Annual Report 2011

Biological control of Russian knapweed, *Acroptilon repens*

U. Schaffner, K. Dingle, C. Swart and M. Cristofaro

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Summary

1. This report summarizes the results of investigations carried out in 2011 on the ecology and host specificity of potential biological control agents of Russian knapweed, *Acroptilon repens*, as well as on the biology of the target weed. The work was done in close collaboration with the Biotechnology and Biological Control Agency (BBCA), Rome, Italy, Montana State University, Bozeman, USA, the Uzbek Academy of Sciences, Tashkent, Uzbekistan, and the University of Mashhad, Iran.

2. Galls of the gall wasp *Aulacidea acroptilonica* (Hymenoptera, Cynipidae) and the gall midge *Jaapiella ivannikovi* (Diptera, Cecidomyiidae) were collected in Uzbekistan in autumn 2010 and spring 2011 and sent to quarantine facilities in North America. Both biological control agents have established and successfully overwintered in the field.

3. In early April, a field experiment was set up at the University of Shirvan, Iran, to further explore the host range of the mite *Aceria* sp. near *acroptiloni*. None of the test plants, including three *Centaurea* spp., revealed signs of mite attack. In contrast, all inoculated Russian knapweed plants were damaged by *Aceria* sp. near *acroptiloni*.

4. Additional impact studies were carried out with the mite *Aceria* sp. near *acroptiloni* in Iran. The different experimental approaches used in the impact study revealed that mite attack can reduce above-ground biomass by 50–75%, the number of flower heads by 80% and seed production by >95%.

5. Preliminary morphological characterization of the mites extracted from test and control plants used in an open-field host-range test in 2010 revealed that at least three different *Aceria* species are associated with *Acroptilon repens* and that the original description of the species *Aceria acroptiloni* may need revision. Therefore, it is planned to collect mites from different parts of Russian knapweed plants (leaves, flower buds, belowground vegetative buds) in 2012 and to characterize them by morphological and molecular means.

6. During field surveys carried out in May in Uzbekistan no larvae of the chrysomelid *Galeruca* sp. were found. Therefore, continuation of field host-range testing had to be postponed to 2012.
1. Introduction

Russian knapweed, *Acroptilon repens*, a long-lived perennial native to Asia, was accidentally introduced into North America in the late 19th century as a contaminant of alfalfa seed. To date, Russian knapweed has spread to 45 of the 48 contiguous states of the USA (Zimmerman and Kazmer, 1999) and is considered noxious in 18 states (USDA, 2012) and in one Canadian province (Alberta).

Its clonal, vegetative growth form and extensive underground root system make Russian knapweed a difficult weed to control. Moreover, despite the fact that an earlier paper claiming to have identified a secondary metabolite in Russian knapweed with allelopathic properties was retracted (Stermitz et al., 2009), there is growing ecological evidence that many North American plants are poorly adapted to Russian knapweed. For example, the biomass of native species in *A. repens* stands was 25–30 times lower in the non-native range than in the native range (Callaway et al., 2011). Experimental addition of native species as seeds significantly increased the abundance of natives at one North American site, but the proportion of native biomass even with seed addition remained over an order of magnitude lower than that of native species in *A. repens* stands in Uzbekistan (Callaway et al., 2011).

*A. repens* propagates by vegetative and sexual means (Watson, 1980). 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. While recruitment of seedlings within established *A. 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 appear to play an important role in spreading *A. repens* (R. Lang, unpublished report).

Since large-scale chemical control is detrimental to the environment and uneconomic on low-value land, first attempts to control this invasive species by biological means were made in the 1970s. The first agent released against Russian knapweed was the nematode *Subanguinea picridis*. Unfortunately, even though laboratory experiments suggested that this agent can have considerable impact on growth and seed output of Russian knapweed, it has not proven to be successful in the field (Coombs et al., 2004). No other biological control candidates were considered for field release at that time.

