



Weed Biological Control

Progress Report 2017

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



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Notes from the section leader

For those of you who might not have the time to read through the whole report I summarized some of the project highlights, especially in terms of petitions, from this spring below.

A joint petition for the field release of the root-feeding hoverfly *Cheilosia urbana* for invasive hawkweeds was submitted to the USDA-APHIS, Technical Advisory Group (TAG) and the Canadian Biological Control Review Committee in December 2014. The agent was approved for release in Canada by the Canadian Food Inspection Agency (CFIA) in April 2016 and recommended for release by TAG for the U.S. in May 2016. This spring, we sent eggs and adults of *C. urbana* to Rosemarie De Clerck-Floate (Agriculture and Agri-Food Canada lab in Lethbridge, Alberta), to infest hawkweed plants in view of field releases in 2018.

We were very satisfied to learn in February 2017 that TAG finally recommended release of the root-mining weevil *Ceutorhynchus scrobicollis* in the w.U.S. This decision came after we had submitted the first petition for field release of this insect in 2008 (!) and it took us three submissions to satisfy TAG reviewer comments. Our U.S. collaborators at the University of Minnesota (Roger Becker and Jeanie Katovitch) and at the Minnesota Department of Natural Resources (Laura van Riper) will now have to facilitate the further administrative steps before the weevil can actually be released, namely section 7 consultation with the U.S. Fish and Wildlife Service (FWS). So keep fingers crossed!

Host-specificity tests with the eriophyid mite *Aceria angustifoliae* on Russian olive have been completed and confirmed that the mite is only able to develop on Russian olive. All data has been summarized and sent to our North American collaborators in March 2017 for the preparation of a petition for field release.

Additional host range tests had been requested by TAG after our first submission of a petition of the gall-forming weevil *Ceutorhynchus cardariae* on hoary cress. These tests were completed this spring and convinced us that the weevil is specific enough to be considered for field release. We are planning on submitting a revised petition in winter 2017/18.

At the beginning of May, Andrew McConnachie from the Department of Primary Industries in New South Wales, Australia, visited the Centre to get acquainted with the root-mining moth *Dichrorampha aeratana* on oxeye daisy. A project for this weed is ongoing since 2008 for Canada and the U.S., but it is also a problem in Australia. Andrew hand-carried back eggs of the moth to establish a rearing in quarantine and conduct additional host-specificity tests in view of potential field release in Australia. A petition for field release of the moth in North America will likely start to be prepared this winter.

Further petitions in the pipeline include the two noctuid moths, *Archanara geminipuncta* and *A. neurica*, for common reed, the gall-forming weevil *Rhinusa rara* on Dalmatian toadflax and the seed-feeding weevil *Mogulones borraginis* on houndstongue.

In addition, I just learnt that the Environmental Assessment for the noctuid moth *Hypena opulenta* for invasive swallow-wort control has been placed on the Federal Register for comment (see <https://www.regulations.gov/docketBrowser?rpp=50&so=DESC&sb=postedDate&po=0&dt=PS&D=APHIS-2017-0053>). This means that it finally passed the section 7 consultation with the US FWS! The large majority of comments are very positive and in complete support of the release, so hopefully we will see this agent finally being released in the U.S. in 2018.

At the end of June, Al Cofrancesco, Technical Director at the Army Corps of Engineers and chair of TAG visited us to get an overview of our ongoing weed biocontrol projects. He encouraged us and our North American collaborators to submit new petitions to TAG. As mentioned above, several are on their way!

Finally, since we do not prepare an Annual Centre Report anymore, I would like to encourage you to visit our website at www.cabi.org/about-cabi/cabi-centres/switzerland/ which now features a description of each of our sections, project highlights and a new video about the Centre.

