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The impact of the flower mite *Aceria acroptiloni* on the invasive plant Russian knapweed, *Rhaponticum repens*, in its native range

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Abstract *Rhaponticum repens* (L.) Hidalgo is a clonal Asteraceae plant native to Asia and highly invasive in North America. We conducted open-field experiments in Iran to assess the impact of the biological control candidate, *Aceria acroptiloni* Shevchenko & Kovalev (Acari, Eriophyidae), on the target weed. Using three different experimental approaches, we found that mite attack reduced the biomass of *R. repens* shoots by 40–75 %. Except for the initial year of artificial infestation by *A. acroptiloni* of *R. repens* shoots, the number of seed heads was reduced by 60–80 % and the number of seeds by 95–98 %. Morphological investigations of the mite complex attacking *R. repens* at the experimental field site revealed that *A. acroptiloni* was by far the dominant mite species. We conclude that the mite *A. acroptiloni* is a promising biological control candidate inflicting significant impact on the above-ground biomass and reproductive output of the invasive plant *R. repens*.

Keywords Above-ground biomass · Acari · *Acroptilon* · Asteraceae · Classical biological control · Pre-release studies · Seed production

Introduction

The aim of pre-release studies in classical biological weed control projects is not only to experimentally

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assess the host-range of biological control candidates, but also to identify those specialist herbivores that impose significant damage upon the target weed and thereby have the potential to reduce weed densities in the invaded range (Pearson and Callaway 2003). Predicting the efficacy of biological control candidates is far from an easy task (Müller-Schärer and Schaffner 2008), but observational and manipulative field experiments in the native range, paired with information on the population dynamics of the weed in the invaded range, can provide some estimates on the candidate's potential impact on the invasive weed. Comparing quantitative data on the impact of the biological control agents in the native range with those collected post-release can also help refining and improving pre-release efficacy studies in biological control.

Russian knapweed, *Rhaponticum repens* (L.) Hidalgo (*Acroptilon repens* (L.) DC.) is a clonal Asteraceae plant. The native range includes Armenia, Turkmenistan, Uzbekistan, Kazakhstan and parts of Turkey, Iran, Russia, China and Mongolia (Watson 1980). It was accidentally introduced into North America in the late 19th century as a contaminant of alfalfa (*Medicago sativa* L.) seed (Watson 1980). To date, *R. repens* has spread to 45 of the 48 contiguous states of the USA (Zimmerman and Kazmer 1999) and is a declared noxious weed in 23 US states and four Canadian provinces (Rice 2013). There is growing ecological evidence that many North American plants are poorly adapted to *R. repens* (Ni et al. 2010). For example, the biomass of native species in *R. repens* stands was 25–30 times lower in northwestern USA than in Uzbekistan (Callaway et al. 2012). Experimental addition of native plants as seeds significantly increased their abundance in *R. repens* stands in northwestern USA, but the proportion of their biomass relative to total biomass remained over an order of magnitude lower than that of native Asian species in *R. repens* stands in Uzbekistan (Callaway et al. 2012).

In the 1970s, first attempts to control *R. repens* by biological means led to the release of the gall-forming nematode *Subanguina picridis* Kirjanova (Tylenchida, Anguinidae). However, although laboratory experiments suggested that the agent can have a considerable impact on growth and seed output of *R. repens*, it has not proven to be successful in the field (Coombs et al. 2004). In 2010, two additional biological control agents were released in the USA and Canada, i.e. the gall wasp *Aulacidea acroptilonica* V.Bel. (Hymenoptera, Cynipidae) and the gall midge *Jaapiella ivannikovi* Fedotova (Diptera, Cecidomyiidae). Both agents can significantly reduce the reproductive output of *R. repens* (Djamankulova et al. 2008). While the two species have established in North America, their population densities have not yet reached a level where they significantly impact *R. repens* at local or even regional scale (L. Baker, personal communication).