In 1997, CABI in Switzerland started surveying various areas in the native range to assess the herbivores associated with Russian knapweed, and studying the biology and host specificity of new biological control candidates. Two of the herbivores found in Asia, i.e. the gall wasp *Aulacidea acroptilonica* and the gall midge *Jaapiella ivannikovi*, proved to be highly specific and impose significant impact on Russian knapweed (Djamankulova et al., 2008). Both herbivores were approved for field release in the USA and Canada. However, since it is not clear yet whether the two agents will be able to build up high enough densities to prevent further spread of Russian knapweed in North America, exploration in the native range of Russian knapweed to find new biological control candidates is being pursued. This report summarizes the work conducted in 2011.
2. Work Programme for Period under Report

In line with the results obtained during the field season 2010, it was proposed that work in 2011 should focus on the following elements:

**Aulacidea acroptilonica** (Hymenoptera, Cynipidae)
- Collect galls in Uzbekistan;
- Send galls to US quarantine facilities.

**Jaapiella ivannikovi** (Diptera, Cecidomyiidae)
- Collect galls in Uzbekistan;
- Send galls to US quarantine facilities.

**Aceria sp. near acroptiloni** (Acari, Eriophyoidae) from Iran
- Continue host-range testing in the field;
- Continue impact experiment in the field;
- Collect mites and start host-range testing at CABI.

**Urophora spp.** (Diptera, Tephritidae) from Uzbekistan
- Collect gall flies and send them to a US quarantine facility.

**Galeruca sp.** (Coleoptera, Chrysomelidae) from Uzbekistan
- Collect adults and larvae in Uzbekistan;
- Continue with host-range testing.

Field surveys
- Survey western regions in Iran;
- Survey central China.

The work in 2011 was carried out in close collaboration with the Biotechnology and Biological Control Agency (BBCA), Rome, Italy, Montana State University, Bozeman, USA, the Uzbek Academy of Sciences, Tashkent, Uzbekistan, and the University of Mashhad, Iran.

3. **Aulacidea acroptilonica** TYUREBAEV (Hym., Cynipidae)

3.1 Activities in 2011

In early spring 2011, a shipment of approximately 400 galls was made to the quarantine facility of AAFC (Agriculture and Agri-Food) Canada, Lethbridge, Alberta, to support continued rearing of this biological control agent. In contrast to previous years, good emergence of gall wasps was obtained, which allowed several field releases to be made in Alberta.

New gall collections were made in Uzbekistan in fall 2011, and the galls were shipped to CABI in Switzerland for overwintering.

3.2 Summary and outlook

According to our colleagues in North America, the gall wasp *A. acroptilonica* has successfully established at a few sites in the USA. At one site in Montana, a relatively large population has built up – 1600 galls were counted – that may allow
redistribution of the gall wasp from 2012 onwards (J. Littlefield, personal communication).

Additional shipments will be made in 2012 to maintain the ongoing quarantine rearing in North America.

4. **Jaapiella ivannikovi** FEDOTOVA (Dipt., Cecidomyiidae)

This biological control agent was first released in 2009 at several sites in Wyoming and Montana. It successfully established and overwintered at most of the release sites.

4.1 Activities in 2011

In May, some 300 *J. ivannikovi* galls were collected in Uzbekistan and shipped to the quarantine facility in Bozeman, Montana, to support continued rearing of this biological control agent.

4.2 Summary and outlook

In 2011, additional releases of the gall midge were made in the USA and in Canada. For example, the USDA-APHIS-PPQ-CPHST (Center for Plant Health Science and Technology) Fort Collins Lab initiated a greenhouse-based rearing programme that was designed to produce galls for field release. In spring and summer 2011, *J. ivannikovi* was provided for release at 19 sites in seven western region states. In 2012, the release sites will be monitored to confirm overwintering and establishment, and more galls will be provided for field releases (R. Hansen, personal communication).

While the overwintering of the gall midge in North America appears to be unproblematic, the build-up of high population densities, and consequently the impact, may depend on the availability of Russian knapweed regrowth during late spring and summer, when the second and potentially third generations of *J. ivannikovi* are active. Observations from the native range indicate that females prefer to oviposit on bolting stems. Also, the impact of the gall midge is highest when eggs are laid into the meristematic tissue of the main shoot.