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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 262 (57.3%) adults of the stem-gall forming weevil, *R. pilosa*, and 140 (45.3%) *R. rara* survived hibernation. The survival rate of both gall inducing species was higher than in 2016. The adults of *R. pilosa* were set up in cages planted with *Linaria vulgaris* for mass rearing. A total of 1090 galls were formed, yielding 3200 adults. In addition, to increase genetic diversity of the laboratory population of *R. pilosa*, a total of 367 galls were collected between 26 April and 1 May from 6 different locations in northern and eastern Serbia. A total of 207 *R. pilosa* adults emerged from the field collected galls, in contrast to >2000 adults of the inquiline weevil, *R. eversmanni*.

To record genetic diversity of the weevils reared under cage conditions and those collected from the field, a total of 55 specimens were extracted from selected galls (one specimen per gall) and analyzed on the mitochondrial COII gene. Both COII genotypes (Rp-A and Rp-B) were recorded in the analyzed specimens selected from different sources and locations. After molecular analysis, the adults from selected galls were separated in batches according to the particular COII gene haplotype and set up separately for aestivation at 24 ± 1 C°.

In addition, a total of 211 galls of *R. rara* were produced on 120 *L. genistifolia* and *L. dalmatica* plants. The induced galls yielded 584 adults. In addition, some 104 *R. rara* galls were field collected between 10 and 11 May at three locations in eastern Serbia. From these galls, 213 *R. rara* adults emerged and 533 *R. eversmanni*. All emerged adults of *R. rara* were set up for aestivation at 24 ± 1 C°.

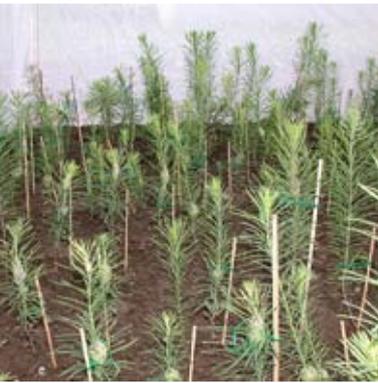
1.2 *Mecinus* spp. (Col., Curculionidae)

A total of 262 (85.3%) adults of the stem-borer weevil *Mecinus heydenii* and 104 (55.6%) adults of *Mecinus peterharrisi* survived hibernation. Because only 16 adults of *Mecinus laeviceps* survived hibernation, an additional 44 adults of this species were field collected on 1 April.

A test was set up with two critical North American test species, *Nuttallanthus canadensis* and *Sairocarpus virga*, to see to which degree *M. heydenii*, *M. laeviceps*, and *M. peterharrisi* can survive and reproduce on these species in the absence of their natural field host. On *N. canadensis*, a total of 166 adults of *M. heydenii* developed, but only two and 17 adults of *M. laeviceps* and *M. peterharrisi*, respectively. No development occurred on *S. virga*. Adults obtained ex *N. canadensis* were set up in batches of five specimens on *N. canadensis* shoots for feeding and aestivation. *M. heydenii* that developed on *L. vulgaris* were set up as a control.

No-choice oviposition and larval development tests with *M. peterharrisi* were continued, exposing 25 plant species and populations, 18 native to North America. Larval development was recorded only on control plants, *L. genistifolia* and North American Dalmatian toadflax populations, confirming the narrow host range of this species.

In additional single-choice (*N. canadensis* only) and multi-choice tests (*Sairocarpus virga* and *N. canadensis*) with *M. heydenii*, *M. laeviceps* and *M. peterharrisi* larval development only occurred on control plants.



Mass rearing of *Rhinusa pilosa* on *Linaria vulgaris* in mesh cage, May 2017



Multiple-choice test with *Mecinus peterharrisi*. *Linaria dalmatica* control (right), *Nuttallanthus canadensis* (middle) and *Sairocarpus virga* NA (left)



Mecinus peterharrisi feeding damage on *Linaria dalmatica*, Kamloops, British Columbia, Canada

2 Houndstongue (*Cynoglossum officinale*)

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2.1 *Mogulones borraginis* (Col., Curculionidae)

Current work on houndstongue is focused on the seed-feeding weevil *M. borraginis*. It has proven to be the most specific agent acting on houndstongue thus far and a petition for field release is in preparation.

