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Weed Biological Control

Progress Report 2016

CABI in Switzerland
Rue des Grillons 1, CH-2800 Delémont, Switzerland

contents

Notes from the programme leader

In the first half of 2016, we had some more staff changes and subsequent changes in project responsibilities. Sonja Stutz successfully completed her PhD on oxeye daisy this spring and is now employed as a project scientist. Her thesis not only greatly contributed to prioritizing biological control agents for oxeye daisy, but also elucidated factors responsible for the invasion success of the plant in North America. This information can also contribute to the development of integrated management strategies for oxeye daisy. We are happy to announce a new member of the oxeye daisy consortium, Australia, via Andrew McConnachie, Department of Primary Industries, New South Wales. Andrew is planning to visit us this autumn.

Hariet Hinz

At the beginning of June, we were finally able to fill the position for a new weed scientist, and welcomed Philip Weyl in our Team. Phil has grown up in various African countries (Zimbabwe, Malawi and South Africa) and did his MSc and PhD at Rhodes University in South Africa with Prof. Martin Hill and Dr. Julie Coetzee, and then worked as a Postdoctoral Fellow with Martin Hill. Phil brings with him a lot of experience in different areas of weed biocontrol, which will perfectly supplement the existing Team. In addition, Phil is a very nice person to work with and I am sure you will enjoy collaborating with him. He is currently taking over the Russian Olive and Russian knapweed projects from Urs Schaffner, who will reduce his time working in the weed biocontrol section to concentrate on a large six year project focussing on Woody Invasive Weeds in East Africa. Phil will also gradually be taking over my project responsibilities for hoary cress and dyer's woad.

Philip Weyl

Sadly, at the end of July, our colleague André Gassmann retired, after 39 years at CABI! I would like to use again the opportunity to thank André for his significant contribution to the weed biocontrol Team in Switzerland and to the weed biocontrol community as a whole. Please remember that thanks to André's work, the Aphthona flea beetles for leafy spurge control were introduced to North America; one of the best documented successes, also in monetary terms, in weed biocontrol. In respect to project responsibilities, Sonja will take over the common tansy project from André and Ghislaine Cortat the swallow-worts project. André will still contribute to the Linaria project together with Ivo Toševski and some of my input. In recent years, André has also taken over additional responsibilities as Assistant Director, especially developing and maintaining links to the local government. This is harder to replace in the short term and so I was very happy when André agreed to keep on working for CABI as an external consultant as needed.

André Gassmann

I would also like to take the opportunity to draw your attention to the Nagoya Protocol, which aims to provide a legal framework for the *Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilisation (ABS)*. "Utilisation of genetic resources" is therein defined as "to conduct research and development on the genetic and/or biochemical composition of genetic resources". This therefore directly impacts our work when searching for, collecting and studying natural enemies in their native range as potential biological control agents for invasive weeds in North America. CABI should not and does not ignore its obligations in relation to the Nagoya Protocol, and so this spring I contacted various National Focal Points and at least within Europe, most countries are not restricting access to their genetic resources. So this is good news. However, from other countries we received contradictory, unclear or no responses. We are currently working with our local collaborators to resolve these issues, but it is clear that at least in some cases, this issue will further complicate and potentially prolong the time we need for certain projects.

In terms of permitting field release of weed biocontrol agents in North America, the US is still a headache for us, with no agents released in the western US since 2011. However, the TAG meeting this spring made us cautiously optimistic that at least some of the petitions stuck since several years will now be moving forward. Let's keep our fingers crossed!

Finally, in contrast to last year we had a very wet and cold spring, which unfortunately negatively influenced some of our rearing colonies and common garden experiments.

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Gall by *Rhinusa rara* on *Linaria genistifolia*

1 Dalmatian and Yellow Toadflax (*Linaria* spp.)

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1.1 *Rhinusa pilosa* ex *L. vulgaris* and *R. rara* ex *L. genistifolia* (Col., Curculionidae)

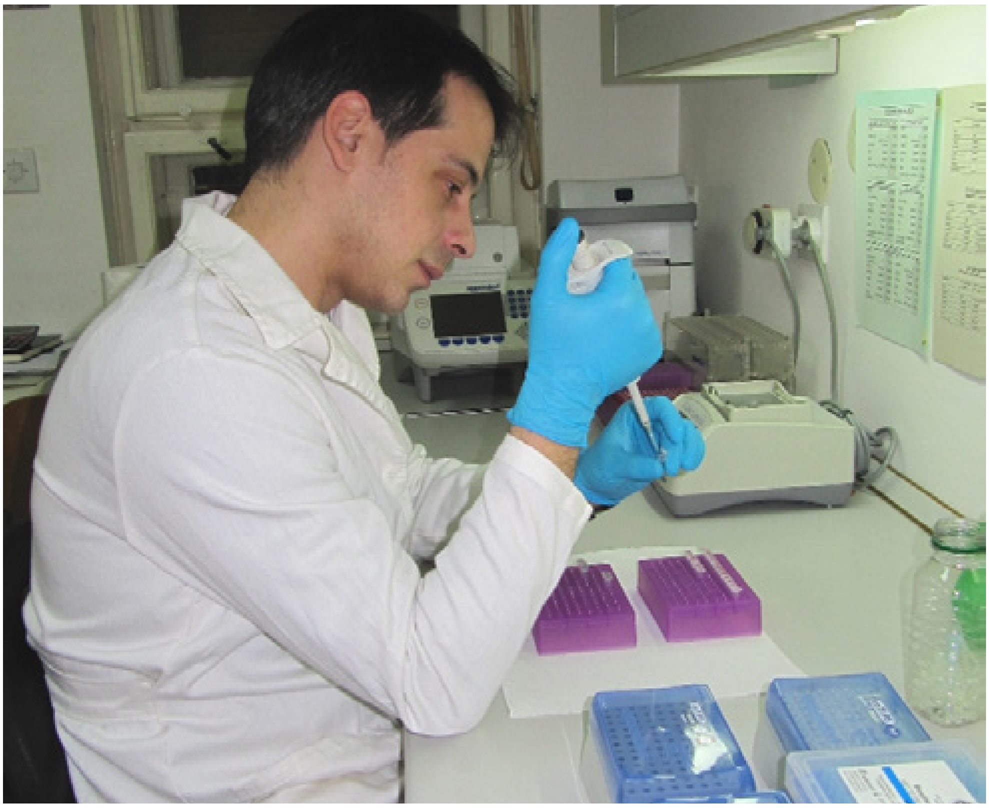
A total of 206 *R. pilosa* galls were collected at three different locations in central and eastern Serbia in order to increase the genetic diversity of the laboratory reared population of this weevil. In addition, some 253 *R. pilosa* galls were collected from two field cages set up in the Plant Protection Institute's garden at Belgrade. Rearing in field cages and field collections yielded 140 galls of *R. rara*. Adults of both *Rhinusa* species started to emerge in mid-June. These weevils will be used for mass rearing and potential shipment to North America.