Additional collections will be made in Uzbekistan in 2012 to maintain the ongoing quarantine rearing in North America.

5. **Aceria** sp. near *acroptiloni* (Acari, Eriophyoidae) from Iran

In 2006, an eriophyoid mite species was found attacking *Acrop tilon repens* in north-eastern Iran. Since the damage inflicted by this mite differs from that described for *Aceria acroptiloni* (Kovalev et al., 1975), the taxonomic status of the population found in north-eastern Iran was questioned (R. Sobhian, personal communication).

At the sites where the mite occurs naturally, numerous closely related native plant species grow interspersed with mite-attacked *Acrop tilon repens* plants, including *Cen tau rea depressa*, *C. squarrosa*, *C. iranicus*, *Carduus acanthoides* and *Carthamus lanatus*. However, careful inspection of these plants did not reveal any evidence for mite damage. Moreover, attacked Russian knapweed plants were significantly stunted and had far fewer flower heads than healthy plants. It was therefore
concluded that *Aceria* sp. near *acroptiloni* is a promising candidate for the biological control of Russian knapweed in North America.

In spring, Russian knapweed shoots attacked by *Aceria* sp. near *acroptiloni* show a characteristic deformation and discoloration (from greenish to yellowish) of the young leaves of the main or side shoots. Dissection of the buds in May revealed relatively low numbers of eggs, crawlers and adults per infested bud. All stages are usually found next to the youngest, softest tissue inside the buds. Later in the season, adults as well as thousands of eggs and crawlers can be found inside newly formed flower heads. These observations suggest that the mite has two generations per year. How and when freshly emerging shoots are infested by the mite was, however, unclear. Therefore, a field site with a natural infestation of *A. sp. near acroptiloni* was visited in early spring, i.e. before shoots emerged above-ground, and several rootstocks were dug up. Visual inspection of the rootstocks revealed that the mites overwinter inside or on the dormant shoot buds (Plate 1). Hence, new shoots are already infested by the mite before they appear above-ground. These findings potentially offer a new way to inoculate test and control plants in host-range tests, i.e. by transferring pieces of infested rootstock next to the roots of test and control plants in early spring and monitoring whether mites move from the decaying rootstock onto the test and control plants over the season.

**Plate 1.** Rootstock of *Acroptilon repens* with below-ground buds attacked by overwintering *Aceria* sp. near *acroptiloni* (photo: G. Asadi).

### 5.1 Identification of mites found in open-field test 2010

**METHODS** All test and control plants from the open-field host-range test carried out in the garden of the University of Shirvan were harvested in autumn 2010 and individually stored in paper bags (Schaffner et al., 2011). In early 2011, one hundred of the 200 shoots harvested in autumn 2010 were sent to BBCA, Rome, for quantitative extraction of the mites. Extraction of the mites was performed using a protocol developed by E. de Lillo (University of Bari, Italy).

**RESULTS** The quantitative extraction of the mites revealed that significantly more plants were infested by mites than was apparent from visual inspection of control and test plants (Table 1, below; Schaffner et al., 2011).

During the counting process, first doubts began to emerge regarding the taxonomic identity of the mites found on Russian knapweed and test plant species. Therefore,
mites extracted from individual plants were stored in vials filled with 100% ethanol, and the vials sent to Dr Philipp Chetverikov, Russian Academy of Sciences, St Petersburg, Russia, for identification.

