Weed Biological Control

Progress Report 2015

CABI in Switzerland
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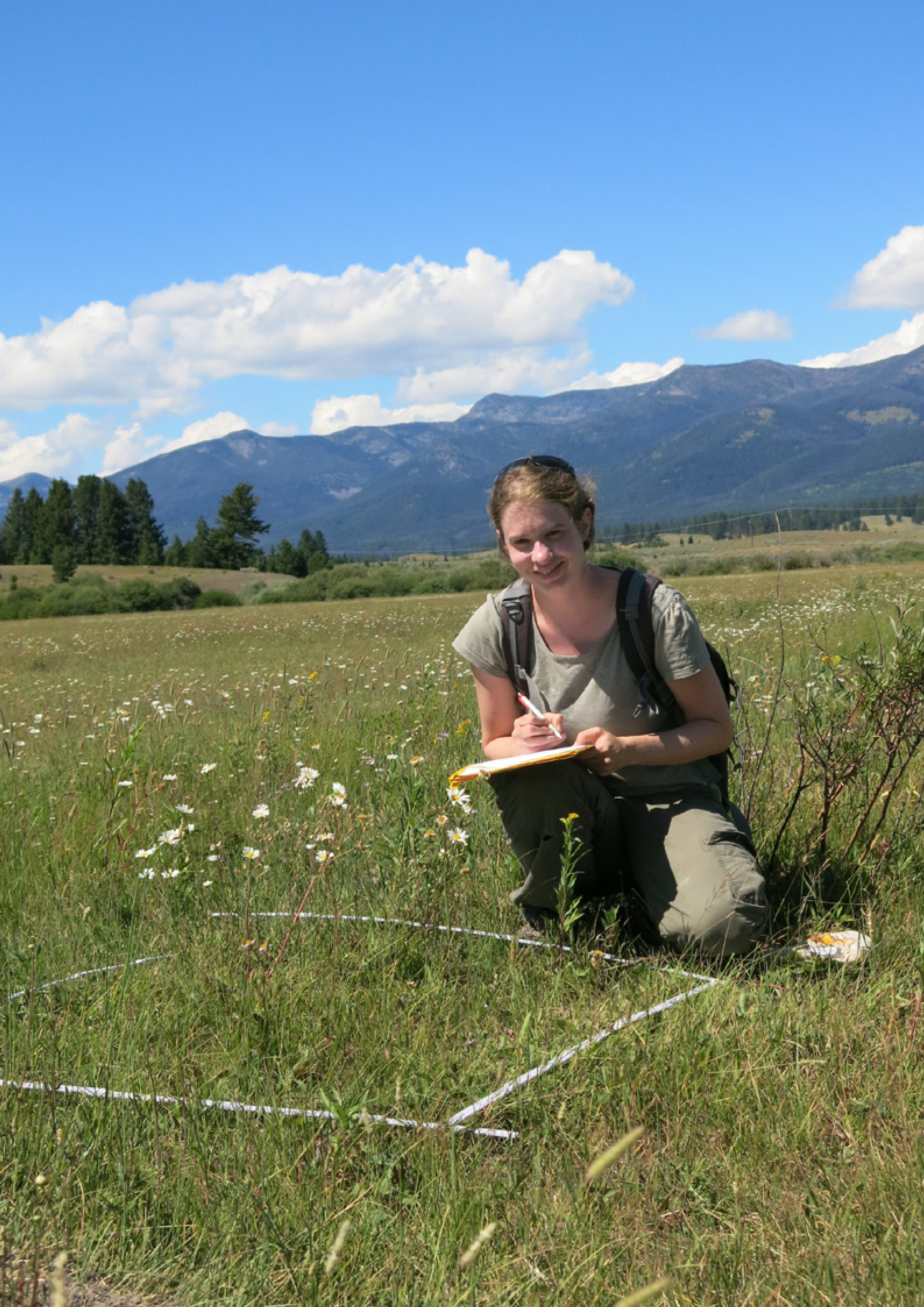
contents



Notes from the programme leader

Hariet Hinz

From 27 to 30 April, Aaron Foster, Chair of the Wyoming Biological Control Steering Committee visited us to get an overview of the biological weed control projects conducted at CABI, especially the projects co-financed by Wyoming. Apart from learning how to sex weevils and seeing the weeds in their native range, we also made sure that Aaron and his wife had a chance to try some typical Swiss foods and drinks. Needless to say visits like those are very useful for both sides and will greatly facilitate our future collaboration.

2015 has seen a few staff changes. Esther Gerber, who has been with us for 17 years, had reduced her time this spring and left us for good as of 30 June to pursue a new direction in her life. Ghislaine Cortat will take over the garlic mustard project from her (see page 7) and Sonja Stutz the perennial pepperweed project (see page 11). Both Ghislaine and Sonja have several years of experience in classical weed biocontrol, which guaranteed a smooth hand-over. I would like to thank Esther for her commitment, enthusiasm and friendship during all these years, and wish her all the best in her new endeavours. I myself will take over the position of Country Director Switzerland from Ulli Kuhlmann as of 1 October. For the time being I will stay responsible for the Biological Weed Control Programme, but we will soon advertise a position to take over some of my direct project responsibilities.

Since two projects (tutsan for New Zealand and Japanese knotweed for Switzerland) came to an end in 2014, we are currently looking into developing 1-2 new projects. The most likely project to start is on camelthorn, *Alhagi maurorum,* a perennial shrub in the leguminose family with prickly thorns, which is a new invader in the northwest of the USA. A very preliminary literature survey and opportunistic field surveys have already revealed a number of interesting insects and at least one damaging eriophyid mite species. In case you are interested in more information I would be happy to send you our flyer. If it materializes we will conduct this project in collaboration with our two long-term collaborators Massimo Cristofaro at BBCA in Italy and Radmila Petanovic from the University of Belgrade. Our North American partner would be Mark Schwarzländer from the University of Idaho. Another potential new project is *Halogeton* (saltlover), which had already been targeted for biological control in the 1970s.