Due to a lack of suitable, reproducing houndstongue plants in 2016, we were able to overwinter only 60 larvae of *M. borraginis*. At the beginning of March 2017, we were able to retrieve 34 adults (22 females and 13 males), which were set up on houndstongue rosettes. At the end of May, 16 females were found back. Two females and one male each were placed onto eight houndstongue plants that had started to produce fruits. After seven days, weevils were retrieved and placed onto new plants, this time only placing one female onto each plant. This set up was repeated twice, resulting in a total of 54 rearing plants. At the end of June, fruit bearing inflorescences were covered individually with gauze bags and vials attached to the end of each bag to collect mature larvae leaving the fruits. A total of 650 *M. borraginis* larvae emerged. We were therefore able to get our rearing back on track despite the low number of egg laying females available.



Set up of rearing of *Mogulones borraginis* at CABI



Summer student Christian Mathias collecting larvae of *Mogulones borraginis* from our rearing

3 Hawkweeds (*Pilosella* spp.)

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3.1 *Aulacidea pilosellae* (Hym., Cynipidae)

Two populations of this gall wasp are being investigated. One population is *A. pilosellae* ex *Pilosella 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 2016, over 2000 galls of *A. pilosellae* ex *P. officinarum* were harvested after plants were exposed to adults obtained from Dr. Rosemarie De Clerck-Floate (Agriculture and Agri-Food Canada (AAFC), Lethbridge). From these about 300 females emerged in May and June 2017. From 7 May to 12 July, 22 species were exposed in no-choice tests, including *P. officinarum* of different origins, three North American (NA) *Hieracium* species and five other NA natives, resulting in a total of 122 plants. Gall harvest is ongoing and so far, galls were only found on *P. officinarum*. In single-choice tests exposing *H. argutum* or *H. gronovii* together with *P. officinarum*, all control plants developed galls and no galls were found on *H. gronovii*. Galls were found on *H. argutum*, but at a much lower level, i.e. only 10% of plants were attacked with about three galls each vs. 25 galls on controls. Additional *P. officinarum* plants are being exposed for rearing.

In order to advance with host range experiments, Dr. De Clerck-Floate (AAFC, Lethbridge) kindly agreed to send us more galls of both southern and northern populations from their cultures. The shipment of about 1000 *A. pilosellae* ex *P. officinarum* and 425 *A. p.* ex *Pilosella* spp. arrived mid-June. Emergence is ongoing, but at a low rate. So far, only a few additional test plants could be exposed with the additional *A. pilosellae* ex *P. officinarum*. With *A. pilosellae* ex *Pilosella* spp. that emerged from the AAFC galls, we were able to set up 15 no-choice tests and two rearing plants.

On 27 and 28 June 2017, *Pilosella* plants with galls of *A. pilosellae* were collected in the Czech Republic and Poland in the Krkonoše area. The plants were brought back to the lab, potted and kept in a greenhouse until galls were mature for harvest. The plants will be kept until summer 2018 to observe flower colour and confirm identification. In Sosnowka (Poland), we collected 63 plants with about 700 galls to augment our rearing and to use in host range tests in 2018. Over 700 galls were collected from 121 plants of *P. aurantiaca*, *P. caespitosa*, *P. glomerata* and seemingly a hybrid of *P. officinarum* from five different field sites. Larval or pupal wasp samples will be sent to Dr. Kevin Floate (AAFC) for sequencing and leaves will be sent to Dr. John Gaskin (USDA, ARS) for molecular analysis. Plants from which wasps will be analysed will be kept as herbaria for later morphological identification.

3.2 *Cheilosia urbana* (Dipt., Syrphidae)

In 2017, activity of *Cheilosia urbana* in the field was delayed owing to a late period of cold and frost. Three field collections in the Swiss Jura Mountains in April were unsuccessful. In May, we surveyed known sites in the Black Forest, Germany and were able to find 42 hoverflies, of which 34 were *C. urbana*. Of these, 24 were sent to Dr. DeClerck-Floate together with 500 eggs harvested from these females in the lab prior to shipment. The larvae were transferred to potted plants at the AAFC quarantine facility in view of field releases in 2018. Additional eggs harvested from the females kept at CABI were used to infest a patch of *P. officinarum* in the CABI garden to secure future on-site collections.