The eriophyoid species *Aceria acroptiloni* was originally described by Kovalev *et al.* (1975) based on material collected from *Acroptilon repens* in Uzbekistan and Ukraine. According to Kovalev *et al.* (1975), *Aceria acroptiloni* has one form of male and three morphologically different forms of females (winter, spring and summer forms). Using light and laser confocal scanning microscopy (LCSM), at least three different species of the genus *Aceria* were found in the samples from the open-field host-range test. All of them were represented by numerous females and single males, confirming that they are separate species. All three species were found on *Acroptilon repens* and on one or a few test plant species. After comparing measurements and LCSM images of the *Aceria* taxa with the original description of *A. acroptiloni*, it is provisionally concluded that: (i) females of *Aceria* sp. 1 are morphologically identical to the winter female form of *A. acroptiloni* (Plates 2 and 3), (ii) females of *Aceria* sp. 2 to the spring female form, and (iii) females of *Aceria* sp. 3 to the summer female form described by Kovalev *et al.* (1975). Hence, Kovalev *et al.* (1975) might have mistakenly described females of three different *Aceria* species as different female forms of a single species (*A. acroptiloni*). First morphological investigations of the mite samples extracted from the test plants revealed that most of the mites found on *Cirsium arvense* belonged to *Aceria* sp. 3. None of the other test plants revealed signs of damage, including *Carthamus tinctorius* (safflower) and *Cynara scolymus* (artichoke) on which a few specimens of *Aceria* sp. 1 were found (P. Chetverikov, personal communication).

**Table 1.** Number of mites extracted from control and test plants from the open-field host-specificity experiment carried out with the mite *Aceria* sp. near *acroptiloni* in 2010 in Shirvan, Iran.

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Replicates</th>
<th># of replicates with mites</th>
<th># of mites per replicate (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acroptilon repens</em></td>
<td>10</td>
<td>10</td>
<td>1058 ± 234b</td>
</tr>
<tr>
<td><em>Centaurea iranica</em></td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>C. squarrosa</em></td>
<td>10</td>
<td>1</td>
<td>1 ± 1</td>
</tr>
<tr>
<td><em>C. depressa</em></td>
<td>10</td>
<td>4</td>
<td>50 ± 39</td>
</tr>
<tr>
<td><em>Cirsium arvense</em></td>
<td>9</td>
<td>9</td>
<td>3631 ± 1074c</td>
</tr>
<tr>
<td><em>Carthamus tinctorius</em></td>
<td>10</td>
<td>8</td>
<td>275 ± 104d</td>
</tr>
<tr>
<td><em>C. lanatus</em></td>
<td>10</td>
<td>2</td>
<td>17 ± 11</td>
</tr>
<tr>
<td><em>Cynara scolymus</em></td>
<td>10</td>
<td>1</td>
<td>17 ± 17d</td>
</tr>
<tr>
<td><em>Lactuca serriola</em></td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Silybum marianum</em></td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

a Crop species.

b Specimens mainly assigned to *Aceria* sp. 1, but also to *Aceria* spp. 2 and 3 (see text).

c Specimens assigned to *Aceria* spp. 2 and 3 (see text).

d Specimens assigned to *Aceria* sp. 1 (see text).
Plates 2 and 3. Female (left) and male (right) shields of *Aceria* sp. 1 collected from *Acroptilon repens* plants in an open-field host-specificity test set up in Shirvan, Iran (photos: P. Chetverikov).

5.2 Laboratory host-range test

**METHODS** In early May 2011, mite-infested Russian knapweed shoots were hand-carried from Iran to CABI's quarantine facility in Switzerland to assess different methods for artificially inoculating potted Russian knapweed and test plants under quarantine conditions. Inspection of the main and lateral buds of the field-collected shoots under the microscope revealed that adults and crawlers were still alive and active. The following approaches were examined for their suitability for inoculating potted Russian knapweed plants: (i) lateral buds with signs of mite attack (curved, discolored leaf tips) were transferred onto leaf axils of Russian knapweed plants; (ii) wrapper leaves of the lateral buds were carefully removed until the meristematic tissue, where adults, eggs and crawlers are found, was exposed – the open buds were then transferred to leaf axils of potted Russian knapweed plants; (iii) crawlers and adults were individually placed next to lateral buds of potted *Acroptilon repens* plants. In parallel to the tests conducted with Russian knapweed, 15 different test plant species were inoculated using either approach (i) or (ii).

In late June, mite-infested flower buds were hand-carried to CABI to repeat efforts to set up a no-choice host-range test under quarantine conditions, but for unknown reasons the millions of mites that were found inside the flower buds were all dead by the time the plant material arrived in Delémont.