Another specialist natural enemy considered for its potential as a biological control agent against *R. repens* is the eriophyid mite *Aceria acroptiloni* Shevchenko & Kovalev (Acari, Eriophyidae) (Schaffner et al. 2013). Here we report the results of open-field experiments on the impact of *A. acroptiloni* on the target weed conducted in the native range. Since several other mite species are reported from *R. repens* (Schaffner et al. 2013), we compared the morphology of the mites found on the experimental plants to clarify whether any impact observed in these studies could indeed be attributed to *A. acroptiloni*.

Materials and methods

The target weed

Rhaponticum repens is a long-lived, rhizomatous perennial that propagates by vegetative and sexual means (Watson 1980). In North America, *R. repens* attains significantly higher above-ground biomass and cover than in Uzbekistan (Callaway et al. 2012). Also, North American populations produce about four times more seeds than those in the native range (J. Littlefield and U. Schaffner, unpublished results), which is at least partly due to significant herbivore pressure on seed output in the native range (Callaway et al. 2012). While recruitment of seedlings within established *R. repens* patches is rare, it is the most important mechanism by which new sites are colonized within the invaded range. Viable seeds are a common occurrence in the faeces of cattle and wildlife which appears to play an important role in spreading *R. repens* (R. Lang, unpublished report).

The mite species

Aceria acroptiloni was originally described by Shevchenko & Kovalev (Kovalev et al. 1974), based on specimens collected in Uzbekistan and the Ukraine. The host-range of this biological control candidate appears to be restricted to *R. repens* (Schaffner, unpublished results). According to our observations, during the vegetative period all life stages of *A. acroptiloni* can be found on above-ground plant parts, first in lateral vegetative buds and later mainly in flower/seed buds. In October, no live mites were found in the dry seed buds, but some mites were recorded below-ground on dormant root buds, indicating that *A. acroptiloni* hibernates below-ground.

Preliminary morphological studies of mites found on *R. repens* revealed that in Iran more than one mite species are associated with *R. repens* (Chetverikov et al. 2012). We therefore randomly collected 20 infested seed heads from *R. repens* plants in a field margin 5 km NE of Shirvan, Iran, from where the mites were collected for the impact experiment (see below), and morphologically analyzed 25–65 mites per flower head. Identification of the mites was conducted at the Center of Microscopy and Microanalysis of Saint-Petersburg State University (Russia) using a phase contrast light microscopy (PC LM) and confocal laser scanning microscopy (CLSM). CLSM was carried out using a Leica TCS SP2 spectral confocal and multiphoton system microscope at an excitation wavelength of 405 nm, an emission wavelength range of 415–750 nm and at 8 % intensity according to the protocols described in Chetverikov (2012) and Chetverikov et al. (2013). Between 12 and 42 optical slices were recorded from each studied mite specimen. The digital images of the confocal stacks were processed using the Fiji-win32-20091014 Open Source Image Processing Package to obtain maximum intensity.

Experimental design

In spring 2010, the impact of the mite was assessed using three different experimental approaches (referred hereafter as approaches A, B and C). Approaches A and B were set up in late April at a well-protected field with no naturally occurring mite population on the campus of Shirvan University. The field was an abandoned alfalfa field with a mixed vegetation dominated by *R. repens*, *Medicago sativa* L., *Alhagi maurorum* Medik and various *Bromus* spp. In approach A, 20 randomly selected *R. repens* shoots growing at the experimental site were selected for mite infestation, and another 20 were selected as control plants and kept free of mite attack by spraying once every two weeks with the acaricide Propargite (Omite ® 57E; Shimagro-Yazd, Iran; concentration 0.001 %), a non-systemic acaricide with the active ingredient 2-(4-tert-butylphenoxy)cyclohexyl prop-2-yne-1-sulfonate which is widely used for controlling phytophagous mites in crop fields.

All infested shoots were sprayed with an equivalent amount of water. Shoots assigned to the ‘mite attack’ treatment were experimentally inoculated with *A. acroptiloni* in late June 2011 by shaking three mite-infested flower heads over each of the 20 shoots. Inspection under the microscope revealed that flower heads were usually colonized by hundreds or thousands of mites (Asadi and Schaffner, unpublished results).