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



Female of *Cheilosia urbana*



Summer student Jérôme Schneuwly collecting *Cheilosia urbana* in the Black Forest, southern Germany

4 Russian Knapweed (*Rhaponticum repens*)

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

In collaboration with Dr Ghorban Ali Asadi, Mashhad University, we continued with the host-range testing in Iran. This mite has been extremely challenging to work with, not only is the species extremely sensitive but we have not yet had long term establishment on control plants under experimental conditions. In the past few years we have put in a tremendous amount of effort to improve our understanding of this species and our understanding of the host-range test results.

The open-field host range test included several closely related North American as well economically important species. As in 2016, 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. In contrast to previous years, about 10 days after the last inoculation the plants were harvested on a daily basis and the mites extracted from fresh material. This allowed us to determine whether mites were alive or dead on the test and control plants. Live mites were recorded on seven of the 10 control plants and unfortunately on several test plant species including, *Solidago nemoralis*, *Artemisia ludovicana*, *Carthamus tinctoria* and *Cynara scolymus* (artichoke). However, at the moment we do not know the exact identity of these live mites and it is possible that they are different to *Aceria acroptiloni*. The mite samples are currently being analysed by our collaborators, Dr. Biljana Vidović and Prof. Radmila Petanović at the University of Belgrade, Serbia.

To collect quantitative data on the field host range of the mite, in 2015 and 2016, test plants, especially close relatives of *R. repens* were collected from field sites near Shirvan, Iran, where *R. repens* was heavily infested with mites. *Aceria acroptiloni* was recorded on several of these test plant species despite no mite attack symptoms ever being recorded. This year we attempted to quantify the natural dispersal of mites from naturally occurring infested *R. repens* plants. We setup 10 pan traps filled with collection liquid in and around the *R. repens* stand and left them overnight for a period of 14 hours. Due to the day temperatures being in excess of 40°C we did not leave the traps for a full 24 hour period. We were able to collect several mites in all the pan traps, however, as above the identity of the mites is still unknown. The samples are in Serbia for analysis. If these mites turn out to be *A. acroptiloni*, this could explain the presence of mites on other plant species growing close to *R. repens* with the absence of damage symptoms, as the mites are randomly dispersing and could be considered vagrant visitors.

Mite-infested plants were also hand-carried from Iran to the quarantine facility at CABI in Delémont to continue with no-choice host-range testing. This year the mites did survive the journey and quarantine tests for 2017 are ongoing. Detailed results will be presented in the Annual Project Report.



Philip Weyl collecting and preparing *Aceria acroptiloni* infested Russian knapweed in Shirvan, Iran, for transport to the quarantine facility at CABI



Francesca Marini (BBCA) preparing a plant sample for mite extraction



Quantifying dispersal of *Aceria acroptiloni* using simple pan traps in Iran



Adult *Ceutorhynchus constrictus*

5 Garlic Mustard (*Alliaria petiolata*)

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5.1 *Ceutorhynchus constrictus* (Col., Curculionidae)

Between 27 March and 4 April, 170 adults of the seed-feeding weevil *Ceutorhynchus constrictus* emerged from our rearing colony established in 2016.

On 16 May, only 22 additional adults were collected at field sites in Switzerland.

Between 8 May and 6 June, 21 no-choice tests were conducted, using six test plant species (five native to North America) and *A. petiolata* as controls. All but one of the controls was attacked and 28.2 ± 0.6 mature larvae emerged from the pods in June. On the test plants, three eggs were found in one replicate of the native *Barbarea orthoceras*, but no mature larvae emerged from the pods. Only a few superficial feeding marks were recorded from *Erysimum cheiranthoides*. Feeding damage to the seeds occurred on *Thelypodium laciniatum* (0.3% of seeds damaged), *Braya alpina* and *Barbarea orthoceras* (both 10.5%). In comparison, 33% of the seeds were damaged in the dissected pods of *A. petiolata*. It also needs to be considered that this was under no-choice conditions. It is very likely that damage would be minimal when these two test plants are exposed together with *A. petiolata*.