1.2 *Mecinus* spp.

In 2016, no larval development of *M. heydeni* ex *L. vulgaris* was recorded on the native North American (NA) species *Nuttallanthus canadensis* in a sequential no-choice test. In a multiple-choice test exposing *L. vulgaris* and the native NA species *N. canadensis* and *Sairocarpus virga*, only one larva was recorded on *N. canadensis*. In contrast, 85 larvae were recorded on *L. vulgaris*. No attack was found on *S. virga*.

No-choice tests have been completed with *M. laeviceps* ex *L. genistifolia* with 13 plant species and populations, of which five are of NA origin, in spring 2016. Results will be available in early August. A sequential no-choice and a multiple-choice test with the same plant species as for *M. heydeni* indicate no attack on the native NA species.

A total of 129 females of *M. peterharrisi* ex *L. dalmatica* ssp. *macedonica* were used in no-choice oviposition and larval development tests with 41 plant species and populations, 29 of which are of NA origin. Results will be available in early August. When exposing the critical native NA species *N. canadensis* and *S. virga* together with *L. dalmatica* ssp. *macedonica* in a multiple-choice cage test, attack was restricted to the latter.



Oliver Krstić, IPPE, Zemum-Belgrade, Serbia

2 Houndstongue (*Cynoglossum officinale*)

Hariet L. Hinz, Cornelia Closca, Emma Dieudonné and **Ljupcho Vasilev**
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Current work on houndstongue is focused on the seed-feeding weevil *Mogulones borraginis.* It has proven to be the most specific agent acting on houndstongue thus far and a petition for field release is in preparation.

Of a total of 1914 larvae that we collected from our rearing in summer 2015, over 250 *M. borraginis* adults already emerged in autumn. In addition, we sifted out adults from the soil and were able to send 200 naïve females each on 20 November and 18 December 2015 to the quarantine facility in Pullman, Washington for additional investigations on the host choice behaviour of the weevil. On 8 April 2016, an additional 266 naïve females were shipped to Pullman. On 24 March, 229 *M. borraginis* (143 females and 86 males) were set up on houndstongue rosettes. In mid May, only few houndstongue plants were starting to flower at the Centre and we received a shipment of 46 bolting/flowering plants from the University of Idaho. On 2 June, we set up two females and one male each on 43 of these plants. After about 12 days we placed them onto new shoots or onto shoots on which we did not find signs of oviposition. Despite these efforts, only 60 *M. borraginis* larvae have emerged so far, which is the lowest number we ever had for our rearing. Luckily enough, our colleagues at the University of Idaho were able to establish a successful rearing for the first time in quarantine and currently have about 1'600 larvae incubated. We will look into options to potentially transport *M. borraginis* back to Switzerland.

PhD student Ikju Park at the University of Idaho is currently writing up his thesis on the host selection behaviour of *M. borraginis.* He is planning to submit his PhD later this year.

Adult of *Mogulones borraginis*

Feeding damage of adult *Mogulones borraginis*

Summer student Emma Dieudonné collecting larvae of *Mogulones borraginis* from our rearing

Female of *Ceilosia urbana*

3 Hawkweeds (*Pilosella* spp.)

Ghislaine Cortat, Anouchka Perret-Gentil and **Sara Visentin**
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3.1 *Aulacidea subterminalis* (Hym., Cynipidae)

Two populations of this gall wasp are being investigated. One population is *A. pilosellae* ex *P. officinarum* pooled from wasps collected in the southern range (Switzerland and southern Germany) and the second population is *A. pilosellae* ex *Pilosella* spp. pooled from wasps from the northern range (eastern Germany, Poland and the Czech Republic). Of the latter we also maintain rearing colonies separate by site and host species.

In 2015, few galls of *A. pilosellae* ex *P. officinarum* developed to maturity from tests and rearing plants, and only 30 galls were overwintered. In an attempt to push the development and in order to obtain several generations, we took the galls out of cold storage early March and placed them in an incubator at 22°C. Only six females emerged after a period of 17 days. *Pilosella officinarum* plants were each exposed to one female in the incubator. Only two plants developed galls (N=37) which were harvested 12 May. From these, only one female emerged after 63 days.

To restore our colony, Rosemarie De Clerck-Floate kindly agreed to send us galls of the same population reared at AAFC Lethbridge, Canada. A shipment was planned at the beginning of the summer. It was a complex and time consuming process, although the wasps are native to Switzerland, but after one month, we were finally able to receive about 2000 galls on 14 July. Emergence started two days later. Adults are being used in host range tests and rearing. Rearing will be maintained by keeping some plants in our common garden under natural conditions and others in an incubator at 20-25°C until mature galls are harvested.

Female *Aulacidea pilosellae* on *Pilosella officinarum* (photo: T. Haye)

Only 42 females and 101 males of *A. pilosellae ex Pilosella* spp. emerged from host range and field collected galls. From 83% of galls contamination (by a species not identified yet) or parasitoids emerged. No-choice tests were conducted with five native North American *Hieracium* species and *Lactuca pulchella*. So far, galls were only found on *P. caespitosa* (control). Detailed results will be presented in the Annual Report.

3.2 *Cheilosia urbana* (Dipt., Syrphidae)

In spring 2016 we received the good news that the field release of the hoverfly *C. urbana* was approved by the Canadian Food Inspection Agency and that USDA APHIS Technical Advisory Group recommended its release in the US.

In March 2016, additional voucher specimens of *C. urbana* were sent to Drs. Rosemarie DeClerck-Floate and Jeff Littlefield (Montana State University), for the Canadian and US National Collections, respectively. In view of field releases in 2017, we visited sites where the fly was known to occur and new sites in the Jura. The weather during the syrphid oviposition period (mid-April to mid-May) was cold and wet. During the first collections on 20-21 April, we only found three *Cheilosia psilophtalma*, the other hawkweed hoverfly species. On 12 May, the weather was improving and we could start field surveys again. On 26 May, two *C. urbana* were found, but they died within a couple of days without laying eggs. During surveys beginning of June, we did not find any further syrphids, and we assume that the oviposition period was over.