In approach B, 20 infested and 20 non-infested shoots from site 5 km NE of Shirvan with a natural mite population were transplanted to the experimental site in a completely randomized design on the campus of Shirvan University. This approach was chosen because in previous years the site with the natural mite population was heavily grazed by sheep and goats, and it was therefore uncertain whether we would find enough naturally infested plants at this site in autumn to compare the impact between experimentally and naturally infested plants. All plants were transplanted in late April when the first signs of mite attack became apparent. The plants were collected at the site with the natural mite infestation by randomly selecting a shoot with first signs of mite attack and then selecting a shoot without signs of mite attack 1 m away in a randomly chosen direction. As above, control shoots were treated once every two weeks with an acaricide and the infested shoots with an equivalent amount of water.

Both experimental approaches A and B have advantages and disadvantages. In the first approach, *R. repens* plants were randomly allocated to the treatments ‘mite attack’ and ‘control’, but the colonization by mites occurred significantly later than under natural conditions. In the second approach, shoots were naturally colonized by the mites, but the transplanting of *R. repens* plants fragmented the rhizomal network of this clonal species.

To compare the results of the two experimental approaches with the impact of *A. acroptiloni* on *R.*

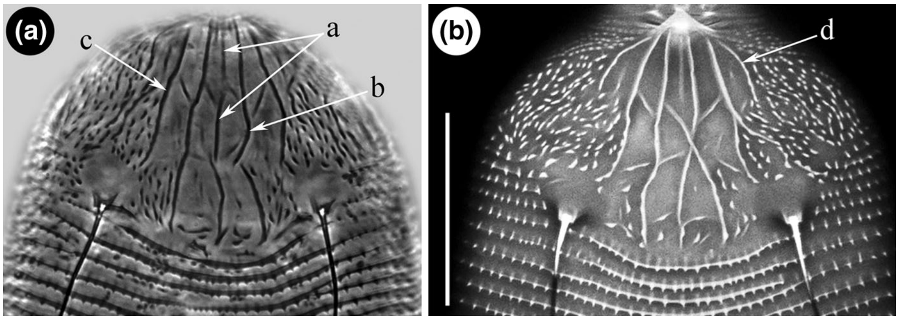

Fig. 1 Phase contrast light microscopy (**a**) and confocal laser scanning microscopy (**b**) images of female prodorsal shield of *Aceria acroptiloni*. Scale bar = 30 μm. Notation: a – median line, b – admedian line (arrow indicates invagination in the middle part of the line), c – submedian-I line, d – submedian-II line

repens under natural conditions, we revisited the field site 5 km outside Shirvan where the mite occurs naturally in late August. Despite some browsing damage, we were able to harvest 20 non-browsed shoots that were attacked by the mite and 20 that showed no signs of mite attack (approach C). At the same time, all 80 shoots from the two experiments (approaches A and B) set up on the campus of Shirvan University in spring 2010 were also harvested and individually bagged. All shoots were dried at 60 °C and weighed, and the number of seed heads and fully developed seeds determined.

In 2011, all three approaches were set up again in an identical manner. However, when harvesting the shoots of the test and control plants all labels that were brought out to mark the experimentally inoculated shoots (i.e. approach A) were left in place. This allowed us to assess the impact of the mite on *R. repens* also in 2012, one year after inoculation, i.e. when the mites had to naturally colonize the new shoots resprouting in spring from below-ground.

The effect of mite attack on shoot biomass, number of seed heads and seed output was assessed using one-way analysis of variance (ANOVA) with herbivory as a fixed factor. The raw data met the assumptions of ANOVA (i.e. normality and homogeneity of variances) and were therefore not transformed. All analyses were carried out using SPSS Statistics 20.

