practitioners are: (i) which species of agent are safe to introduce; (ii) what types of agents are the most likely to be effective controls; and (iii) how many different species of agents should be introduced? Here I describe the biological control programme against diffuse knapweed, *Centaurea diffusa*, which involved the introduction of 13 species and the establishment of 11 agents over a 20-year period (Bourchier *et al*., 2002). This is a story of a programme based on the premise that multiple species of agents would be necessary for successful control (Harris, 1981). After 30 years of monitoring diffuse knapweed, my students and I have observed that populations have declined following the establishment of the last agent to be introduced, the weevil, *Larinus minutus* (Fig. 9.1). The effectiveness of this species is demonstrated by the decline of diffuse knapweed density at sites with the beetle and continued high density of knapweed at sites where the beetle has not yet established. The diffuse knapweed programme provides an excellent case study for the evaluation of whether multiple species of biological control agent are required for success, or if single agent

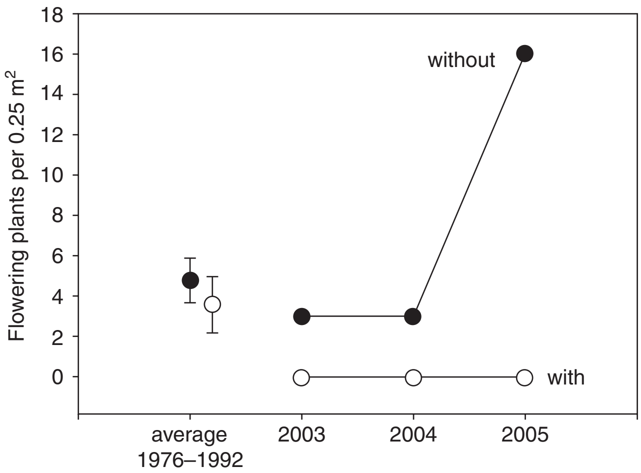

Fig. 9.1. Average number of flowering diffuse knapweed plants at a site where the weevil *L. minutus* was not established (solid circles) and a site where beetles became established in the late 1990s (open circles). Densities in recent years are compared to average densities measured between 1976 and 1992, prior to beetle establishment. Error bars are 2 SE. The return of average rainfall following several years of dry conditions was associated with the increased plant density in 2005 at the site lacking beetles.

species can be successful (Denoth *et al.*, 2002). It also provides the opportunity to study interactions among different agents and shows the value of quantitative monitoring of population densities for the evaluation of biological control success.

The Diffuse Knapweed System

Diffuse knapweed (*C. diffusa*) is an aster of Eurasian origin that was introduced to British Columbia prior to 1930 in contaminated lucerne seeds. Diffuse knapweed is a serious rangeland pest because it displaces forage grasses and has attained very high densities in dry rangelands in many areas of western North America. Knapweeds are poor forage for cows because of their bitter taste, and their dense stands are unpleasant to even walk through. Story *et al.* (2000) reported that in North America over 3 million ha of rangelands have been invaded by diffuse and spotted knapweed, *Centaurea stoebe* ssp. *microanthos* (formerly *Centaurea maculosa*).

The biological control programme for diffuse knapweed began in 1970 in Canada and, over the next 20 years, 12 insect species that attack different parts of knapweed plants (Fig. 9.2) were introduced, and nine of these are now widely distributed in British Columbia. The first two species to be introduced were gall-forming flies in the family Tephritidae, *Urophora affinis* and *Urophora quadrifasciata* (Harris and Myers, 1984), which have markedly reduced the production of seeds