Summer students Sara Visentin and Anouchka Perret-Gentil searching for *Cheilosia urbana* on *Pilosella officinarum* at a field site in Delémont

4 Russian Knapweed (*Rhaponticum repens*)

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Aceria acroptiloni infested Russian knapweed flower buds at the collection site in Shirvan, Iran

As already mentioned in the Introduction, we have a new staff member, Philip Weyl, who is currently taking over project responsibilities from Urs Schaffner for Russian knapweed and other projects.

4.1 *Aceria acroptiloni* (Acari, Eriophyoidae) from Iran

In collaboration with Dr Ghorbanali Asadi, Mashhad University, we continued with the open-field host-range test in Iran. North American test species that were planned to complement the field plot were held up by Iranian customs and did not survive. Nevertheless, the open-field host range test included several closely related North American as well economically important species. As in 2015, two methods were used to inoculate the test and control plants. First, mite infested flower buds were cut into two pieces and pinned to the shoots or rosette leaves of the test and control plants. Second, mite-infested Russian knapweed plants from a natural field site near Shirvan, Iran, were randomly transplanted together with soil to the experimental plot. For a period of three weeks, leaf samples were collected from both the test and control plants every two days to assess early mite mortality and establishment.

We decided to inoculate an additional 10 individuals from test plants where mite survival under quarantine conditions was longer than two weeks (artichoke) or where a positive identification had occurred during molecular identification (*Aster laevis, Calendula officinalis*). These additional plants were either cultivated or were growing wild on the experimental farm. Again, infested flower buds were used for inoculation. Every two days a shoot onto which mites were released was collected and inspected for mite survival and establishment.

In both experiments, early mite mortality was very high on all test plant species and after just 4 days live mites were only recorded on *R. repens*. All above-ground parts of the test and control plants from the field plot will be harvested in August 2016 and sent to Dr Radmila Petanovic and Dr Biljana Vidović, University of Belgrade, Serbia, for mite extraction and identification.

To collect quantitative data on the field host range of the mite, test plants, especially close relatives of *R. repens* were collected from field sites near Shirvan, Iran, where *R. repens* was heavily infested with mites. The plants will also be sent to Dr Radmila Petanovic and Dr Biljana Vidović, University of Belgrade, Serbia, for mite extraction and identification.

Mite-infested plants were also hand-carried from Iran to the quarantine facility at the CABI Centre in Delémont to continue with no-choice host-range testing. Unfortunately, this consignment did not survive the journey and thus far quarantine tests for 2016 have not been possible. We are looking into options to get material hand carried from Iran to Europe later this field season.

Dr Ghorbanali Asadi and Philip Weyl collecting *Aceria acroptiloni* infested Russian knapweed in Shirvan, Iran

5 Garlic Mustard (*Alliaria petiolata*)

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Release of *Ceutorhynchus scrobicollis* onto *Alliaria petiolata* 'release plant' during the open-field test

5.1 *Ceutorhynchus constrictus* (Col., Curculionidae)

Between 4 and 19 April, 434 adults of the seed-feeding weevil *C. constrictus* emerged from our rearing colony established in 2015. On 9 May, a shipment of 200 *C. constrictus* was made to Jeanie Katovich and Roger Becker at the University of Minnesota to supplement their rearing colony in quarantine and to conduct additional tests with native North American (NA) species.

On 2 and 3 June, 215 additional adults were collected at field sites in Switzerland. Between 23 May and 6 June, 39 no-choice tests were successfully conducted, using 10 test plant species (nine native to NA) and *A. petiolata* as control. All controls were attacked and from the test plants only one larva emerged from a replicate of *Braya alpina.* In previous no-choice tests, *Boechera hoffmannii* was accepted for oviposition. We therefore conducted single-choice tests simultaneously exposing *Boechera hoffmannii* and *A. petiolata* to *C. constrictus*. All control plants were attacked, and none of the test plants received eggs.

5.2 *Ceutorhynchus scrobicollis* (Col., Curculionidae)

Thlaspi arvense wild type (left) and crop variety (right) from the open-field experiment with *Ceutorhynchus scrobicollis*, harvested March 2016

In autumn 2015, an open-field experiment was set up on arable land close to a forest in the area of Delémont. Twelve rows of *Thlaspi arvense*, half of wild origin and the other half a commercial variety selected as an oil crop, were sown. We assured that sowing conditions and management of the plot were as close as possible to a commercial crop field. Six potted *A. petiolata* plants each were embedded in the ground at two opposite sides of the rows. Adult *C. scrobicollis* were released onto *A. petiolata* ('release plants') situated on the side opposite to the forest. The other *A. petiolata* plants acted as 'trap plants'.

Almost all plants sampled and dissected on 9 November and 14 December 2015 contained eggs and first instar larvae. At the end of March 2016, one *A. petiolata* each of the 'release plants' and of the 'trap plants' were dissected together with 16 plants each of the two *T. arvense* populations. All plants but one *T. arvense* of wild origin contained eggs and/or larvae of all three stages. The remaining five *A. petiolata* 'release' and 'trap plants', as well as twelve plants each of the two *T. arvense* populations were covered with gauze bags and kept in an unheated glasshouse for adult emergence. Adult *C. scrobicollis* emerged from all *A. petiolata*, but one 'release plant', ten *T. arvense* of the crop variety and seven of wild origin. However, the mean number of emerged adult per plant was much lower on *T. arvense* compared to *A. petiolata* (mean ± SE: *T. arvense* wild 1.8 ± 0.7; crop 2.1 ± 0.4; *A. petiolata* release 6.8 ± 2.3; trap 20.8 ± 3.6). The number of adults emerging reflects the attack found in March and represents between a third and half of the number of eggs and larvae.

Summer student Anouchka Perret-Gentil setting up host range tests with *Ceutorhynchus constrictus*

6 Common Reed (*Phragmites australis*)

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6.1 Rearing of stem-mining moths

Work in 2016 is focussing on maintaining the rearing of the two noctuid moths *Archanara geminipuncta* and *A. neurica*. We were able to produce 2500 eggs of *A. neurica*. However, we are experiencing every year more and more problems with the rearing of *A. geminipuncta*. In 2016, nearly all larvae died before pupation, and we only obtained about 300 eggs so far. We were able to collect 20 additional pupae in the field and hope to increase the number of eggs with the emerging adults.

