

# Two shoot miners as potential biological control agents for garlic mustard: should both be released?

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## Summary

Two shoot-mining weevils, *Ceutorhynchus alliariae* and *C. roberti*, both potential biological control agents for *Alliaria petiolata* in North America, show high temporal and spatial niche overlap. To select an appropriate future release strategy, we investigated the capacity of different weevil combinations to attack the target plant. We tested *C. alliariae* alone and *C. alliariae* in combination with *C. roberti*, both in field sites and under experimental conditions. The comparison of attack levels as an indirect estimate of their potential to damage garlic mustard revealed that in both cases, *C. alliariae* is at least equally as effective in attacking garlic mustard alone as in combination with *C. roberti*. Under experimental conditions, *C. alliariae* alone reached even higher infestation levels than the mixed species treatments. However, the higher attack levels did not result in a higher impact on garlic mustard. Provided *C. alliariae* and *C. roberti* prove to be equally specific once host-range tests are completed, two release strategies can be envisioned: a) only one of the two species will be released to minimize potential non-target effects. Its establishment and impact will be closely monitored, and the second species will only be released if the first species fails to establish in all habitats or does not provide the expected impact. b) Both species will be released together. Replicated releases of different combinations of the two species, i.e. *C. alliariae* alone, *C. roberti* alone, and both together, would provide us with a unique opportunity to test the conclusions from our pre-release investigations and thereby to test the predictive power of pre-release studies.

**Keywords:** biological weed control, *Alliaria petiolata*, *Ceutorhynchus alliariae*, *Ceutorhynchus roberti*, pre-release studies.

## Introduction

It is a matter of controversy whether successful biological control results from the impact of a single agent (Myers 1985) or the combined effect of multiple agents (Harris 1981, Schröder & Goeden 1986). A recent tendency in biological control is to reduce the number of insects released, since each additional introduction adds an increment of environmental and economic risk (McEvoy & Coombs 1999). We agree with McEvoy &

Coombs (1999) that only a subset of the most promising organisms in terms of safety and effectiveness should be released. Presuming that for each weed biocontrol system, there is a “certain” number of agents required to achieve control, each species released in addition to that number is redundant. In the best-case scenario, releasing an extra species has little or no influence, in the worst case, the additional introduction may reduce the overall impact on the target plant (Myers 1985). The risk of redundancy among biological control agents is particularly high if several insects occupying similar feeding niches on the target plant are co-introduced.

Two shoot-mining weevils, *Ceutorhynchus alliariae* Brisout and *C. roberti* Gyllenhal, are currently being investigated as potential biological control agents for *Alliaria petiolata* (Bieb) Cavara & Grande in North America. Studies on their biology and ecology revealed high temporal and spatial niche overlap (Gerber &

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Hinz, unpublished data). In addition, host-range tests have so far not shown any major differences in their specificity (Gerber *et al.* 2002).

We will therefore face the selection of the appropriate release strategy. Should both species be released? Or is one species sufficient to successfully control garlic mustard – and which one of the two species should we choose for release? One important criterion for this decision is the potential impact of the two weevils on garlic mustard. In general, only agents that reach high population levels have the potential to successfully control a target weed (Gassmann 1996). We therefore considered weevil attack levels as an indirect estimate of their potential to damage garlic mustard and therefore their effectiveness.

In this paper, we compare the results of two different approaches to investigate the effectiveness of *C. alliariae* alone and together with *C. roberti*. Attack levels on *Alliaria petiolata* were measured: 1) at field sites where both species occur and sites where only *C. alliariae* occurs, and 2) in a manipulative experiment, where both species or only *C. alliariae* were released onto potted plants in a common garden. The latter approach was also used to collect quantitative data on the impact of, and potential competitive interactions between, the two species.