As each year, we have a dynamic group of 11 summer students, this time from nine countries to help with practical work and data entry during the field season. Please keep this opportunity in mind in case you know of an adventurous undergrad or graduate student, ideally with some entomological background, who would like to gain some practical experience abroad. Thank you.

Hariet L. Hinz
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From right to left: Aaron Foster, Sonja Stutz, André Gassmann, Hariet Hinz, Irene Castellan, Wilke Heijs, Amanda Stahlke, Sergio Ribeiro

Picture opposite side: Sonja Stutz recording data on oxeye daisy

Gall induction by *Rhinusa pilosa* originating from Denmark on North American *Linaria vulgaris*

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

Ivo Toševski, Oliver Krstić, Jelena Jović and **André Gassmann**
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1.1 *Rhinusa pilosa* ex *L. vulgaris* and *R. rara* ex *L. genistifolia* (Col., Curculionidae)

A total of 37 adults of the stem-gall weevil *R. pilosa* (31.9%), and 62 *R. rara* (37.8%) emerged from overwintering cages set-up in 2014. The survival rate for both species was better than in 2014. Mass rearing of *R. pilosa* resulted in a total of 270 galls induced on 230 *L. vulgaris* planted in one field cage. In contrast, a total of only 35 galls of *R. rara* were induced on 64 *L. genistifolia* / *L. dalmatica* planted in two field cages. The low number of *R. rara* galls was probably caused by the inadequate phenological stage of the host plants and highly fluctuating temperatures in early April.

In addition, one female of *R. pilosa* originating from Denmark was set up for oviposition on *L. vulgaris* EU and *L. vulgaris* NA to obtain individuals from a more cold adapted clade of this species. A total of 35 galls were induced on both plants. The galled plants are kept at 23 °C during gall development.

1.2 *Mecinus* spp.

A total of 280 *M. heydenii* adults have been reared this year. Numbers for *M. laeviceps* will be available soon. A no-choice sequential oviposition and larval development test with five *M. heydenii* females was set up on *Nuttallanthus canadensis* NA and *N. texanus* NA with *L. vulgaris* as control plant. Larval development was recorded on *L. vulgaris* only. In parallel, a multiple-choice field cage test was set up with *N. canadensis* NA, *N. texanus* NA, *Sairocarpus virga* NA and *Maurandella antirrhiniflora* NA. Larval development was recorded on *L. vulgaris* only.

No-choice oviposition and larval development tests with *M. peterharrisi* were conducted with 104 overwintered females. A total of 30 plant species and populations, of which 19 species were native to North America, were included in the test. Results will be available at the beginning of August after all plants have been dissected.

Ivo Toševski, IPPE, Zemum-Belgrade, Serbia

2 Houndstongue (*Cynoglossum officinale*)

Hariet L. Hinz, Irene Castellan, Cornelia Closca, Wilke Heijs and **Stéphanie Morelon**
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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 4709 larvae that we collected from our rearing in summer 2014, over 500 *M. borraginis* adults already emerged in autumn. Of these, 285 females were sent on 15 December 2014 to the quarantine facility in Pullman, Washington for additional investigations on the host choice behaviour of the weevil. On 11 May 2015, an additional 600 *M. borraginis* adults were shipped to Pullman, 300 naïve females, 200 females that had been on potted houndstongue plants and 100 males, while some 700 adults were placed onto 88 potted gauze-covered houndstongue plants. At the end of May, when houndstongue plants started to reproduce, weevils were retrieved from the 88 plants and placed (1-3 females and 1-2 males) onto new flowering/reproducing plants. A total of 80 rearing plants were set up in this way. Until mid July, 1914 larvae emerged, which were transferred into sifted soil for pupation and adult emergence in 2016.

PhD student Ikju Park at the University of Idaho continues to collect very interesting data on the host selection behaviour of *M. borraginis.* He recently concentrated on more detailed studies of the visual cues female weevils use to distinguish houndstongue from other closely related species and found that both flower colour and size are important.

Adult of *Mogulones borraginis* on houndstongue flower

Multispectral images of *Cynoglossum officinale* (300 nm to 700 nm) simulating the vision of *Mogulones borraginis*

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

3 Hawkweeds (*Pilosella* spp.)

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In 2015, we changed our gall overwintering protocol in order to control temperature and humidity. Based on procedures used at Agriculture and Agri-Food Canada (AAFC), Lethbridge, galls of both *Aulacidea* sp. were stored in an incubator at 2-5°C. During April and May, galls were moved to an incubator at 10°C for a week before temperature was raised to 21-22°C to trigger emergence.

3.1 *Aulacidea subterminalis* (Hym., Cynipidae)

Between 11 and 18 May 2015, 19 females emerged from about 50 overwintered galls. Although the number of wasps emerging this year was still low, the adults were in better condition than in 2014. The wasps were released onto seven large rearing pots (diameter 34 cm) containing several *Pilosella officinarum* plants to maintain our rearing colony. Plants will be checked for galls later this summer.

3.2 *Aulacidea pilosellae* (Hym., Cynipidae)

Two groups 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). Separate populations from the northern range are also kept for rearing.

Single-choice test with left: *P. officinarum* and right: *Hieracium albiflorum*

A. pilosellae ex *P. officinarum* emergence occurred two to four weeks after the galls were taken out of overwintering and placed at 22°C and lasted up to 22 days. A total of 189 females emerged and 84 no-choice host range tests were established. Plants exposed included three native North American (NA) *Hieracium* species and nine other species (crops, grasses and forbs), three of which are native to NA. Nine single-choice tests were also established, exposing one *P. officinarum* plant and one *Hieracium albiflorum, H. argutum* or *H. gronovii* in a pot to two females each.

Of *A. pilosellae* ex *Pilosella* spp., 52 adults emerged and 15 no-choice host range tests were established. Plants exposed included five NA *Hieracium* spp.