6.2 Egg overwintering experiment

In the past, we were able to show that the native subspecies *Phragmites australis* ssp. *americanus* is not attractive for oviposition for both *Archanara* species. However, we were not able to clearly show this for the other subspecies, *P. australis* ssp. *berlianderi*, occurring on the Gulf Coast. We assume that the distribution of *A. geminipuncta* and *A. neurica* is limited to the south and that the moth will not reach the Gulf Coast. To test whether this could be due to missing the obligatory cold induced diapause, we setup nearly 500 eggs of *A. neurica* and 155 eggs of *A. geminipuncta* in an incubator under Florida conditions in July and August, respectively.

Archanara geminipuncta adult

Archanara neurica adult

Cages setup for oviposition of *A. geminipuncta* and *A. neurica*

A gall and larval emergence hole (see arrow) from *Ceutorhynchus assimilis* on *Lepidium draba*

7 Whitetop or Hoary Cress (*Lepidium draba*)

Hariet L. Hinz, Cornelia Closca, Emma Dieudonné, Ljupcho Vasilev, and **Philip Weyl**
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7.1 *Ceutorhynchus cardariae* (Col., Curculionidae)

Of the 339 adults of the gall-forming weevil *C. cardariae* that overwintered at CABI, 74% survived. To boost the populations used for rearing and host range tests after relatively low overwintering survival an additional 141 adult weevils were field collected in Romania in March. Another consignment of 110 *C. cardariae* females were sent to the quarantine facility at the University of Washington, Pullman, where PhD student Jessica Rendon (University of Idaho) is conducting bioassays on the host finding and acceptance behaviour of the weevil.

We continued conducting additional host-specificity tests as recommended by the USDA, Technical Advisory Group (TAG). During no-choice development tests with 15 species or subspecies, four of which had not been tested before, nine species developed galls to some extent and some adults emerged. We also established a cage impact experiment exposing two plant species (*Streptanthus anceps* and *S. flavescens*) that had been attacked under multiple-choice conditions by *C. cardariae.* Detailed results will be presented in the Annual Project Report.

7.2 *Ceutorhynchus turbatus* (Col., Curculionidae)

Streptanthus anceps with galls or gall attempts by *Ceutorhynchus cardariae* in a cage impact test

On 6 June about 2500 adults of the seed-feeding weevil *C. turbatus* were collected in southern Switzerland. On 7 June, we established no-choice oviposition tests with 54 plants; 9 *L. draba* control plants and 1-5 replicates of 14 test species. Apart from *L. draba,* eggs were found on three native North American species *Stanleya pinnata, Braya alpina* and *Lepidium alyssoides*. In addition, we established no-choice larval development tests with test species that had been accepted for egg laying in previous tests. Mature larvae only emerged from the target species *L. draba.*

7.3 *Ceutorhynchus assimilis* (Col., Curculionidae)

Between 8 October and 2 December 2015, we established 216 no-choice oviposition and development tests with 33 test species. Plants were kept in the greenhouse over winter then placed outside in a garden bed in spring 2016 and were regularly checked for adult emergence. Thirteen test species supported adult development. We are planning to set up a multiple-choice cage test with nine of these species and two additional closely related species (*Lepidium appelianum* and *Lepidium chalepense*) in autumn.

On 17 February and 19 March 2016, 141 galls of *C. assimilis* were collected at field sites close to Montpellier, France by our colleagues at USDA, ARS, EBCL and sent to CABI. From 3 to 22 June, 40 adults of *C. assimilis* emerged, which were placed in cylinders for aestivation. Including these and the adults that emerged from host-specificity tests and our colony, we currently have a total of about 750 *C. assimilis* in aestivation for additional tests in autumn 2016.

Summer student Emma Dieudonné collecting *Ceutorhynchus turbatus* in southern Switzerland

8 Dyer's Wood (*Isatis tinctoria*)

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8.1 *Ceutorhynchus peyerimhoffi* (Col., Curculionidae)

Between 22 April and 13 July 2016, 2549 adults of the seed-feeding weevil *C. peyerimhoffi* emerged from our rearing colony. These adults were used in tests or transferred onto flowering dyer's wood plants for rearing. From the rearing plants and dyer's wood plants used in the 2016 tests, 4500 larvae were kept for adult emergence in 2017.

Since *C. peyerimhoffi* were able to oviposit on the federally listed *Boechera hoffmannii*, an additional oogenesis test was set up. Newly emerged weevils that had not fed on dyer's wood were placed directly onto either *B. hoffmannii* or dyers wood on 27 April and retrieved one month later. Eggs were recorded on both species, but the mean number of eggs was much lower on *B. hoffmannii* (27.2±10.2) compared to dyers wood (577.2±191.4).

Between 6 May and 8 July, no-choice oviposition tests were established with 67 plants: 15 test species with 1-7 replicates each, and 18 dyer's wood control plants. Apart from dyer's wood, eggs were found on only two test species, the native North American *Braya alpina* and *Streptanthus lasiophyllus*. These species were subsequently tested for larval development. Only one mature larva was found on *B. alpina*.

We repeated the multiple-choice field cage test with *B. hoffmannii* and *S. heterophyllus*, native NA species that had supported development under no-choice conditions in 2014. A few eggs were recorded on one of the six *B. hoffmannii* exposed, which was unexpected and might have been due to a short period in which the corresponding dyer's wood control plants were too advanced for oviposition, creating a "no-choice situation".

Tube attached to a no-choice development plant to collect emerging larvae of *Ceutorhynchus peyerimhoffi*

8.2 *Ceutorhynchus rusticus* (Col., Curculionidae)

A total of 162 plants from 18 test plant species and the control were exposed to *C. rusticus* in a no choice oviposition and development test. Test plants were regularly checked, and any dying plants were dissected. Three quarters of our dyer's wood control plants supported adult emergence and a much higher number of adults than in the last years (an average of 6.7) emerged per plant. Only four test species, including two new ones (the native North American *Eutrema salsugineum* and the European *Lepidium sativum*), supported limited adult development. We are planning to expose these in an open-field test in autumn 2016.

Adults of *Ceutorhynchus rusticus* on *Isatis tinctoria*

8.3 *Metaculus sp.* (Aceria, Eriophyidae)

In 2006 and again in 2007, we found an eriophyid mite on dyer's wood in Turkey, causing deformed, shriveled leaves. Identified as an oligophagous species at the time, it is now suspected to be a species new to science and likely specific to dyer's wood. Massimo Cristofaro from BBCA in Italy collected more mites this spring and Radmila Petanović from the University of Belgrade, Serbia, is currently analysing the material. In parallel, we set up an open-field test in Turkey with nine test plant species. Material was harvested in July and is currently being analysed for the presence and the identity of mites by Radmila in Serbia.