## Materials and methods

### Study organisms

*Ceutorhynchus alliariae* and *C. roberti* are sibling species in the family Curculionidae. They have almost identical life histories. They overwinter as adults and start to lay eggs in spring. We found no differences in average fecundity or oviposition period (Gerber *et al.*, unpublished data). Larvae of both species mine during April and May in shoots of bolting plants, but also in petioles of rosettes of garlic mustard. No spatial or temporal niche segregation for larvae was found, and they cannot be distinguished morphologically (Gerber & Hinz, unpublished data). Mature larvae leave the plant to pupate in the soil, and adults of the F1-generation emerge about four weeks later. The two species do differ, however, in their geographical distribution. *C. alliariae* and *C. roberti* occur both in geographically isolated (allopatric) and associated (sympatric) populations. Both are considered as monophagous on garlic mustard, a plant of Eurasian origin that was introduced into North America in the 19th century (Cavers *et al.* 1979). The plant has since become one of the most serious invaders in forested areas of the north-eastern and mid-western United States (Blossey *et al.* 2002). Garlic mustard is a strict biennial in the family Brassicaceae that reproduces entirely by seed. Seedlings emerge in early spring and form rosettes over summer. These start to bolt in March/April of the following year and siliques form by June.

### Field data

We collected and compared attack data between field sites where only *C. alliariae* occurs (allopatric,  $n = 6$ ) and where both occur (sympatric,  $n = 10$ ). At each site, we randomly collected 12–332 bolting garlic mustard plants along a transect and brought the plants back to the laboratory for dissection under a stereo microscope. The number of larvae and exit holes were recorded separately for each shoot. To calculate attack levels (i.e. average number of larvae per shoot), one exit hole was counted as one larva that had left the shoot.

### Experimental data

A manipulative experiment was conducted in a common garden at the CABI Bioscience Centre Switzerland, in Delémont, Switzerland, (47°21'N, 7°22'E) in 2000. Potted, bolting plants of garlic mustard were dug into the ground about 50 cm apart on 12 April 2000. We covered each plant individually with gauze bags (55 cm diameter, 150 cm high), and applied the following treatments: 1, 2, 4 and 8 pairs of *C. alliariae*, 1, 2, 4 and 8 pairs of *C. roberti* and 2, 4 and 8 pairs of the combination of both species in a frequency of 1:1. Plants without weevils were established as controls. Each treatment was replicated 10 times. Adults of both species were collected at garlic mustard sites in Switzerland and southern Germany. The fertility of females was tested and fertile weevils placed on the plants according to treatments on 14 and 24 April. Between 14 and 19 June, we cut the shoots of all plants and stored them at 2°C. Between 15 June and 20 July, we dissected all shoots and recorded the number of larvae still present in shoots and the number of exit holes. We also recorded the impact of the different weevil combinations and densities on the growth and reproductive output of garlic mustard as well as the effect on competitive interactions between the two species. These data will be presented in forthcoming papers. In this paper, we will only present and compare data of attack levels from treatments with 2, 4 and 8 pairs of *C. alliariae* and 2, 4 and 8 pairs of the combination of both species.

## Results

Data collected at field sites revealed that garlic mustard shoots are extensively utilized resources. We recorded equally high proportions of attacked shoots and plants at sites where only *C. alliariae* occurred and at sites where both species were present. In the allopatric area, on average  $86.9\% \pm 4.5$  (mean  $\pm$  SE) of plants (range: 70–100%) and  $74.7\% \pm 8.6$  of shoots (range: 40.8–100%) were attacked, while  $87.1\% \pm 5.4$  of plants (range: 40.0–100%) and  $78.0\% \pm 5.9$  of shoots (range: 37.7–100%) were infested in the sympatric area (independent samples t-test: plants:  $t = -0.27$ ,  $df = 14$ ,  $P = 0.979$ ; shoots:  $t = -0.331$ ,  $df = 14$ ,  $P = 0.746$ ). About

four larvae were found mining in each attacked shoot, irrespective of whether only *C. alliariae* or both species were present (Fig. 1, independent samples t-test:  $t = -0.552$ ,  $df = 14$ ,  $P = 0.589$ ).