**RESULTS** Leaves and buds of the Russian knapweed and test plants inoculated in May 2011 in CABI's quarantine facility in Switzerland were repeatedly inspected under the microscope, but no living mites were found.

5.3 Open-field host-range test

**METHODS** In early April 2011, i.e. at a time when the results from the morphological identification of the mite samples were not yet available, a new field experiment was set up at the University of Shirvan (about 200 km west of Mashhad) in collaboration with Dr Ghorbani and Dr Asadi, Mashhad University. One hundred $50 \times 50$ cm plots were set up in ten rows of ten plots each, and the top 10 cm of each plot was ploughed and all vegetation removed. In May, ten replicates each of *A. repens* and nine test plant species (Table 2) were planted into the plots in a Latin
square. Seeds of *Carthamus tinctorius* were germinated in pots filled with garden soil, and the rosettes transplanted to the experimental plots in mid May 2010. At the same time, rosettes or bolting plants of all other test plant species and of Russian knapweed were collected nearby and transplanted into the experimental plots. In June, flower heads of Russian knapweed infested with the mite were collected from a site outside Shirvan where there was a large population of the mite, taken back to the laboratory and checked under the microscope for the presence of the mite. Flower heads infested with the mite were cut open, and three buds were shaken over each control and test plant. In addition, healthy artichoke plants growing in the medicinal garden of the experimental farm of Mashhad University (Plate 4) were inoculated with *Aceria* sp. *near acroptiloni* by shaking opened mite-infested flower buds over the plants. As a control, Russian knapweed plants growing at the edge of the medicinal garden were inoculated using the same method.

During summer 2011, all plants were regularly checked for signs of mite infestation. In mid August, when the plants started to show senescence symptoms, the experiment was stopped and the test and control plants harvested. The plants were individually bagged in paper bags, taken back to Mashhad University and air-dried.

**RESULTS** In the established open-field test plots, all inoculated Russian knapweed plants were attacked by *Aceria* sp. *near acroptiloni* (Table 2). In contrast, none of the test plants revealed signs of mite attack.

Neither the artificially inoculated artichoke plants growing in the medicinal garden of the experimental farm nor the control plants revealed signs of mite attack. This test will therefore be repeated in 2012.
Table 2. Results of the open-field test with the mite *Aceria* sp. near *acroptiloni* carried out in 2011 in Shirvan, Iran.

<table>
<thead>
<tr>
<th>Plant Species</th>
<th># of plants exposed</th>
<th># of plants attacked</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acroptilon repens</em></td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td><em>Centaurea iranica</em></td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td><em>C. squarrosa</em></td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td><em>C. depressa</em></td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td><em>Cirsium arvense</em></td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td><em>Carthamus tinctorius</em> a</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td><em>C. lanatus</em></td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td><em>Cynara scolymus</em> a</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td><em>Lactuca serriola</em></td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td><em>Silybum marianum</em></td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

a Crop species.

5.4 Impact experiment

Methods. In collaboration with Dr Ghorbani and Dr Asadi, Mashhad University, a new field experiment was set up near Shirvan, to assess the impact of the mite on above-ground biomass and seed output of *Acroptilon repens*. As in 2010, we took three different approaches to quantify the impact of the mite on *A. repens*. The first two experimental designs were set up at a well-protected field site on the campus of Shirvan University with no naturally occurring mite population.

In the first experiment, 20 randomly selected *A. repens* shoots growing at the experimental site were either inoculated with the mite, or kept free of mite attack by spraying an acaricide (control shoots). Russian knapweed shoots were experimentally inoculated with *Aceria* sp. near *acroptiloni* in late June by shaking three mite-infested flower heads over each of the 20 shoots that were assigned to the ‘mite attack’ treatment.

In a second experiment, 20 infested and 20 non-infested shoots from a site with a natural mite population were transplanted to the experimental site of the campus of Shirvan University in a completely randomized design. This approach was chosen because the site with the natural mite population is regularly grazed, and we assumed that most Russian knapweed plants at this site would be heavily damaged by grazing at the end of the season. 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 bi-weekly with an acaricide and the infested shoots with an equivalent amount of water.