Summer student Ljupcho Vasilev exposing test and dyer's wood plants in the multiple-choice cage set up for *Ceutorhynchus peyerimhoffi.*

Ekaterina Nikolova inspecting PPW shoots collected in Kazakhstan for the presence of the eriophyid mite *Metaculus lepidifolii*

9 Perennial Pepperweed (*Lepidium latifolium*)

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9.1 Field surveys

In May, we conducted a field trip to Kazakhstan. The main goal of this field trip was to collect a population of the eriophyid mite *Metaculus lepidifolii* that is adapted to the most common perennial pepperweed (PPW) genotype that is invasive in the US. In addition, we took the opportunity to look for new potential biological control agents. Shoots at a total of 16 sites were collected and checked for eriophyid mites under a microscope in the field. In addition, plants were dug up from nine sites and checked for root herbivores. Eriophyid mites were found at seven sites and at one site a weevil larva mining in the roots. All specimens and samples will be forwarded to specialists for identification. In addition, shoots infested with mites were collected from one site and imported into the quarantine facility at CABI to establish a rearing colony and develop methods for host-range testing (see below).

9.2 *Metaculus lepidifolii* (Acari, Eriophyidae)

An open-field test with the eriophyid mite *M. lepidifolii* was established at the Erciyes University in Kayseri, Turkey. On 23 June, a total of four PPW populations representing all four genotypes that are invasive in North America as well as one PPW population from Turkey were inoculated by placing bouquets of cuttings of PPW infested with *M. lepidifolii* close to the young leaves of each potted plant. On 17 July, the plants were harvested and sent to Dr Biljana Vidović University of Belgrade, Serbia for mite extraction and identification.

A similar experiment was conducted under laboratory conditions where mite-infested flowers and shoots were attached to leaves of potted plants of four different PPW genotypes invasive in the US as well as on one PPW population from the native range. In addition, a few test plants were also included in these studies. All plants were regularly checked for the presence of mites. Detailed results will be presented in the Annual Report.

Set-up of the open-field test with *Ceutorhynchus marginellus* in Russia

9.3 *Ceutorhynchus marginellus* (Col., Curculionidae)

In March, 2015, weevils were taken out of overwintering incubators. In contrast to last year, overwintering survival was relatively low and from the 418 adults only 114 females and 89 males successfully overwintered.

A large scale (15 x 15 m) open-field test was set up with *Lepidium crenatum*, *L. eastwoodiae*, *L. huberi*, *L. virginicum* and PPW as controls in Russia by Dr Margarita Dolgovskaya and her team (Russian Academy of Sciences, Zoological Institute, St Petersburg). On 14 May a total of 60 females and 45 males were released. All plants were checked for galls in mid-June. Apart from PPW, galls were found on *L. eastwoodiae* and *L. virginicum,* though in lower numbers than on PPW. Plants without galls were dissected while plants with galls were covered with gauze bags and are regularly being checked for adult emergence. Detailed results will be presented in the Annual Report.

Francesca Marini and Matthew Augé collecting PPW at a field site in Kazakhstan

10 Swallow-Worts (*Vincetoxicum* spp.)

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10.1 *Chrysochus (Eumolpus) asclepiadeus* (Col., Chrysomelidae)

We are currently maintaining a rearing colony of the root-mining beetle *C. asclepiadeus* from a tested population originating in Ukraine (UKR) and one population from southern Switzerland (CH). In June 2016, a total of 25 females and 17 males of the Ukrainian population emerged from 625 larvae (6.7%) transferred onto *V. nigrum* in 2014. No beetles have emerged from larvae transferred in 2015 (UKR and CH). Additional adults were collected in southern Switzerland in July 2016 to complement the existing colony.

The same rearing methods are used as in previous years. Adults are placed into rearing containers to collect eggs and are fed with cut shoots of *V. nigrum* (UKR) or *V. hirundinaria* (CH). Rearing containers consist of transparent ventilated plastic boxes (32 x 18 x 12 cm) containing swallow-wort shoots inserted into moist blocks of florist foam with parallel lines engraved into them to serve as an oviposition substrate. Eggs are collected twice a week and plant shoots are changed when necessary.

Rearing of *Chrysochus asclepiadeus* from Ukraine

10.2 *Euphranta connexa* (Dipt., Tephritidae)

Pupae (N=319) of this seed feeding fly collected in summer 2015 and were overwintered at 5°C. Between 19 April and 14 June, a batch of about 50-60 pupae were taken out of cold storage each week and placed into an incubator at 22°C to synchronise adult emergence with the wide range of pod production periods of test species. The containers were regularly checked for emergence and a few mouldy pupae were removed. In 2016, only five flies have emerged. Dissection of pupae from the three first batches taken out of cold storage revealed that some of the pupae were empty although the case did not show any sign of damage; others were flattened, dry and dark. We are in contact with Alicia Leroux (former Master student on the project) to try and elucidate the factors leading to this unusually low survival and to improve the methods for this winter. Seed pods of *V. hirundinaria* infested with *E. connexa* were collected on 24 and 25 July in southern Switzerland. This was slightly too early and a second trip is planned for mid-August. Pupae will be overwintered and emerging flies used for host-specificity tests in 2017.

Flowers and pods of *Asclepias physocarpa* one of the test species for *Euphranta connexa*

Ghislaine Cortat, Anouchka Perret-Gentil and Sara Visentin checking for pests on *Vincetoxicum* spp. in the CABI garden

Set-up of no-choice tests with *Microplontus millefolii* in Russia

Chrysolina eurina collected in the Czech Republic on *Tanacetum vulgare*

11 Common Tansy (*Tanacetum vulgare*)

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11.1 *Microplontus millefolii* (Col., Curculionidae)

Work on the shoot-mining weevil *Microplontus millefolii* is being conducted in Russia by Dr Margarita Dolgovskaya and her team (Russian Academy of Sciences, Zoological Institute, St Petersburg). In June, additional no-choice oviposition and development tests were conducted with 13 test plant species. Individual shoots of test and control plants were exposed to females for several days and the shoots were dissected 20 to 25 days after exposure. Larvae were found in 81% of the *T. vulgare* plants exposed, but only one live larva was found in each of the native *T. camphoratum* and *T. huronense* and in the medicinal plant *T. parthenium.* No larvae were found in any of the other plant species exposed.