Results

Taxonomic remarks

Some 96 % of the mites found in flower heads of *R. repens* were identified as *A. acroptiloni*. Individuals of *A. acroptiloni* have a distinct design of the prodorsal shield (Fig. 1). Morphologically, *A. acroptiloni* from Iran is very similar to the winter form of *A. acroptiloni* described in Kovalev et al. (1974). The other 4 % of the mites found in flower heads were morphologically distinct from *A. acroptiloni* and resembled *Aceria* cf. *centaureae* (Vidović 2012).

Impact

In 2010, all three approaches revealed a similar impact of *A. acroptiloni* on total above-ground biomass of *R. repens*. On average, mite attack reduced above-ground biomass by 40–60 % (Fig. 2). In contrast, the impact of the mite on seed head production and seed output differed among the different approaches. While the number of seed heads was significantly lower on mite-infested plants in approaches A and C, i.e. in those approaches where *R. repens* plants were not transplanted, both infested and non-infested transplanted plants in approach B produced very few flower heads. In terms of seed output, no effect was found in approach A where the plants were artificially inoculated with *A. acroptiloni*. In contrast, transplanted *R. repens* shoots with natural infestation of *A. acroptiloni* and infested shoots collected at the natural site at the end of the season produced on average only 1 % and 6 % of the seeds produced by the shoots in the control group, respectively (Fig. 2).

In 2011, all three approaches revealed a highly significant reduction in shoot biomass, number of seed heads and number of seeds produced. Mite attack reduced shoot biomass by 50–75 %, the number of seed heads by 60–80 % and the number of seeds by 95–97 % (Fig. 3). In 2012, shoots emerging beside