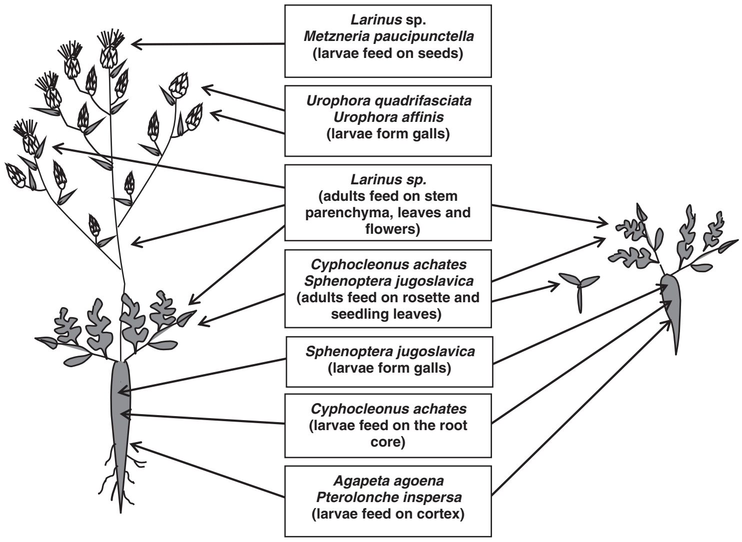

Fig. 9.2. Nine species of biological control agents attack various parts of knapweed plants. Both adult beetles and larvae of *C. achates*, *S. jugoslavica* and *L. minutus* feed on knapweed plants, while for other species only larvae attack the plants. Arrows indicate plant parts where agents oviposit and feed.

but not the density of knapweed plants (Myers and Risley, 2000, Myers and Bazely, 2003). *Metzneria paucipunctella*, a moth originally from European spotted knapweed, was also introduced in the early 1970s, and the larvae feed on seeds of both knapweed species but little is known of its impact (Bourchier *et al*., 2002).

Next the root-boring beetle, *Sphenoptera jugoslavica*, was introduced and widely distributed in the 1980s, and these further reduced knapweed seed production (Powell and Myers, 1988; Powell, 1990), as well as the survival of seedlings and rosettes, and delayed the flowering of plants. A model based on density-dependent birth and death rates of the plants and including the impact of the beetles, however, showed that the knapweed populations were resilient to this attack (Powell, 1990).

In the 1980s to mid-1990s, three species of root-feeding Lepidoptera were introduced to and distributed in British Columbia. *Agapta zoegana* was first introduced in 1982, distributed through the 1990s and has become widely established (Bourchier *et al*., 2002). *Pelochrista medullana*, introduced in 1992, has not thrived (Bourchier *et al*., 2002). *Pterolonche inspersa* apparently became established following its initial introduction in 1986, although it has not been monitored (P. Harris, Lethbridge, Alberta, 2006, personal communication). Although initially collected from spotted knapweed, the weevil, *Cyphocleonus achates*, was widely distributed following its original introduction to British Columbia in 1987. It can exist on large rosettes of diffuse knapweed and now occurs at many sites in the southern Okanagan Valley of British Columbia, even though adult beetles are not capable of flight. Larvae of this weevil feed on the root core and reduce the growth of flowering plants and can kill rosette plants (Story *et al*., 1996; Bourchier *et al*., 2002).

Most recently, beginning in 1991, two species of weevil, *Larinus minutus* and *L. obtusus* were introduced as biological control agents on diffuse and spotted knapweed (Groppe, 1990) (Fig. 9.3). The adult weevils damage and can kill plants by feeding on the epidermis of stems and branches of the knapweed as well as feeding on flower buds, thereby causing their abortion. The larvae feed on developing achenes. The *Larinus* species were widely distributed in British Columbia in 2000 and 2001 and they appear to be good dispersers.

Two additional species that were introduced, but did not establish, are the ovary-feeding fly, *Chaetorellia acrolophi*, and the soft achene-feeding fly, *Terellia virens*. A fungus, *Puccinia jaceae diffusae*, was accidentally introduced to British Columbia, and this can kill seedlings and rosettes as well as infect flowering plants. Although it is widely spread it is not known to have an impact on plant density (P. Harris, Lethbridge, Alberta, 2006, personal communication).

The same species of potential biological control agents were introduced to knapweed infestations in the USA and a recent overview is given by LeJeune *et al*. (2005).