11.2 *Platyptilia ochrodactyla* (Lep., Pterophoridae)

Between 10 and 12 May, about 1000 shoots attacked by the shoot-mining moth *Platyptilia ochrodactyla* were collected in western Germany. Because *P. ochrodactyla* females oviposit in flowers and in previous years adults emerged long before any *Tanacetum vulgare* were flowering, larvae and pupae were kept at several different temperatures (10°C, 15°C, 20°C and 25°C) to extend the period of adult emergence. From 29 May to 3 August, a total of 203 females and 324 males emerged. Adult emergence was high at all temperatures except when larvae were constantly kept at 10°C. No-choice oviposition and development tests were carried out with 14 plant species, with 1-13 replicates each. Since many test plants were already flowering in June whereas *T. vulgare* only started flowering in mid-July we were not able to simultaneously expose control- and test plants. All flower heads will be dissected about two months after the exposure to *P. ochrodactyla.*

11.3 *Chrysolina eurina* (Col., Chrysomelidae)

The leaf beetle *Chrysolina eurina* is a new potential agent selected because it is only recorded from common tansy in the literature. From 26 June to 3 July a field trip was conducted by our collaborator Dr. Alecu Diaconu, Biological Control Institute Iaşi, in Western Romania. A total of 25 sites were surveyed but no *Ch. eurina* were found. An additional survey was conducted in Austria, the Czech Republic and Slovakia from 4 to 7 August. A total of 15 sites were surveyed and two beetles were found at one site in the Czech Republic. We are planning to go back to this area at the beginning of October to hopefully find more beetles.

Summer student Ekaterina Nikolova releasing adult *Platyptilia ochrodactyla* on a test plant

12 Russian Olive (*Elaeagnus angustifolia*)

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As already mentioned in the Introduction, we have a new staff member, Philip Weyl, who is currently taking over project responsibilities from Urs Schaffner for Russian Olive and other projects.

12.1 *Aceria angustifoliae* (Acari: Eriophyoidae)

In collaboration with Dr. Ghorbanali Asadi, the open-field host-range test for *Elaeagnus angustifolia* at the experimental farm of Mashhad University was continued in 2016. In May 2016 additional test plants were planted into the field plot. As in previous years mite-infested leaves of *E. angustifolia* were pinned to the young leaves of the test plants so that they were touching to allow for easy mite transfer. Leaves were sampled every two days to assess early survival and establishment of the mites on both the target and closely related test species. In addition, all trees were assessed for symptoms of mite attack. There was a very high level of mite mortality on closely related species and live mites were only recorded on *E. angustifolia*. For the first time, all eight of the *E. angustifolia* trees are showing symptoms of mite attack, in some cases high levels, while none of the test species, including closely related species native to North America, are showing any signs.

Symptoms of *Aceria angustifoliae* attack on young shoots of Russian olive

In early June, mite-infested Russian olive branches were collected in Iran and hand-carried to the quarantine facility at CABI Switzerland. Mite-infested leaves were pinned to leaves of *E. angustifolia* and closely related test species that are either native or ornamentals in North America. Test and control plants will be monitored for mite attack at weekly intervals. Results will become available towards the end of summer 2016.

The impact study at the experimental farm of Mashhad University is still ongoing and the plants are regularly inoculated, usually at the same time as the open-field host-range test. Despite the repeated attempts to inoculate the plants since 2010, infestation levels remain low. Additional impact experiments have been setup in Turkey, where 20 *E. angustifolia* plants have been transplanted into a common garden experiment, 10 have been infested with the mite and 10 remain un-infested and healthy. The second experiment is using wild populations of *E. angustifolia*, where 10 branches that were infested with the mite and 10 branches that were not, have been labelled and the number of leaves, flowers and fruits counted on a regular basis throughout the 2016 field season. These data will become available in winter 2016.

Julie Klötzli inspecting leaves of Russian Olive and other test plants that were inoculated with *Aceria angustifolia* under quarantine conditions

Provided, the additional host-range tests be successful, we will start preparing a petition for field release for the mite in winter 2016/17.

12.2 *Aceria eleagnicola* (Acari: Eriophyoidae)

In collaboration with Drs. Radmila Petanovic and Biljana Vidovć, University of Belgrade, Serbia, we setup a rearing of this second mite to study its development and biology. Unfortunately the rearing was not as successful as we hoped, but we are working towards rectifying the rearing problems and hope to have a successful method by spring 2017.

Dr. Ghorbanali Asadi inspecting Russian Olive for mite attack

13 Oxeye daisy (*Leucanthemum vulgare*)

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Adult *Dichrorampha aeratana*

13.1 *Dichrorampha aeratana* (Lep., Tortricidae)

Unlike in previous years, overwintering survival of *Dichrorampha aeratana* was very low and only 13 females and 34 males emerged from the more than 80 potted oxeye daisies that had been infested with five to ten larvae each in 2015. To increase the number of adults and larvae available for host-range tests, a field trip to southern Switzerland was conducted on 16 May and an additional 21 females and 20 males were collected. This enabled us to set up no-choice larval development tests with 11 different test plant species, with 1-10 replicates each. Five first instar larvae were transferred onto each of the plants. We also set up a multiple-choice cage test with four species that had supported development under no-choice conditions (*Matricaria chamomilla*) or had ambiguous results in previous tests (*M. discoidea*, *Achillea ptarmica*, *Ismelia carinata*) and *L. vulgare* as controls. Between 13 May and 5 June, six females were released into each of four field cages containing three plants of each species. All plants will be dissected for larvae in autumn.

13.2 *Oxyna nebulosa* (Dipt., Tephritidae)

From 14 to 15 April and from 6 to 10 June several field sites where galls of the root-galling tephritid fly *Oxyna nebulosa* had been found in 2013 were visited in Regensburg (Germany) and in the Czech Republic. Only one gall was found in Germany but a total of 378 galls were collected from three sites in the Czech Republic. From these galls a total of 114 females and 96 males emerged from 17 June to 22 July and females started to lay eggs about four days after emergence. Between 27 June and 28 July we set up no-choice oviposition and development tests with 17 plant species and varieties, with 1-4 replicates each. In addition, we set up a multiple-choice cage test with three Shasta daisy varieties and oxeye daisies as controls. On 15 July, seven females and four males were released into one field cage containing six potted Shasta daisies and oxeye daisies each. All plants will be dissected in spring 2017.

Galls induced by *Oxyna nebulosa* on field collected oxeye daisy

Summer student Sara Visentin releasing *Oxyna nebulosa* in a field cage with oxeye daisy and Shasta daisy

14 Field bindweed (*Convolvulus arvensis*)

Ghislaine Cortat, Anouchka Perret-Gentil, Sara Visentin and **Ivo Toševski**
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The spring 2016 was particularly cold and wet. This caused a delay in the development of most of our insects which had a negative impact on our work.