Results of all tests will be available later this summer.

3.3 *Cheilosia urbana* (Dipt., Syrphidae)

Five dried specimens of *C. urbana* were sent to Dr. Rosemarie DeClerck-Floate, AAFC, Lethbridge, to be used as vouchers since we submitted a petition to field release this insect in North America. Additional specimens were sent to Ulrich Schmid, Staatliches Museum für Naturkunde Stuttgart Germany, for identification.

4 Russian Knapweed (*Rhaponticum repens*)

Urs Schaffner, Allison Seidel and **Massimo Cristofaro**
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Open-field host-range test with artichoke, *Cynara scolymus*, growing next to mite-infested Russian knapweed plants

4.1 *Aulacidea acroptilonica* (Hymenoptera, Cynipidae)

During winter 2014/15, some 800 galls were collected in Uzbekistan. However, due to the fact that this biocontrol agent has now built up high densities at some of the release sites in the USA and in Canada, the field-collected galls were not shipped to quarantine facilities in North America.

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

In collaboration with Dr Asadi, Mashhad University, we continued with the open-field host-range test in Iran. The test design was identical to the tests set up in previous years, but the field plot was complemented by shipping an additional eight test plant species from Switzerland to Iran. Because the mite infestation rate on the Russian knapweed control plants was very low in 2014, we decided to use two methods to inoculate test and control plants. First, mite infested flower buds were cut into two pieces and pinned to the shoots or rosette leaves of the transplanted 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. All above-ground parts of the test and control plants will be harvested in August 2015 and sent to Dr Radmila Petanovic and Dr Biljana Vidović, University of Belgrade, Serbia, for mite extraction and identification. The results of the open-field host-range test will become available in winter 2015.

Mite-infested plants were also hand-carried from Iran to the quarantine facility at the CABI Centre in Delémont to continue with the no-choice host-range testing under quarantine conditions. In total, ten different test plant species and the control *R. repens* were artificially inoculated by pinning mite infested leaves onto the healthy leaves of the potted plants. All plants will be weekly inspected by randomly selecting a leaf from the potted test and control plants and inspecting it under the microscope for the presence of live mites. The final results of the different host range tests will become available in autumn 2015.

Dr Ghorbanali Asadi inspecting sunflowers for the open-field host-range test with *Aceria acroptiloni*

Group of *Cirsium* spp. plants exposed during the open-field test

The native North American *Cirsium undulatum* infected by *Pustula spinulosa* during the open-field test

5 Canada Thistle (*Cirsium arvense*)

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In 2013 and 2014, three native North American (NA) *Cirsium* species/subspecies, *C. undulatum*, *C. undulatum var. tracyi* and *C. ochrocentrum*, were found to be weakly susceptible when infected with the white blister rust *Pustula spinulosa*, currently investigated as a biological control agent for *Cirsium arvense*. From August to September 2014, an open-field test was undertaken in Urumqi, Xinjiang Province, China, to test the susceptibility of the three native *Cirsium* species to natural infection by. *P. spinulosa*. Two fully susceptible populations of *Cirsium arvense* (from Urumqi and Colorado, USA) as well as a few plants of *C. vulgare* were also included in the field trial. Both populations of *C. arvense* became infected with *Pustula* sp. under field conditions, while *C. vulgare* was found to be immune. Unexpectedly, also all three NA native *Cirsium* species became infected with *Pustula* sp. and the level of infection was comparable to the infection of the *C. arvense* control plants.

To verify that infection on test species really originated from *P. spinulosa*, molecular analysis was conducted in 2015. At least one of the infections on test species (ex *C. undulatum*) was identical to *Pustula spinulosa* ex Urumqi. Infections on *C. arvense* ex Urumqi and Colorado were also identical to *P. spinulosa* ex Urumqi. These results are in contrast to most other examples in weed biological control where weakly susceptible non-target plants, grown and inoculated under greenhouse conditions are not susceptible under natural field conditions. The reason could be that the mycoparasite that attacked *P. spinulosa* under laboratory conditions has no impact on its infection under low humidity conditions in the field.

In summary, we had to conclude that the NA native species will be at risk from infection by *P. spinulosa* from Urumqi, China if it was to be released as a classical biological control agent for *C. arvense* in North America.

Preliminary results of this research were presented at the XIVth International Symposium on the Biological Control of Weeds, 2 - 7 March 2014 in Kruger National Park, South Africa. Work in 2015 will focus on publishing the results of this project in a peer-reviewed Journal.

Wan Huanhuan inspecting *Cirsium* plants to be exposed in the open-field test with the white rust *Pustula spinulosa*

6 Garlic Mustard (*Alliaria petiolata*)

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Adult of *Ceutorhynchus alliariae*

6.1 *Ceutorhynchus alliariae* (Col., Curculionidae)

Between 12 March and 9 April 2015, adults of the stem-mining weevil *C. alliariae* ($N = 162$) were collected at field sites in Switzerland to conduct additional host-specificity tests. In no-choice oviposition and development tests with the North American (NA) *Streptanthus inflatus,* two larvae were found and preserved in ethanol for confirmation of identification. Five to six larvae were found on garlic mustard.

In single-choice tests with the two native NA species, *Rorippa subumbellata* and *Streptanthus glandulosus* ssp. *niger*, garlic mustard was highly preferred for oviposition, i.e. only 0.8 % and 14.8 % of eggs were found on the two test species, respectively.

On 6 May, an open-field test was set up with the native NA *Nasturtium gambelli* and *Alliaria* plants, to compare the host-choice behaviour of *C. alliariae* in the presence and absence of its normal host. Six wooden frames filled with sawdust were established, three with *N. gambelli* only and three boxes with both *N. gambelii* and garlic mustard. Unexpectedly, we found a high number of eggs and larvae on *N. gambelli*. Since this could also be due to contamination from other weevil species, the material will be sent for molecular analysis.