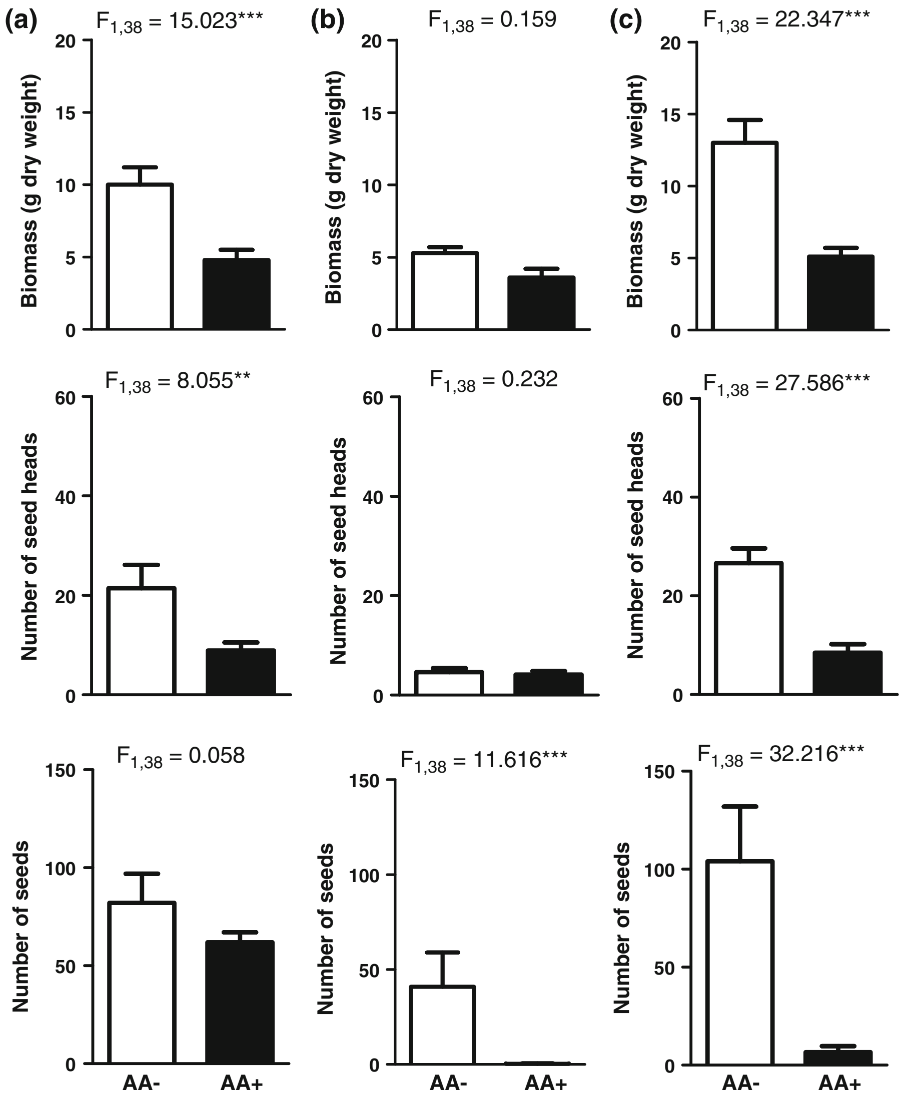

Fig. 2 Biomass (top), number of seed heads (middle) and number of seeds (bottom) of *Rhaponticum repens* shoots inoculated with *Aceria acroptiloni* (black bars), or kept free of mite attack (white bars) in 2010. **a** *R. repens* shoots growing on the experimental site were either inoculated with the mite or kept free of mite attack by spraying an acaricide, **b** Naturally infested and non-infested shoots were transplanted from a natural site to an experimental site in a completely randomized order in early spring 2011; non-infested shoots were sprayed with acaricide, **c** Infested and non-infested shoots were randomly collected at a field site with a natural population of *A. acroptiloni*. AA+ = with *A. acroptiloni*, AA− = without *A. acroptiloni*. *Bars* are means + S.E. ** $P < 0.01$, *** $P < 0.001$ (ANOVA)

fourteen out of twenty labels that indicated the position of the previous year's inoculated shoots were infested by *A. acroptiloni*. Infestation of the re-sprouting shoots reduced the shoot biomass by 75 %, the number of seed heads by 78 % and the number of seeds by 97 %, compared to non-infested re-sprouting *R. repens* shoots (Fig. 4).

Discussion

Mites of the genus *Aceria* are commonly regarded as highly specialized herbivores, and they often inflict significant damage on their host-plants (Skoracka and Kuczynski 2012). For example, *Aceria salsolae* de Lillo and Sobhian reduced the size of the invasive