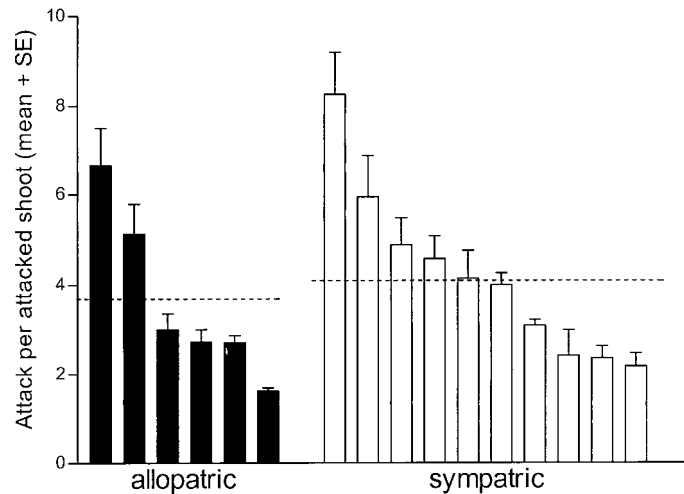
Under experimental conditions, *C. alliariae* reached higher attack levels than the combination of *C. alliariae* and *C. roberti* (Fig. 2, Mann-Whitney test:  $U = 300.00$ ,  $P = 0.027$ ). Because increasing weevil densities did not increase attack levels (Kruskal-Wallis: *C. alliariae*:  $\chi^2 = 0.919$ ,  $df = 2$ ,  $P = 0.632$ ; both species:  $\chi^2 = 0.000$ ,  $df = 2$ ,  $P = 1.000$ ), data were pooled over the different densities.

## Discussion

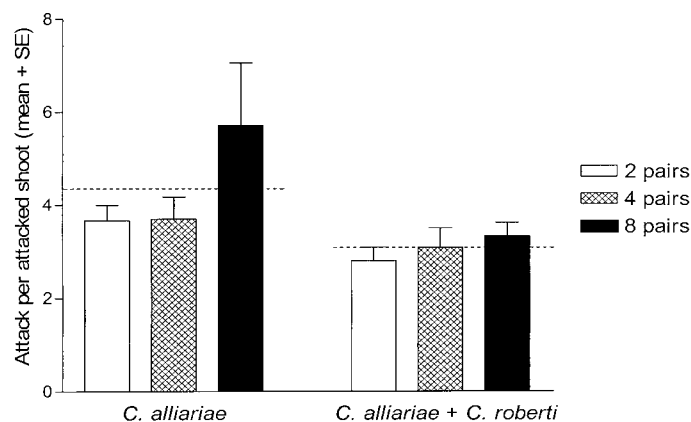
Considering the results from our field and experimental studies, we reach the same conclusion. In both cases, *C.*

*alliariae* alone is at least equally as effective in attacking garlic mustard as in combination with *C. roberti*. Under experimental conditions, *C. alliariae* alone reached even higher attack levels compared to the mixed species treatments. However, the higher attack levels did not result in a higher impact on garlic mustard (Gerber & Hinz, unpublished data). Adults as well as larvae of *C. alliariae* tend to be smaller than *C. roberti*, which might explain this result.

The manipulative experiment also allowed us to investigate potential interactions between the two species. Competitive interactions between agents have been documented in several biological weed control programs (Briese 1991, Woodburn 1996, Story *et al.* 2000). In the worst-case scenario, the more effective agent is displaced by a more competitive, but less effective, agent, thereby reducing the overall impact on the



**Figure 1.** Attack of *Alliaria petiolata* at different field sites – allopatric = field sites in the range where only *C. alliariae* occurs; sympatric = field sites in range where both species occur; black bars = attack by *C. alliariae* at six field sites, white bars = attack by both species at 10 field sites.