6.2 *Ceutorhynchus constrictus* (Col., Curculionidae)

Between 23 March and 28 April, 355 adults of the seed-feeding weevil *C. constrictus* emerged from our rearing colony established in 2014. On 27 April, a shipment of 200 *C. constrictus* was made to Jeanie Katovich and Roger Becker at the University of Minnesota to supplement their rearing colony and to conduct additional tests with indigenous native NA species in quarantine.

On 2 and 3 June, 215 additional adults were collected at field sites in Switzerland. At CABI, no-choice oviposition and development tests were established with four test plant species, none of which were attacked.

6.3 *Ceutorhynchus scrobicollis* (Col., Curculionidae)

In February 2015, no-choice oviposition and development tests were established with the indigenous NA *Streptanthus glandulosus* ssp. *niger.* Since plant quality deteriorated, plants were dissected about one month after set-up. One to three eggs or larvae were found on three of the five test plants, while up to 41 eggs and larvae were found on garlic mustard. The eggs and larvae found on *Streptanthus* will be sent for molecular analysis. If they are confirmed as *C. scrobicollis*, development and single choice tests will be conducted this autumn.

Amanda Stahlke releasing *C. alliariae* in the open-field test

Elinor Smith setting up the open-field oviposition test

7 Common Reed (*Phragmites australis*)

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7.1 Rearing of stem-mining moths

Our rearing of *Archanara geminipuncta* and *A. neurica* is on track. We produced enough pupae of *A. neurica* and *A. geminipuncta* to maintain our rearing colony and to setup another open-field oviposition test.

7.2 Open-field oviposition test

In tests conducted in 2013 and 2014, we exposed native North American (NA) reed (*Phragmites australis* spp. *americanus*), and populations of European and NA invasive reed (*P. australis*) to females of *A. geminipuncta* and *A. neurica* in an open-field setting. We were able to show that both moth species highly prefer European and NA invasive reed for oviposition under these conditions. Less than 5 % of the eggs were laid on native NA reed. In 2015, we are establishing a similar open-field oviposition test including the golf coast strain of *Phragmites australis* (haplotype I), which is also supposed to be native to NA. Four plots were established with 14 pots each of European, introduced, native, and haplotype I reed. We already released 13 mated females of *A. neurica* on each plot. Releases of *A. geminipuncta* will follow.

Setup of the open-field oviposition test

8 Whitetop or Hoary Cress (*Lepidium draba*)

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8.1 *Ceutorhynchus cardariae* (Col., Curculionidae)

Of the 715 adults of the gall-forming weevil *C. cardariae* overwintered at CABI, 91% survived, which were used for rearing and host range tests. Twohundred *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 28 species or subspecies, 12 of which had not been tested before, six of the new species developed galls to some extent and from one adults emerged so far. In a multiple-choice cage test exposing two plant species that had supported development in no-choice tests in 2014, one test species (*Paysonia densipila*) formed galls and one adult emerged. Finally, we established another open-field test with four non-target species that had supported relatively high adult emergence in previous tests. Detailed results will be presented in the Annual Project Report.

The no-choice development tests included a series of bioassays with 15 *Streptanthus* species and 27 populations. From these, leaves for chemical analysis were collected and will be analysed at the University of Neuchâtel with the aim to correlate the chemical profile of plants with their susceptibility to *C. cardariae.*

Plot with the four native North American test species exposed during the open-field test with *Ceutorhynchus cardariae*

Lepidium draba heavily attacked by *Ceutorhynchus cardariae* during the open-field test

8.2 *Ceutorhynchus turbatus* (Col., Curculionidae)

On 27 May 2015, about 1600 adults of the seed-feeding weevil *C. turbatus* were collected in southern Switzerland. On 28 May, we established no-choice oviposition tests with 59 plants; eight *L. draba* control plants and 1-5 replicates of 14 test species. Apart from *L. draba,* eggs were found in the native NA *L. integrifolium* and in the European *L. heterophyllum.* In addition, we established no-choice larval development tests with five test species that had been accepted for egg laying in previous tests. Mature larvae only emerged from the two target species *L. draba* and *L. chalepense.*

8.3 *Ceutorhynchus assimilis* (Col., Curculionidae)

Between 20 October and 5 November 2014, we established 127 no-choice oviposition and development tests with 22 test species. Plants were placed outside in a garden bed over winter and were regularly checked for adult emergence in spring 2015. Six test species supported adult development. We are planning to set up a multiple-choice cage test with these six species in autumn.

On 26/27 February 2015, over 900 galls of *C. assimilis* were collected at field sites close to Montpellier, France, and taken back to CABI. From 26 May to 10 June, 306 adults of *C. assimilis* emerged, which were placed in cylinders for aestivation. Including adults emerged from host-specificity tests and our rearing, we currently have a total of 600 *C. assimilis* in aestivation for additional tests in autumn.

Set up of open-field test with *Ceutorhynchus cardariae* in 2015.

Set up of impact experiment with *Ceutorhynchus peyerimhoffi*

9 Dyer's Wood (*Isatis tinctoria*)

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9.1 *Ceutorhynchus peyerimhoffi* (Col., Curculionidae)

Between 13 April and 21 May 2014, 3141 adults of the seed-feeding weevil *C. peyerimhoffi* emerged from our rearing colony. Adults were transferred onto flowering dyer's wood plants for rearing. From rearing plants and dyer's wood plants used in tests and for an impact experiment (see below) 8800 larvae emerged, but we only kept about half for adult emergence in 2016.

Between 5 May and 13 June, no-choice oviposition tests were established with 97 plants: 26 test species with 1-5 replicates each, and 16 dyer's wood control plants. Apart from dyer's wood, eggs were found in three test species. Plant species accepted for egg laying were subsequently tested for larval development. Mature larvae only emerged from control plants.

Since some *C. peyerimhoffi* larvae emerged from the federally listed *Boechera hoffmannii* last year, we set up an oogenesis test with this species. On one of the two *B. hoffmannii* plants established, females were apparently able to develop and lay eggs, but only dead first or second instar larvae were found upon dissection.