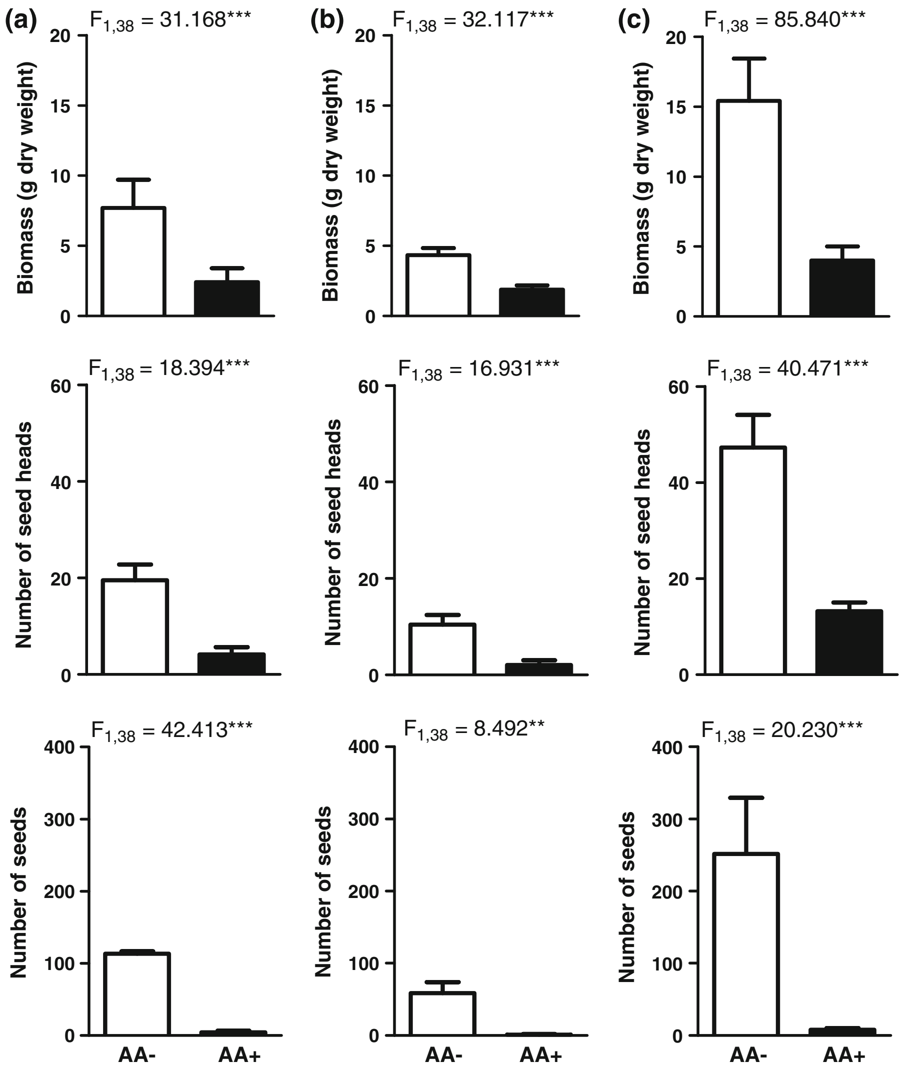

Fig. 3 Biomass (top), number of seed heads (middle) and number of seeds (bottom) of *Rhaponticum repens* shoots inoculated with *Aceria acroptiloni* (black bars), or kept free of mite attack (white bars) in 2011. For further details see Fig. 2

plant *Salsola tragus* L (Chenopodiaceae) by 66 % within 25 weeks after infestation (Smith 2005). *Aceria* mites have therefore been repeatedly considered for use as classical biological control agents of weeds (Smith et al. 2010; Urban et al. 2011). The results of our open-field impact studies indicate that the mite *A. acroptiloni* is also a promising biological control candidate, inflicting significant impact on the above-ground biomass and particularly on the reproductive output of the invasive plant *R. repens*. In our experiment, transplanted shoots (approach B; Fig. 2b, c) grew smaller and produced fewer seed heads than undamaged *R. repens* shoots. In 2010, the impact of *A. acroptiloni* on above-ground biomass and number of seed heads of transplanted *R. repens* shoots was non-significant, suggesting that the mite causes particularly high damage on vigorously growing *R. repens* plants. Nevertheless, *A. acroptiloni* was able to colonize the seed heads of transplanted shoots and significant reduce seed production in both years where shoots were transplanted.

Morphological investigations of the mites attacking *R. repens* at the experimental field site revealed that in Iran at least three mite species can be found on *R. repens* (Chetverikov et al. 2012). However, *A. acroptiloni* was by far the dominant mite species,

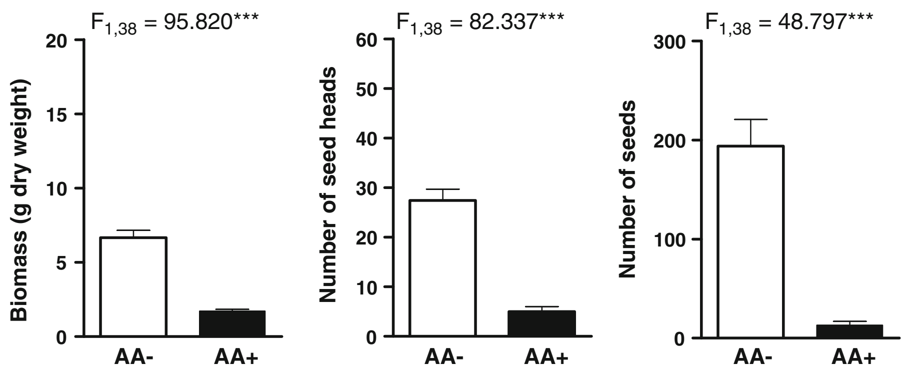

Fig. 4 Biomass (left), number of seed heads (middle) and number of seeds (right) of *Rhaponticum repens* shoots in 2012, i.e. one year after inoculation with *Aceria acroptiloni* (black bars), compared with shoots kept free of mite attack (white bars). For further details see Fig. 2