**Figure 2.** Attack of *Alliaria petiolata* under experimental conditions. Bars are means of 10 replicates (plants) each – dotted lines = overall mean for each weevil composition. Attack corresponds to the sum of all larvae and exit holes found upon dissection.

target weed (Woodburn 1996). In the case of *C. alliariae* and *C. roberti*, the release of increasing weevil densities did reduce the number of offspring produced per female, however the reduction was the same, whether females of the same or females of both species were released, i.e. intra- and interspecific competition were equally strong (Gerber & Hinz, unpublished data). We therefore do not expect that the two species would negatively affect each other's establishment or impact if co-released. In a similar way to *C. alliariae* and *C. roberti* on garlic mustard, the two leaf beetles *Galerucella californiensis* L. and *G. pusilla* Duftschmid occupy the same fundamental niche on purple loosestrife (*Lythrum salicaria* L.) and have identical competitive abilities (Blossey 1995a). They were jointly released in 1992 for the control of this invasive weed in North America. Up to now, no signs of competitive exclusion or negative effects on their establishment or impact have been observed (B. Blossey, pers. observ.).

For the biological control of leafy spurge, *Euphorbia* spp., five flea beetle species, *Aphthona* spp., were released in North America (Gassmann *et al.* 1996). Larvae and adults of the different species have the same feeding niche on their host plant, but the species differ in their habitat preferences (i.e. three species predominate in open dry habitats, two prefer moister sites), which led to differential establishment according to habitat (Nowierski *et al.* 2002). In the case of the two weevils investigated, *C. alliariae* is reported to prefer shaded habitats, while *C. roberti* is supposed to occur more frequently in open habitats (Pencke 1928, Strejcek 1969). Our investigations confirmed the latter, but we did not find evidence for habitat preferences of *C. alliariae* (Gerber *et al.*, unpublished data). Nevertheless, such subtle differences might contribute to ultimate differences in the impact of these two weevils in different microhabitats.

The advantage of comparing attack levels of two potential agents at field sites is that the species can freely move between plants and choose which shoots to infest, leading to realistic results obtained under natural environmental conditions. In the case of our investigation, the comparison of attack levels is however confounded by the fact that the two ranges, i.e. allopatric and sympatric, lie in different geographical regions that differ in climatic conditions. We cannot exclude the possibility that the conditions in the allopatric area are intrinsically more suitable for the development of *C. alliariae*. In addition, the population of *C. alliariae* in the allopatric area might be higher due to the absence of a key predator or parasitoid. These potential differences are excluded in our experimental approach, conducted under controlled and standardized conditions. Data on the attack levels of *C. alliariae* alone and in combination with *C. roberti* are therefore directly comparable. The disadvantage is, however, that females were confined to individually potted plants, which in itself might influence the outcome of

the experiment if, for instance one of the species is more sensitive to these conditions. Hence, results of such experiments cannot necessarily be extrapolated to natural conditions. In addition, if one of the two species is, for example, more vulnerable to parasitoids or predators, its population size could be limited, and in turn its effectiveness as a biological control agent. Such differences would not have been detected under the experimental conditions used.

Provided *C. alliariae* and *C. roberti* prove to be equally specific once host-range tests are completed, two release strategies can be envisioned:

1. Only one of the two species is released to minimize the danger of potential non-target effects. At the moment, we would suggest that *C. alliariae* should be released first, because it is found equally often in all habitat types (Gerber & Hinz, unpublished data). Its establishment and impact would be closely monitored, and *C. roberti* would be only released if *C. alliariae* fails to establish in all habitats or does not provide the expected impact.
2. Both species are released together. In this case, we would make replicated releases of different combinations of the two species, i.e. *C. alliariae* alone, *C. roberti* alone, and both together, thereby testing the conclusions from our pre-release investigations with the following predictions: firstly, both species will establish and co-exist, and secondly, each species alone will have a similar impact as both species together. Such carefully planned release experiments provide a unique opportunity to test the predictive power of pre-release studies conducted in the area of origin of the target weed and are essential if we want to improve the success rate and credibility of biological weed control programs (Malecki *et al.* 1993, Blossey 1995b).

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