We also established a multiple-choice field cage test with *B. hoffmannii* and *Streptanthus heterophyllus*, another native NA species that had supported development under no-choice conditions in 2014. We found eggs in controls and unexpectedly also a few in *S. heterophyllus*. Since eggs on *S. heterophyllus* could have been due to contamination, we will send the material for molecular analysis for confirmation.

Finally, we established an experiment to test the impact of larval mining by *C. peyerimhoffi* on reduction in seed output. Fifty homogenous dyer's wood plants were assigned to one of five weevil densities, 0, 4, 8, 16 or 32 females and an equal number of males for 3 weeks. Data on seed output is currently being collected.

9.2 *Ceutorhynchus rusticus* (Col., Curculionidae)

All plants that had been exposed to the root-crown mining weevil *C. rusticus* during no-choice oviposition and development tests set up in autumn 2014 were regularly checked, and dying plants were dissected. Adults only emerged from dyer's wood plants, and no mines typical for *C. rusticus* were found in any of the test plant species.

Stéphanie Morelon and Allison Seidel taking down the impact experiment with *Ceutorhynchus peyerimhoffi.*

10 Perennial Pepperweed (*Lepidium latifolium*)

Sonja Stutz, Amanda Stahlke, Esther Gerber, and Hariet L. Hinz (CABI) and Massimo Cristofaro, Matthew Augé, Daniele De Simone, Francesca Di Cristina, and **Francesca Marini (BBCA)**
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10.1 *Metaculus lepidifolii* (Acari, Eriophyidae)

In 2015, we conducted two field trips to Georgia, one at the end of April and one at the beginning of June. The aim of the first field trip was to collect the eriophyid mite *Metaculus lepidifolii* from a field site near Tbilisi where the mite had been found in 2014. During the second field trip we searched for additional field sites around Tbilisi and in northern Georgia, an area not surveyed so far. Deformed plant material potentially attacked by *M. lepidifolii* was collected in ethanol and has been or will be forwarded to Dr Radmila Petanovic, University of Belgrade, Serbia, for mite extraction and identification.

Metaculus lepidifolii on PPW

During both field trips to Georgia, shoots and whole plants of perennial pepperweed (PPW) with signs of mite attack were collected from the site near Tbilisi and imported into the quarantine facility at CABI to establish a rearing and develop methods for host-range testing. Although all plants and shoots were immediately checked under a microscope, no mites were observed on any of the plant material.

To find out more about the biology and phenology of *M. lepidifolii,* several visits were made between April and July to PPW sites in central Turkey where this eriophyid mite had been found in previous years. In addition, an open-field test was established using eight test plant species and PPW as controls. On 22 June, bouquets of cuttings of PPW infested with *M. lepidifolii* were placed close to the young leaves of each potted test species and PPW (see picture below). The plants are currently being harvested and will be sent to Dr Petanovic for mite extraction and identification.

10.2 *Ceutorhynchus marginellus* (Col., Curculionidae)

On 4 March 2015, weevils were taken out of overwintering incubators. Similar to last year, overwintering survival was high for weevils reared on PPW (86%), while survival was much lower for weevils reared on *L. eastwoodiae* (11.4%, only females survived) and *L. virginicum* (45.2%). We continued with population viability tests that had been established with these two native North American test plant species in 2014. Females reared on *L. eastwoodiae* laid a similar number of eggs and a similar number of larvae and adults emerged as from females reared on PPW. Oviposition of females reared on *L. virginicum* was delayed for 12 days and only a few adults emerged. However, this might have been caused by low plant quality. Currently, we keep about 800 adults from PPW, 30 from *L. eastwoodiae* and six from *L. virginicum* in cylinders and/or on potted plants.

Ceutorhynchus marginellus on a PPW flower head

Bouquet of PPW cuttings infested with *Metaculus lepidifolii* placed on a potted PPW plant in the open-field test in central Turkey

Chrysochus asclepiadeus on *Vincetoxicum hirundinaria*, Ticino, 18 June 2015

11 Swallow-Worts (*Vincetoxicum* spp.)

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

We are currently maintaining a rearing of the root-mining beetle *C. asclepiadeus* from a tested population originating in Ukraine at CABI. From 1-20 June 2015, a total of 94 females and 69 males emerged from 750 larvae (21.7%) transferred onto *V. nigrum* in 2013. No beetles have emerged from 500 larvae transferred in 2014.

Adults are reared as in previous years. All adults are placed into separate rearing containers to collect eggs and are fed with cut shoots of *V. nigrum*. Rearing containers consist of transparent ventilated plastic boxes (32 x 18 x 12 cm) containing shoots of *V. nigrum* 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.

In total, 750 newly hatched larvae have been transferred onto *V. nigrum* to ensure maintenance of a small colony for future potential research. All plants are being kept in the Centre's garden to monitor adult emergence in 2016.

A preliminary molecular identification based on the mtDNA COI gene of 19 *C. asclepiadeus* collected from Ukraine, France and Switzerland in 2010 found that there was quite a large genetic divergence between specimens from France/Switzerland and those from Ukraine suggesting at least the existence of well-defined subspecies. However, our experience with *Rhinusa pilosa* from *Linaria vulgaris* indicates that an analysis of the nuclear EF-1αgene will be needed to confirm species separation.

With this aim in mind, a collection of 102 beetles has been made on 17-18 June in southern Switzerland. Thirty beetles have been put in ethanol for molecular analysis. A rearing has been started with the remaining ones. On 30 June, 24 beetles have been collected near Poligny in eastern France. These beetles will be used for molecular work as well.

11.2 *Euphranta connexa* (Dipt., Tephritidae)

We are planning to collect seed pods of *V. hirundinaria* infested with this seed feeding fly in early August in southern Switzerland and two weeks later in the Swiss Jura Mountains. Pods will be overwintered and emerging flies used for additional host-specificity tests in 2016.