Interactions among Agents

One reason for being conservative in the introduction of biological control agents is that a potentially effective agent may be suppressed through competition by another and thus reduce the success of the biological control programme. In fact, Zwölfer (1973) recommended the introduction of poor competitors for biological weed control, under the assumption that once released from competition, they would thrive and damage host plants. I expanded this idea and suggested that agents that are rare in their native habitats may have the best potential for biological control success. My students and I reviewed weed biological control programmes to determine if single species of agents could be successful and if competition among species of agents was indicated. We found that the establishment rates of agents was not significantly related to the number of agents introduced, although for single-agent introductions, the success of establishment was 53% and for multiple-species programmes it was 32% (Denoth

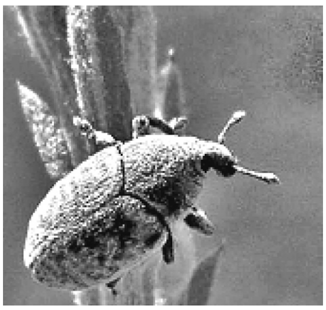

Fig. 9.3. *Larinus minutus* on a knapweed plant. Photo by Shannon White.

et al., 2002). Thus there is no strong evidence that the introduction of multiple species influences the establishment of agents.

We also found that the success rate of biological weed control increased with the number of agents introduced (Denoth *et al*., 2002). We suggested that this result could be explained by either the ‘lottery model’, in which the probability that a successful agent will be introduced increases with the number of agent species introduced, or the ‘cumulative stress model’ (Harris, 1981), in which additive stress from multiple agents promotes host plant decline. Perhaps our most important finding was that in over half of successful programmes, success was attributed to a single agent. This supports the proposal that cumulative stress from multiple agents is not necessary for successful biological control, but in many cases ‘silver bullets’ can achieve success.

The multitude of species introduced on diffuse knapweed allows the study of interactions among agents. The agents introduced on knapweed fall into two categories: those that reduce seed production directly by attacking the flowers and those that reduce seed production indirectly by feeding on the roots of the plants and thus reducing their growth, development and energy stores for seed production (Fig. 9.1).

Seed-feeding Species

Given the high number of agent species introduced, it is interesting to determine if competitive interactions can occur among agent species in the diffuse knapweed programme. The two gall flies attack the knapweed flowers at slightly different times. *U. affinis* lays eggs in flower buds and is reported to be competitively superior to *U. quadrifasciata*, which lays eggs at a slightly later stage of bud development. The two species also differ in that *U. quadrifasciata* has a larger second generation than *U. affinis*, which is primarily univoltine. This might contribute to increased dispersal of the latter, as they must seek out flower buds in the proper stage for oviposition. *U. affinis* also produces a hard gall, which may protect it from predators, while *U. quadrifasciata* produces a potentially more vulnerable soft gall. In territorial interactions between males of the two species, *U. affinis* is dominant (Berube, 1980). *U. quadrifasciata* and *U. affinis* were initially introduced together, with *U. quadrifasciata* being a contaminant of the *U. affinis* stock. However, both species increased rapidly (Harris and Myers, 1984) and they coexist in most locations (Harris, 1990).

Larvae of the moth *M. paucipunctella* will feed on *Urophora* larvae (Story *et al*., 1991); however, given their poor survival, *M. paucipunctella* is not a major player in the species interactions. *L. minutus* larvae kill *M. paucipunctella* larvae in co-inhabited flower heads (Harris, 2005; website). These negative interactions among species in the seed-feeding guild may increase the dispersal of agents as they search for suitable hosts and stages of bud and flower development for oviposition. They have not, however, apparently prevented the establishment of species.

Finally, two species of *Larinus* were introduced to British Columbia, *L. minutus* and *L. obtusus*, and nothing is known about their competitive interactions. Harris (Lethbridge, Alberta, 2006, personal communication) has suggested that the two species may hybridize. The original introductions were based on differences in host plant and habitat preferences, *L. minutus* preferring diffuse knapweed and drier environments, and *L. obtusus* preferring spotted knapweed and moister environments. Understanding the interactions between the two species will require genetic studies.