14.1 *Melanagromyza albocilia* (Dip., Agromyzidae)

Since it has proven difficult to conduct tests with this stem-mining fly under confined conditions, we planned to establish an open-field test in southern Germany, where the fly occurs naturally. In order to select potential sites for an open-field experiment, we surveyed for field bindweed attacked by the fly at the edge of maize fields. With the delayed season, we had to wait until the beginning of July to find visible signs of attack in the field, i.e. late instar larvae and pupae. We found two potential sites, but the process to get into contact with the owners and to obtain permission took much longer than expected and we therefore had to postpose the experiment to 2017.

Convolvulus arvensis in a corn field in southern Germany

In 2015, 786 live and dead larvae (all stages) and pupae were extracted from plants field collected in southern Germany. After emergence of a few flies and parasitoids in autumn 2015, 190 live pupae were placed into Petri-dishes with slightly moist filter paper and stored in an incubator at 5°C for overwintering. The temperature was raised to 10-15°C until July and then to 20°C. Emergence of parasitoids started 18 July and adult flies on 27 July. So far 27 females and 30 males have emerged. Egg laying females will be used to conduct no-choice and single-choice tests on potted plants.

14.2 *Tinthia brosiformis* (Lep., Sesiidae)

We believe that attacking the root system of field bindweed will be critical in successfully controlling the plant, and we therefore will start exploring root-mining clear-wing moths in the genus *Tinthia*. *T. brosiformis* occurs in xerothermic and ruderal habitats in south and south-eastern Europe. We are working with our colleague Ivo Toševski (Institute for Plant Protection and Environment, Zemun, Serbia), a Sesiidae specialist who has extensive experience with clear-wing moths and who has collected both larvae and adults of *T. brosiformis* in the field previously. However, spring in Serbia was also very cold and humid, considerably delaying the field season, and Ivo has not been able so far to collect any moths.

Male of *Tinthia brosiformis* collected in Serbia

Summer students Anouchka Perret-Gentil and Sara Visentin looking for attack by *Melanagromyza albocilia* on *Convolvulus arvensis* in Southern Germany

First instar larva of *Bagous validus*

15 Flowering Rush *(Butomus umbellatus)*

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15.1 *Bagous nodulosus* (Col.: Curculionidae)

Overwintering survival of adults set up on potted, gauze covered plants in autumn 2015, was again very good with nearly 60% surviving to spring 2016 (100 of 170). More than 20 additional weevils were found on plants that were exposed to larvae or ovipositing females in 2015. Between May and June 2016, we unexpectedly found over 80 adult *B. nodulosus* on plants growing in our artificial pond. This means that we must have overlooked adults that emerged in our rearing in 2015 and that our success rate was much higher than thought so far. On various field trips to Slovakia, northern Germany and Serbia, 250 additional weevils were collected. Over 1200 larvae were transferred onto 120 mainly unsubmerged flowering rush plants to continue our rearing.

We made major progress in successfully sexing adults. Instead of measuring ratios of rostrum length and width, we are now able to reliably distinguish male and females in most cases visually.

We also continued with no-choice oviposition tests with *B. nodulosus*. Oviposition was found on North American populations of flowering rush, but so far not on any of the 35 test plant species exposed.

Leaf infested by the white smut *Doassansia niesslii*

15.2 *Bagous validus* (Col.: Curculionidae)

During a field trip in May to eastern Slovakia we found two additional sites with *B. validus,* and together with the site found in 2015, we were able to collect 90 adults. Adults were setup for oviposition in yogurt cups with cut pieces of leaves, in cylinders with *B. nodulosus* plants without soil, and on potted plants covered by gauze bags. However, as in 2015, we found only single eggs randomly laid on the leaf surface. We also found no larvae yet on plants collected on any of the three sites. In mid-July, we found for the first time a female ovipositing nine eggs into cut leaf pieces in a yogurt cup, and one week later we also found four eggs and one first instar larva on a potted plant. This could indicate the start of the oviposition period of *B. validus*. We are planning another field trip to Slovakia in early August and expect to finally also find larvae in the field.

15.3 *Phytoliriomyza ornata* (Dipt.: Agromyzidae)

Our efforts to obtain adults of the agromyzid fly *P. ornata* using emergence traps were not successful yet. We probably did not collect the plants at the right time.

15.4 *Doassansia niesslii* (Basidiomycota)

In mid-June 2016, a two day field trip to northern Germany was made with pathologist Carol Ellison from our UK Centre. The aim was to collect plants infested by *Doassansia niesslii*, a white smut that was identified from leaf samples we had collected in 2013 in the area of Bremen. We found two sites with plants infested by this smut. Leaf samples and plants were taken back to the UK to inoculate flowering rush from various North American and European populations. So far, the white smut has been successfully inoculated onto plants collected from Bremen, Canada and Slovakia. The complicated lifecycle of the pathogen is being investigated and final results will be presented in the Annual Report.

Carol Ellison searching a flowering rush site in northern Germany for the presence of the white smut *Doassansia niesslii*

16 Japanese Knotweed (*Fallopia japonica*) work in the UK

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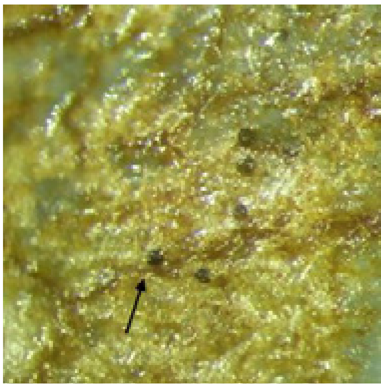
The assessment of selected strains of *Mycosphaerella polygoni-cuspidati* ex Mt Hiko (high altitude site, Kyushu) and ex Omura (coastal low altitude site, Kyushu) as a biocontrol agent of *Fallopia japonica* has continued. This study focuses on evaluating the susceptibility of the most prominent Canadian biotypes of *F. sachalinensis* and *F.* x *bohemica,* as well as critical non-target species such as *F. cilinodes*, *F. scandens*, *Polygonum glaucum* and selected Canadian varieties of buckwheat (*Fagopyrum esculentum*) to *M. polygoni-cuspidati.* The susceptibility of the Canadian biotype of *F. japonica* which is identical to the UK biotype has been previously confirmed as part of the UK research programme.