Sérgio Ribeiro collecting *Chrysochus asclepiadeus* on *Vincetoxicum hirundinaria* in France, 25 June 2015

12 Common Tansy (*Tanacetum vulgare*)

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12.1 *Microplontus millefolii* (Col., Curculionidae)

Work on *M. millefolii* is being conducted in Russia by Dr Margarita Dolgovskaya and her team (Russian Academy of Sciences, Zoological Institute, St Petersburg). In mid-June, 88 females and 82 males of this shoot-mining weevil were collected and used for additional host-specificity tests. No-choice tests are being carried out with 14 plant species. In addition, one multiple-choice field cage test and one open-field test has been set-up. All plants will be dissected mid-July.

Flowering *Tanacetum huronense* at the end of the oviposition period by *Platyptilia ochrodactyla*

12.2 *Platyptilia ochrodactyla* (Lep., Pterophoridae)

On 20 June, 340 shoots attacked by the shoot-mining moth *Platyptilia ochrodactyla* were collected in southern Germany. The collection was made during a similar time period as in 2013. While in 2013, the ratio between the number of larvae and the number of attacked shoots collected was slightly above 1, it was only 0.3 in 2015, which means that about two thirds of the attacked shoots were empty at the time of collection.

The sex ratio of *P. ochrodactyla* has been very inconsistent during the past three years: 2.3 ♂/♀ in 2013, 1.0 ♂/♀ in 2014 and 0.4 ♂/♀ this year. In total, 30 males and 76 females emerged from 110 larvae/pupae collected in 2015.

Set up of open-field test with *Microplontus millefolii*, Russia, 15 June 2015 (photo: Margarita Dolgovskaya)

Another difficulty encountered this year was the absence of flowering *T. vulgare* during the emergence period of the moth. However, because our main plants of concern, i.e. *T. huronense* from North America, *T. parthenium* and *T. corymbosum* were in a good phenological stage for oviposition, we decided to go ahead with oviposition tests with these three species despite the lack of controls.

Sérgio Ribeiro dissecting *Tanacetum parthenium* flowerheads

Dr Ghorbanali Asadi with the test plant *Ziziphus jujuba* in the garden of the experimental farm of Mashhad University, Iran

13 Russian Olive (*Elaeagnus angustifolia*)

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13.1 *Aceria angustifoliae* (Acari: Eriophyoidae)

In collaboration with Dr Asadi, Mashhad University, Iran, the test and control plants in the common garden at the experimental farm of Mashhad University were again inoculated with *A. angustifoliae* by pinning infested leaves to healthy leaves of each test and control species. Throughout the growing season, all plants will be regularly inspected for signs of mite attack.

We also continued with the impact experiment set up at the experimental farm of Mashhad University. Inspection of the ten Russian olive trees that have been experimentally inoculated with *A. angustifoliae* since 2010 revealed that the level of infestation remained very low.

During the summer, we managed to re-establish an impact experiment in central Turkey. In collaboration with Dr Kahraman Gurcan, Erciyes University, Kayseri, Turkey, twenty 50 cm tall Russian olive trees were purchased at a tree nursery. Half of the trees were inoculated with the mite, while the other half was kept free of mite attack (control plants). As in the other impact experiment, these young trees will be repeatedly inoculated over the next years to monitor impact of *A. angustifoliae* on young Russian olive plants.

In early June, mite-infested Russian olive branches were collected in Iran and hand-carried to the quarantine at the CABI Centre in Delémont, Switzerland. Mite-infested leaves were pinned to leaves of 10 test plant species and to Russian olive. Test and control plants will be monitored for mite attack at a weekly interval. The results of this bioassay will become available in winter 2015/16. If the host-range testing will be successful in 2015, the compilation of a petition for field release of this biological control candidate can be envisaged for 2016.

13.2 *Aceria eleagnicola* (Acari: Eriophyoidae)

In collaboration with Dr Radmila Petanovic and Dr Biljana Vidović, University of Belgrade, Serbia, field studies have been initiated in Serbia to study the biology of this second mite species under open-field conditions. It is also planned to evaluate methods to experimentally inoculate potted Russian olive plants under contained conditions which would allow to start with host-specificity studies in 2016.

Allison Seidel checking test plants for attack by the mite *Aceria angustifoliae*

14 Oxeye daisy (*Leucanthemum vulgare*)

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14.1 *Dichrorampha aeratana* (Lep., Tortricidae)

In spring 2015, we recorded adult emergence from the nine Shasta daisy varieties and oxeye daisies which had been exposed in multiple-choice cage tests with *Dichrorampha aeratana* in spring 2014. An average of 0.2 adults emerged from Shasta daisies and 2 adults from oxeye daisies. Adults emerged from all except for one Shasta daisy variety.

In May, additional no-choice larval development tests were set up with 13 different test plant species, with 1-8 replicates each. Five first instar larvae were transferred onto each of the plants, which will be dissected for larvae in autumn.

Set up of open-field test with *Cyphocleonus trisulcatus*

14.2. *Cyphocleonus trisulcatus* (Col., Curculionidae)

More than 80% of the 540 adults of the root-feeding weevil *Cyphocleonus trisulcatus* that were kept for overwintering in cylinders in an incubator set at 2°C survived until spring. From May to July, we set up no-choice oviposition and larval development tests with 34 test plant species. In total, 220 control and test plants were exposed and about half of the plants have been dissected so far. Larvae were found on one *Achillea ptarmica* plant but not on any of the other test plant species exposed.

We also set up multiple-choice cage tests with *Matricaria chamomilla*, *M. occidentalis* and *Glebionis coronaria*, plant species that had supported adult development in previous tests. Ten females were released into each of four cages containing three plants of each test plant species and controls. In July, all plants were dissected. Larvae were found on all control plants, on 25% of *M. chamomilla*, 17% of *M. occidentalis* but on none of the *G. coronaria* plants.