U. affinis may also have an advantage over the seed-feeding weevil *L. minutus* because buds heavily attacked by *U. affinis* do not open and are not attractive to ovipositing weevils (Harris, 1990). Feeding on the stems, leaves and buds of knapweed plants by *L. minutus* in the early spring, however, can reduce bud development and thus oviposition sites for flies. On the other hand, *L. d*[illegible] larvae will feed on gall-fly larvae if they are in the same flower head (LeJeune *et al.*, 2005). LeJeune *et al.* (2005) found 'no detectable effect of *U. affinis* on seed abundance' of knapweed, but *U. affinis* was reduced by the presence of *L. minutus*.

Root-feeding Species

Associations among insects feeding on knapweed roots were studied in field surveys in Europe, their native habitat, by Muller (1989). He found 12 species of insects attacking five areas of the roots of diffuse knapweed: crown, collar, vascular tissue, cortex and outer surface. Of these, the most relevant to the knapweed biological control programme are the two widely established species that attack the central vascular tissue of the root, the weevil, *C. achates* and the buprestid beetle, *S. jugoslavica*. Larvae of the moth, *A. zoegana*, develop in the root cortex. No evidence for negative interactions among these species was apparent in Muller's study, and *C. achates* and *A. zoegana* were positively associated in roots, an indication that they may have similar preferences for larger plants as oviposition sites. LeJeune *et al.* (2005) found that *C. achates* responded positively to larger knapweed rosettes that resulted from nitrogen fertilization, but that *S. jugoslavica* avoided fertilized plants. *A. zoegana* was not part of this study.

Most of the work on the root-feeding species in North America has been on spotted knapweed. In British Columbia, the most common of the species that attacks diffuse knapweed roots is the buprestid beetle *S. jugoslavica*. This is also the most widely distributed of the root-feeding species in British Columbia, and at one site they attacked half of the knapweed plants (personal observation). This is considerably higher than the 2% level of attack reported by Muller (1989) for one European site. The lower density and more restricted distributions of the other root-feeding species reduce the opportunity for competitive interactions among these species in British Columbia at this time.

What Types of Agents Should be Introduced?

Early in this study, Harris (1974) wrote a paper describing a system for the evaluation of different types of insects as biological control agents. This was based on the type of damage they did. An alternative approach to selecting biological control agents is from the perspective of the plant biology.

Attack by the gall flies and the root-boring beetle *S. jugoslavica* reduced the seed production of diffuse knapweed by over 95% without reducing plant density (Powell, 1990). This may seem quite surprising, but models of the density–survival patterns of diffuse knapweed showed that compensatory survival and seed production buffers population densities from increased seed predation (review in Myers and Bazely, 2003). Survival is reduced when density is high and better when density is low, so populations can be maintained at high densities even with reduced numbers of seeds. In addition, at low density, flowering plants are larger and produce more seeds, and this too helps to buffer population densities. To explore these associations we developed a simulation model that incorporated the impacts of the gall flies, the root-boring beetle *S. jugoslavica*, and a hypothetical agent that killed plants after the stage at which compensatory survival would occur, e.g. the rosette or flowering-plant stage (Myers and Risley, 2000). This model showed that only the latter mortality would reduce knapweed density, and we recommended the introduction of species that could kill plants. Two of the agents that are established in British Columbia can kill plants: *C. achates* and *L. minutus*, and thus these fit the predicted requirement for successful control of diffuse knapweed.

L. minutus was the last of the species to be distributed and established on diffuse knapweed. In many sites it has reached high population densities. Particularly during a period of drought, feeding by beetles killed many bolting plants. This result has also been observed in other areas (LeJeune *et al*., 2005). With this attack, densities of diffuse knapweed have declined precipitously at many locations in British Columbia. While it appears that *L. minutus* caused the decline, the widespread presence of the *Urophora* species and of *S. jugoslavica* make it impossible to evaluate if the weevil *L. minutus* could have achieved biological control success on its own and experiments are necessary.