Both experimental approaches have some advantages and disadvantages: in the first approach, *Acroptilon repens* plants were randomly allocated to the treatments ‘with 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 transplanting *A. repens* plants means destroying the root
system of this plant, and in particular fragmenting and weakening its rhizome. We therefore revisited the natural field site in late August to check whether it was possible to compare the results from the two experimental approaches with non-experimental, observational data at a site with a natural infestation of *A. repens* by *Aceria* sp. near *acroptiloni*. Luckily, we found enough undisturbed plants to harvest twenty shoots each that were either attacked by the mite or that showed no signs of mite attack. The 40 shoots were individually bagged and taken back to the institute. At the same time, all 80 shoots from the two experiments set up on the campus of Shirvan University were also harvested and individually bagged. All shoots were dried at 60°C and weighed. All shoots were transported to the University of Mashhad to count the number of flower heads and mature seeds.

In contrast to 2010, we left all the labels at the site of the first experiment. This will allow us to experimentally assess the impact of the mite on Russian knapweed in the second year after inoculation, i.e. when the mites recolonize the new shoots in spring from below-ground, since artificial inoculation of *Acroptilon repens* shoots in late spring may not lead to significant impact during the same growing season.

**RESULTS**

While the absolute values for above-ground biomass and number of flower heads produced varied among the different approaches, they all revealed a similar relative impact of the mite on above-ground biomass and the production of flower heads by *A. repens*. On average, mite attack reduced above-ground biomass by 55–75% and the number of flower heads by 80% (Figure 1). Moreover, seed production at the natural sites was reduced by more than 95%. The data on seed reduction from the two experimental approaches are still pending.

These results strongly suggest that *Aceria* sp. near *acroptiloni* has a significant impact on above-ground biomass and reproductive output of *Acroptilon repens*. The relatively low biomass and number of flower heads recorded in the second approach is a direct consequence of the transplanting of Russian knapweed rootstocks in spring.

It should be noted, though, that it remains to be shown whether the impact measured in this impact experiment is due to one specific mite species, or whether it is the result of combined impact of two or three of the mite species associated with *A. repens*. The outcome will largely depend on whether the mites found in the flower heads belong to one or several species.
Figure 1. Above-ground biomass, number of flowers and number of seeds of *Acroptilon repens* shoots inoculated with *Aceria* sp. near *acroptiloni* (black bars), or kept free of mite attack (white bars). (A) *Acroptilon 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 complete 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 *Aceria* sp. near *acroptiloni*. Bars are means ± S.E. *** P < 0.001 (ANOVA).
Plate 5. In the mite impact experiment set up in the garden of Shirvan University, Iran, individually labelled *Acroptilon repens* shoots were either inoculated with the mite *Aceria* sp. near *acroptiloni* or kept free of mite attack (approach 1). In autumn, all labels were left in the experimental plot, which will also allow us to assess the impact of the mite in the second year after experimental inoculation.

5.5 Summary and outlook

The new findings obtained in 2011 clearly indicate that there is a complex of mite species attacking *Acroptilon repens*. Therefore, emphasis in 2012 will be put on re-describing *Aceria acroptiloni* and on describing the two apparently new *Aceria* spp. associated with *Acroptilon repens*. In parallel, the biology of the different *Aceria* species needs to be assessed in more detail in order to elaborate experimental approaches that will allow testing the host-range of *A. acroptiloni* and potentially of the other mite species associated with *Acroptilon repens*. Molecular and morphological identification of the *Aceria* spp. found on the rootstocks, inside the main and the lateral buds of the shoots and in the flower heads will help to identify the best methods for inoculating test and control plants with single mite species only. Also, test and control plants will be grown in greenhouses to reduce the risk of unintentional colonization of the plants with mites.