In addition, we set up an open-field test with four Shasta daisy varieties and oxeye daisies as controls. A total of 50 females were released in June and the plants will be regularly checked for adult emergence from September onwards.

The plants of the impact experiment that had been set up in 2014 have been harvested in July. Plants infested with *C. trisulcatus* produced on average 50% fewer shoots and flower heads than non-infested control plants.

Adult of *Cyphocleonus trisulcatus* (photo: T. Haye)

14.3. *Tephritis neesii* (Dipt., Tephritidae)

Of the 778 adult *T. neesii* which were hibernated under different conditions only 47 survived until spring 2015. Only 4% of the 680 adults kept in rearing cages under ambient temperatures survived compared to 18% of adults kept in cylinders in an incubator set at 2°C. Overwintering survival was considerably lower than in 2013 when adults had been kept in cylinders in a wooden or underground shelter.

Amanda Stahlke releasing adults of *Cyphocleonus trisulcatus* in the open-field test

Adult of *Melanagromyza albocilia*

15 Field bindweed (*Convolvulus arvensis*)

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15.1 *Melanagromyza albocilia* (Dip., Agromyzidae)

Between 11 July and 11 September 2014, 2102 plants were collected in southern Germany and brought back to the lab for dissection. From the 430 larvae and pupae that were extracted, 266 live pupae were placed into Petri-dishes with slightly moist filter paper and stored in an underground shelter. In August and September 2014, 13 parasitoids, 13 female flies and six males emerged. The remaining pupae were checked regularly for fungi, cleaned and any dead pupae were removed. After overwintering, 193 pupae were left. In late May 2015, 40 flies emerged, 40% males, and 62% parasitoids (both in line with previous years). Pairs of flies were offered honey and cut shoots of field bindweed in plastic cylinders and females were used in tests when mating was observed or eggs were found. Six females were used in sequential oviposition tests, where cut shoots of test plants are exposed for one to three days, immediately followed by exposing *C. arvensis* (control). No feeding or oviposition was found on *Hyosciamus niger.* We also exposed seven potted hedge bindweed (*Calystegia sepium)* and field bindweed control plants to two females and one male each in no-choice development tests. Hedge bindweed had been accepted for oviposition in previous no-choice oviposition tests. However, we never recorded attack on hedge bindweed plants at sites where we collected infested field bindweed. Plants are currently being checked for feeding and dissected for eggs and larvae.

Further field collections of pupae in southern Germany will be conducted later this summer.

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Calystegia sepium (left) and *Convolvulus arvensis* (right) in a corn field in southern Germany (photo: G. Grosskopf-Lachat)

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

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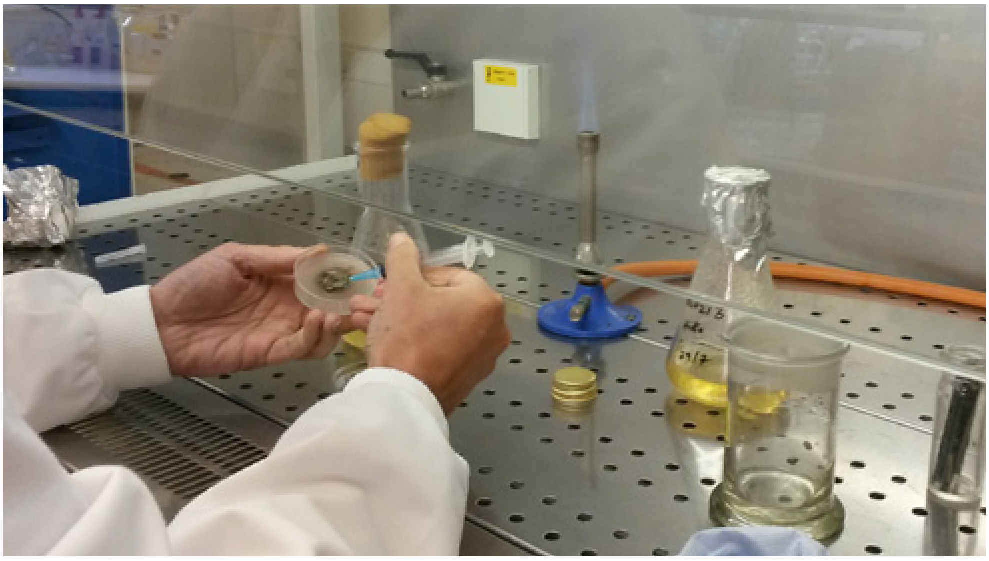
Following on from last year's research undertaken for Canada, virulence assessments of the selected strains of *Mycosphaerella polygoni-cuspidati* ex Mt. Hiko (high altitude site, Kyushu) and ex Omura (coastal low altitude site, Kyushu) were continued. This study focusses on evaluating the susceptibility of the most prominent Canadian biotypes of *Fallopia 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*). 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.

Susceptibility of *F. sachalinensis* towards both pathogen strains was found to be low with few plants showing more than 10 individual small necrotic lesions, per inoculated leaf whilst others showed no symptoms. Compared to that, the susceptibility of *F. x bohemica* (210-307) was slightly higher showing more consistent leaf-spot symptoms on inoculated leaves. The replicate numbers for the other two clones of *F. x bohemica* (Nehalem and Suislaw River) are currently too low to draw definite conclusions as to their susceptibility. To date, no leaf-spot characteristic symptoms have been observed on any of the tested non-target species, although some inoculated plants of both buckwheat varieties showed unspecific chlorosis, possibly due to damage caused during application of the inoculum or by insect pests.

Host-specificity assessments undertaken for *M. polygoni-cuspidati* with respect to its biocontrol potential for the UK have highlighted the UK native species *Polygonum maritimum* as a potentially vulnerable non-target species. Based on these results, it has been recommended to include the rare North American *Polygonum glaucum* into the initial specificity assessments for Canada. *Polygonum glaucum* closely resembles *P. maritimum* and is frequently listed as a synonym of this species. Seeds of *P. glaucum* were collected by Lisa Tewksbury in October 2014 and sent to CABI for propagation. A further shipment of *P. glaucum* seedlings was made in June 2015. Plants are currently establishing in our quarantine facilities and will be assessed as soon as possible.