Conclusions

Over the 30 years of the biological control programme on diffuse knapweed, interest in non-target impacts of introduced biological control agents has increased (Louda *et al*., 2003). In the diffuse knapweed programme, 13 additional exotic species have been introduced in the quest to reduce the density of one non-indigenous host plant. The introduction of multiple agents in biological control programmes raises an interesting philosophical question about the potential modification of species complexes. With each introduction comes a chance of indirect effects on non-target plants or influences on other components of the community, such as by providing new food items for predators.

We anticipated in the early stages of the knapweed programme that seed predators would be unlikely to control diffuse knapweed effectively. And yet *L. minutus* was introduced approximately 20 years after the gall flies, primarily as an additional seed predator. It seems that the main impact of *L. minutus* may be the severe damage caused by adult weevils feeding on flowering plants. This impact received little comment in the studies carried out in Europe prior to the introduction of *L. minutus* to North America (Groppe, 1990). Perhaps further study of this species in its native habitat would have helped to identify its multiple positive characteristics, such as adult feeding, high reproductive potential and good dispersal ability, in addition to its impact as a seed predator.

Although competitive interactions among the multiple biological control agents in the diffuse knapweed programme display occur, these have not apparently caused species exclusion. Variation in the density and phenology of host-plant populations may allow the established species to coexist. Of the root-dwelling species only *S. jugoslavica* is currently widely distributed at moderately high densities. If densities of *C. achates* and *A. agoena* increase, more competitive interactions may occur.

Earlier I posed three questions that are important to biological control and these can be considered retrospectively for the knapweed programme.

1. Were the introduced species safe? No detrimental non-target impacts of the biological control agents in the knapweed programme have been recorded. Thus the safety of the agents appears to have been evaluated appropriately.
2. What types of agents were most likely to be successful? Because knapweed produces many seeds it was thought initially that seed predators or insects that reduced seed production would be effective. However, the ability of knapweed to compensate made populations resilient to seed predation. Although we predicted that only an agent that killed plants in late life-history stages could be successful, this type of agent was not initially identified. Early selection of *L. minutus* may have reduced the number of agents introduced.
3. How many agents should be introduced? It is not clear that this question was considered in the knapweed project. The economics of identifying and testing agents for host specificity is always a consideration. In the knapweed programme, however, the philosophy was to keep introducing agents until success was achieved. While this approach has been common to most programmes in biological control of weeds, recent concerns arising from the attack of native plants by introduced insects may change this philosophy in the future. If a limit on the number of introductions were set, more focus might have to be placed on finding effective agents at the pre-release stage.

Like most biological control projects, this programme has involved concerted efforts by many people in Canada and Europe. It is an example, however, of how difficult it is to maintain long-term monitoring, which is crucial to its evaluation. Measuring only seed production is an insufficient index of biological control success, which is only achieved through reduced plant density. It is unfortunate that other exotic species now await their opportunity to replace knapweed. In particular, the grass *Bromus tectorum* appears to have benefited by the knapweed decline.

Although in almost half of the successful biological control of weeds programmes, success has been attributed to a single species of agent, the general support for the possibility that ‘silver bullets’ exist remains weak among biological control practitioners. I think that a more conservative and predictive approach is necessary for selecting agents in the future programmes, to reduce the number of exotic species introduced. In addition, my suggestion that seed predators are unlikely to successfully control weeds that produce many seeds has not been popular. Field experiments and population-simulation models of target weeds in both exotic and native habitats should be prerequisites for selecting the agents for introduction. And finally, I recommend that more emphasis should be placed on species that are rare in their native environment rather than those that are common and widely spread when picking candidate species for biological control. It is likely that plants are well adapted to the attack of their common insect herbivores. This too has not been a widely accepted suggestion. A more scientific and experimental approach to biological weed control could reduce the economic and environmental costs of introducing unsuccessful agents in future control programmes.

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