The extraction of mites from the test and control plants from the open-field experiment conducted in 2010 revealed that mites were found on various test plants, but usually in significantly lower numbers than found on *Acroptilon repens*. The only test plant on which high numbers of mites were detected was *Cirsium arvense*. The species found on *C. arvense* was *Aceria* sp. 3, which is a different species to that commonly found on *Acroptilon repens* (*Aceria* sp. 1). It remains to be seen how the presence of a few mites on test plants under open-field conditions should be interpreted in terms of non-target risks.

In any case, a continuation of the in-depth studies on *Aceria* sp. 1 associated with *Acroptilon repens* is well justified, since this mite is likely to be the reason for the high reduction in above-ground biomass and reproductive output found in the impact experiment.
6. *Galeruca* sp. from Uzbekistan

Adults reared from larvae collected on *A. repens* near Samarkant, Uzbekistan, were sent to taxonomists, but the identification of this species is still pending. Identification of this species may be difficult because the genus *Galeruca* is likely to include species complexes, consisting of genetically isolated species with distinct life-history traits. Ivannikov *et al.* (1976) reported that a chrysomelid species found feeding on Russian knapweed in Kazakhstan was identified as *Galeruca interrupta* ssp. *armeniaca*.

6.1 Activities in 2011

To assess the host specificity of this beetle under field conditions, we planned to release early-instar larvae onto test plants growing on the campus of the Institute of Zoology, Tashkent, and cover the plants with mesh bags. However, no adults or early-instar larvae were found during three trips to the area of Samarkant in May 2011. The planned field experiment was therefore postponed to 2012.

6.2 Summary and outlook

While the *Galeruca* sp. feeding on *A. repens* in Uzbekistan can defoliate Russian knapweed shoots entirely, its host range appears to be broader than that of the biological control agents that have already been released in North America against Russian knapweed, and most probably also broader than the host range of the mite *Aceria* sp. near *acroptiloni* (see above). Preliminary host-range studies indicated that late-instar larvae feed on species belonging to the genera *Acroptilon*, *Centaurea* and *Saussurea*. So far, we have not been able to verify whether *Galeruca* sp. oviposits and can complete its entire larval development (from first-instar to pupation) on *Saussurea* species. However, the fact that larvae feed on members of this genus is problematic since six species of *Saussurea* are native to North America. Since safflower (*Carthamus tinctorius*) and *Serratula tinctoria*, which belong to the subtribe Centaureinae, are not attacked by this beetle, larval feeding on the more distantly related *Saussurea discolor* (subtribe Carduinae) suggests that *Galeruca* sp. has a disjunct host range. Provided that female adults or early-instar larvae can be collected in early spring 2012, emphasis will therefore be put on testing the host specificity of freshly hatched larvae on safflower and plant species that belong to the subtribe Carduinae.

7. Field Surveys

It was originally planned to conduct additional surveys for new biological control candidates in parts of Asia that have not been surveyed so far, such as western Iran and central China. However, because of the new questions that came up in late 2010/early 2011 regarding the mite *Aceria* sp. near *acroptiloni*, which is currently our prime biological control candidate, we decided to allocate additional time and funding towards resolving the taxonomic and experimental problems described above.

8. Work Programme Proposed for 2012

Based on the work carried out in 2011, the following work plan is being proposed for 2012:
Aulacidea acroptilonica (Hymenoptera, Cynipidae)
• Collect galls in Uzbekistan;
• Send galls to US quarantines.

Jaapiella ivannikovi (Diptera, Cecidomyiidae)
• Collect galls in Uzbekistan;
• Send galls to US quarantines.

Aceria sp. (Acari, Eriophyoidae) from Iran
• Continue host-range testing in the field;
• Continue impact experiment in the field;
• Collect mite and start host-range testing at CABI.

Urophora spp. (Diptera, Tephritidae) from Uzbekistan
• Collect gall flies and send them to US quarantine.

Galeruca sp. (Coleoptera, Chrysomelidae) from Uzbekistan
• Collect adults and larvae in Uzbekistan;
• Continue with host-range testing.

Field surveys:
• Survey western regions in Iran.

9. Acknowledgements

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10. References


# Distribution list

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