Sarah Thomas and Kate Pollard inspecting *Fallopia* species for leaf spot symptoms



Preparation of *Mycosphaerella polygoni-cuspidati* mycelial inoculum for host range testing of *Fallopia* spp

17 Flowering Rush *(Butomus umbellatus)*

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Mature larva of *Bagous nodulosus* mining in flowering rush rhizome

17.1 *Bagous nodulosus* (Col.: Curculionidae)

We found exceptionally high survival rates of *B. nodulosus* adults overwintered on potted plants individually covered by gauze bags in a pond in our common garden (see picture below). Over 80% of the weevils were found again in spring 2015. Over 200 additional adults were collected during field trips in Northern Germany (May 14-15), Czech and Slovak Republics (May 29-June 1) and Serbia (June 7-8).

Between 21 and 27 May 2015, an impact experiment was established by releasing different densities (0, 1, or 3 pairs) of *B. nodulosus* onto individually potted, gauze-covered flowering rush plants. Ten replicates were established per density. Only egg laying females were used. Prior to set up plant size was measured and it was ensured that there were no initial differences in plant parameters between treatments. After two weeks, weevils were removed from the plants and feeding damage quantified. Once weevils have completed their larval development (after about eight weeks), all plants will be dissected, the number of *B. nodulosus* noted and biomass recorded. Results will be available later this year.

Bagous validus female with egg

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 19 test plant species exposed.

The remaining adults were used to test various rearing methods. It seems that we are having more success rearing this species when using plants grown under not submerged conditions.

17.2 *Bagous validus* (Col.: Curculionidae)

During a field trip to the Czech and Slovak Republics from 29 May – 1 June, we finally found *B. validus* at a site in southern Slovakia near the Hungarian border. We were able to collect 25 individuals (12 females, 13 males). Since this species is extremely rare and nothing is known about its biology, we are concentrating on elucidating its life cycle and trying to establish a rearing at CABI. The adults are indeed feeding on flowering rush. However, we were able to obtain only six eggs so far, and eggs were randomly laid on the leaf surface or on the walls of the rearing containers. We went back to the site on 30 June, to collect plant material to be dissected for larvae and mining of *B. validus*.

17.3 *Phytoliriomyza ornata* (Dipt.: Agromyzidae)

In autumn 2014, we had collected several pupae of the agromyzid fly *Phytoliriomyza ornata*. Since only one adult fly emerged, we were not able to work with this species in 2014. We suspect the species to overwinter in the pupal stage. Indeed, a fly emerged from eight overwintered pupae in May 2015. However, only parasitoids or nothing emerged from the remaining pupae. We hope to obtain more larvae and pupae by dissecting plants collected during our field trips in June/July.

Artificial pond at CABI in which *Butomus* plants and potential agents (see gauze-covered plants) are kept

18 Himalayan Balsam (*Impatiens glandulifera*)

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As Japanese knotweed, Himalayan balsam is a 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. One of the most promising agents for Himalayan balsam is the rust *Puccinia komarovii* var. *glanduliferae*. Host range testing for Europe was completed during 2014 and included a total of 75 plant species and varieties. No non-target symptoms were observed on any of the plant species tested with the exception of *Impatiens scabrida*, which was weakly susceptible, and *Impatiens balsamina*, which was shown to be fully susceptible under quarantine conditions. *I. balsamina* is an ornamental species which is only occasionally grown in the UK. Data on the host range, infection parameters and information on the lifecycle of the pathogen was published in the journal Plant Pathology at the beginning of 2015.

In 2014 the Pest Risk Assessment for the rust was approved by UK authorities (FERA/DEFRA) and accepted by the European Commission (EC) Standing Committee of Plant Health, and in September first releases occurred at three sites in southern England. Monitoring at these sites revealed that the rust naturally spread and infected neighbouring Himalayan Balsam plants in the field. The overwintering teliospore stage of the rust was found to develop on leaves as these senesced and to persist in the leaf litter during the winter months. In a subsequent field experiment, the spermogonial spore stage of the rust life cycle was recorded on *I. glandulifera* seedlings resulting from infection with basidiospores originating from germinating overwintered teliospores. Seedlings also displayed symptoms of stem warping indicative of the beginning development of the aecial spore stage (see pictures). This indicates that the rust pathogen is able to complete its lifecycle under field conditions in the UK. During 2015 the rust was released at five additional regions across the UK. Monitoring of the persistence of the rust and its initial spread is currently ongoing and will continue until plant die back in the autumn. These releases include both northern (Northumberland) and southern (Cornwall) counties and will provide valuable data on the potential of the rust to establish and spread under Canadian climatic conditions.

Research during 2015 is continuing to evaluate the host range of the rust against the remaining Canadian specific test plants. Due to partial overlap with the European test plant list, a total of 39 species including *I. namchabarwensis*, *I. flanaganae*, *I. gomphophylla*, *Cyrilla racemiflora*, *Dodecatheon pulchellum*, *Strax americanus* and *Symplocos paniculata* have been tested with no non-target impacts recorded to date. Seeds of *I. ecalcarata* and *I. aurella* sent during 2014 failed to germinate and will be collected again later in the year.

Himalayan balsam seedlings infected by the rust *Puccinia komarovii* var. *glanduliferae*. Aecial development on the stem following cross fertilization of the spermogonia: stem is distorted, and yellow aecial cups erupt from the surface

Stems of Himalayan balsam seedling with first signs of infection by the rust: distortion, reddening and spermogonial development visible

Himalayan balsam seedlings infected with the rust elongate more than the non-infected seedlings, taking the spores above the canopy for release into air currents

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