particularly in the flower heads. This strongly suggests that the impact detected in the different approaches can be attributed to *A. acroptiloni*. One year after experimental transfer of the mite, most shoots emerging next to the labels that marked the position of the shoots that were inoculated in the previous year were infested by *A. acroptiloni*. Dissections of *R. repens* roots in autumn 2013 revealed that at least some *A. acroptiloni* mites hibernated on below-ground buds from which shoots grow out in the next growing season. Interestingly, the impact of the mite at the experimental site reached the same levels as at the natural field site. These results suggest that establishment of new *A. acroptiloni* populations (e.g. in North America) should be relatively easy and should lead to immediate and consistent impact on shoot growth and particularly on reproductive output.

The impact studies were carried out in the native range of *A. acroptiloni*, raising the question whether such studies accurately predict the impact of this biological control candidate in the invaded range. Post-release studies with *Jaapiella ivannikovi* Fedotova (Diptera, Cecidomyiidae), a gall midge that has recently been released in North America as a biological control agent of *R. repens*, revealed that the impact measured in the native range (Djamankulova et al. 2008) accurately predicted the impact of *J. ivannikovi* on growth and reproductive output of the target weed at the shoot level in the introduced range (Baker and Pieropan, unpublished results). While pre-release studies as those carried out with *J. ivannikovi* and *A. acroptiloni* may therefore be suitable to predict impact of biological control candidates at the individual plant level, their impact on the population dynamics of the invasive plant species also depends on the population dynamics of the biological control candidate (Zalucki and van Klinken 2006; Müller-Schärer and Schaffner 2008). The latter is likely to be affected by the new biotic (e.g. release from specialist natural enemies) and abiotic conditions in the new range and therefore more difficult to assess during pre-release studies in the native range.

Its clonal, vegetative growth form and extensive underground root system make *R. repens* a difficult weed to control. None of the specialist insects that have been approved or that are considered for biological control of *R. repens* in North America appears to be able to kill established *R. repens* clones. However, the results from this study indicate that *A. acroptiloni* has the potential to significantly reduce seed output of *R. repens*. Reduced seed output is unlikely to affect the cover of *R. repens* in invaded sites, since seedling recruitment has not been observed in North America inside established *R. repens* stands (Callaway et al. 2012). Yet, a significant reduction of seed output is likely to slow down the spread of this invader. Importantly, *A. acroptiloni* and—to a lesser extent—the gall midge *J. ivannikovi* (Djamankulova et al. 2008) also considerably reduce the plant's above-ground biomass. Whether a reduced standing biomass or cover will promote the recovery of native species depends on the type of competitive interaction between *R. repens* and the native North American plant species. Recently, Sun et al. (2013) suggested that in the case of the closely related *Centaurea stoebe* L., impact in the home range appears to be driven by competition for the same limiting resources, but by other factors in the introduced range, possibly by exploitation of resources that are not used by the new neighbours or by interference competition. Findings by Ni et al. (2010) and Callaway et al. (2012) suggest that the way *R. repens* competes with species from the native and the introduced range also differs. This distinction has important consequences for the management of *R. repens*. If impact by *R. repens* is indeed driven by interference competition, ecosystem recovery is less likely to follow after simple biomass reduction. To promote habitat restoration, a reduction in above-ground biomass of *R. repens* due to biological control may need to be accompanied by sowing of native plant species that are not, or only moderately, susceptible to direct interference by *R. repens*.

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