Disease symptoms caused by the leaf-spot pathogen were found to be greatly enhanced by increasing the humidity within the Perspex cages holding the plants after inoculation; symptom development was accelerated and typically observed 7-10 days after inoculation, compared to 21 days in a lower humidity environment. Furthermore, high humidity favoured the development of necrotic lesions across the whole leaf surface (see picture), often resulting in total leaf abscission. For this reason it was decided to re-test a subset of the test plant species previously assessed. *Fallopia sachalinensis* and *F. bohemica* were found to be more susceptible to the leaf-spot pathogen than previously reported, with necrotic lesions which begin to merge forming across the leaf surface. Incubation of potential symptomatic leaf pieces of *F. sachalinensis* and *F. bohemica* resulted in the formation of fewer spermogonia (the reproductive bodies of the first life cycle stage) on the lower leaf surface and the production of spermatia than on Japanese knotweed control plants. Necrotic lesions identified on *F. scandens* were also incubated and resulted in the formation of spermogonia; however, spermatia were not observed. Fully developed spermogonia were only recorded on medium-aged leaf stages of *F. scandens* (see picture), whilst on the younger leaf stage these bodies appeared to be immature. No spermogonia formed on necrotic older leaves. This is in contrast to results of previous test runs. While unspecific chlorosis was also recorded on inoculated leaves of *F. cilinodis,* no spermogonia developed on this species. Initial assessment of *P. glaucum* using the strain from Omura showed mild necrotic symptoms on the young and medium-aged leaves (see picture) but no spermogonia developed within these lesions. Further assessment of this species, including assessment with the strain from Mt Hiko, is currently ongoing.

Work in 2016 will continue to assess the virulence of the two selected pathogen strains towards the Canadian non-target species using the adjusted methodology, with particular focus on *P. glaucum*. Additional research on the psyllid, *Aphalara itadori* will also take place. Current work in North America considers two different strains of *A. itadori* (i.e. Kyushu and Hokkaido) with differing preference for knotweed species. Prior to the release of both strains it is fundamental to identify any non-target effects of the hybrid which may emerge from the result of both strains being present in field conditions. The host specificity of the *A. itadori* hybrid will be tested against *F. cilinodis* and *F. esculentum*, two non-target species native to North America identified as marginal hosts separately for each psyllid strain. These tests will be complemented through UK projects with the assessment of best release strategies for *A. itadori* in open-field conditions.



Spermogonia (arrowed) on the medium-aged leaf stage of *Fallopia scandens* after inoculation with the Omura strain of *Mycosphaerella polygoni-cuspidati*

Necrotic symptoms on the medium-aged leaf stage of *Polygonum glaucum* 18 days after inoculation with the Omura strain of *Mycosphaerella polygoni-cuspidati*

Whole leaf necrosis on Japanese knotweed control plants after inoculation with the Omura strain of *Mycosphaerella polygoni-cuspidati*

Kate Pollard monitoring the spread of the rust on Himalayan balsam plants in Middlesex, UK

Rust infection on Himalayan Balsam following the first release in June in the UK

17 Himalayan Balsam (*Impatiens glandulifera*) work in the UK

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As Japanese knotweed, Himalayan balsam is an invasive alien weed in both Europe and North America. The project conducted for Canada therefore profits from the biocontrol work for Europe, which is ongoing since 2006. The rust, *Puccinia komarovii* var. *glanduliferae,* is common and damaging in the native range of Himalayan balsam. Since it has also proven to be very specific, release was granted for the UK in 2014.

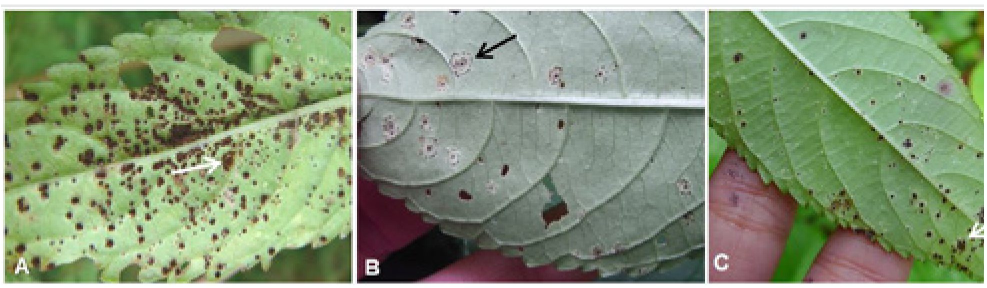
In 2015 the rust was released at five regions (25 sites) across England and southern Wales. Overall there was limited spread of the rust (1-5 m from the rust source plants) and there were low levels of infection. The rust pustule size was small, and urediniospore production within the pustules was reduced, in comparison to infection observed on plants in the field in the Himalayan native range (see pictures below), and under glasshouse conditions. However, there was variation between sites, with rust spread and pustules size being better at some sites than others (see picture below). Environmental data (temperature and humidity) was collected at each site. The results overall suggest that there is a positive correlation between the level of rust spread onto field plants from the rust source plants and the occurrence of optimal conditions for rust infection at a site. However, at some sites where environmental conditions were suitable for infection, infection was poor, and so an investigation into possible genetic variation in the weed has been undertaken in 2016. Himalayan balsam plants from each release site are being compared for their susceptibility towards the rust under field conditions to establish if there have been multiple introductions of the plant to the UK.

Experiments are being conducted to:

- Improving the field adaptation of rust through repeated culturing on plants under natural conditions in the field;
- Testing a new strain of the rust from Pakistan to see if it is more aggressive on UK populations of the weed;
- Investigating the effect of key biotic and abiotic factors on the level of rust infection and spread in the field. The rust has been released at a few sites close to CABI, multiple times and under different microclimatic conditions, and is being monitored for establishment, persistence and spread throughout the growing season (see picture at side). This work is ongoing and will continue until the plants die back in autumn.

It is anticipated that a full county-wide release will take place in 2017.

For Canada, a test plant list containing 51 species specific to North America was compiled by Dr Alec McClay and to date 44 species have been tested with no non-target impacts recorded to date. In 2016, research will continue to evaluate the host specificity of the rust against the remaining seven Canadian specific non-target species; assuming they can be sourced. The project will also benefit from the results of the 2016 experiments and releases of the rust in the UK which aim to optimize the rust release strategy.



Comparison between rust infection (urediniospores) on Himalayan balsam: (A) native Himalayan range, Kullu Valley, India; and invasive range (B) Cornwall, England and (C) Lampeter, Wales. Arrows indicate satellite pustule formation around primary pustule.

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