Summer students Jérôme Schneuwly and Coline Braud preparing plants for a no-choice test with *Ceutorhynchus constrictus*

6 Common Reed (*Phragmites australis*)

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6.1 Rearing of stem-mining moths

In 2017, we mainly reared pupae of the two noctuid moths *Archanara neurica* and *A. geminipuncta* to be shipped to our collaborators in North America. In June, 80 pupae of *Archanara neurica* and 60 pupae of *A. geminipuncta* were sent to Prof. Richard Casagrande and Lisa Tewksbury, University of Rhode Island, USA, to gain experience in mating and oviposition of the moths in quarantine. Two shipments with 60 pupae of *A. neurica* and 50 pupae of *A. geminipuncta* were sent to Dr. Robert Bouchier (Agriculture and Agri-Food Canada, Lethbridge), for analysis of their pheromones. This is in preparation of monitoring the establishment of the moths, should their release should be approved.

With the remaining moths that emerged in our rearing, we produced over 3000 eggs of *A. neurica* and a bit more than 700 eggs of *A. geminipuncta*. They are being kept in a wooden shelter at ambient temperatures and will be available for additional work in 2018. By intensifying our care for the rearing, we were able to avoid the high larval mortality observed for *A. geminipuncta* in 2016.

6.2 Egg survival test under Gulf Coast conditions

In 2016, we kept eggs of both moths in an incubator simulating conditions at Fort Pierce, Florida. We wanted to test whether the eggs would be able to develop and hatch under temperatures that are common in southern latitudes, where the supposedly native North American subspecies *Phragmites australis berlandieri* is growing.

No larva hatched from these eggs in 2017, while larval hatch could be observed from eggs kept in an incubator at 15°C from August to mid-October, and at 2°C from mid-October to March. Thus, we suspect both moth species to be unable to establish and develop in the area of distribution of *P. australis berlandieri* due to too warm autumn and winter temperatures.



Summer student Leslie Mann checking the rearing of the noctuid moths



Pupae of *Archanara neurica*



Phragmites australis

7 Whitetop or Hoary Cress (*Lepidium draba*)

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7.1 *Ceutorhynchus cardariae* (Col., Curculionidae)

Of the 1482 adults of the gall-forming weevil *C. cardariae* that overwintered at CABI, 55% survived. To boost the populations used for rearing and host range tests after relatively low overwintering survival an additional 113 adult weevils were field collected in Romania in March 2017.

We continued conducting additional host-specificity tests as recommended by the USDA, Technical Advisory Group (TAG). During no-choice development tests with 11 species or subspecies, only one, the native North American (NA) *Lepidium austrinum*, developed galls to some extent but no adults emerged. This species had not been tested previously. The federally listed *Sibara filifolia* which had been attacked in the past was set up in a single-choice test. None of the five *S. filifolia* replicates were attacked while galls formed on all replicates of the target, *Lepidium draba*. In addition, we set up a multiple-choice cage test with 14 test species, which had been attacked under no-choice conditions. Only four species (*Streptanthus insignis*, *S. lasiophyllus*, *Euclidium syriacum*, and *Lepidium paysonii*) developed galls, with adults only emerging from the Eurasian species, *E. syriacum*.

Finally, we established another open-field test with three critical NA non-target species (*Lepidium oblongum*, *Streptanthus insignis*, *Lepidium paysonii*) that had supported relatively high adult emergence in previous tests. Only one replicate each from *L. oblongum* and *L. paysonii* were attacked.

Overall, our test results confirm that *C. cardariae* has a very restricted host range under choice and field conditions. We are planning to summarize all data collected since the submission of the last petition in 2011 and re-submit a revised petition this coming winter.

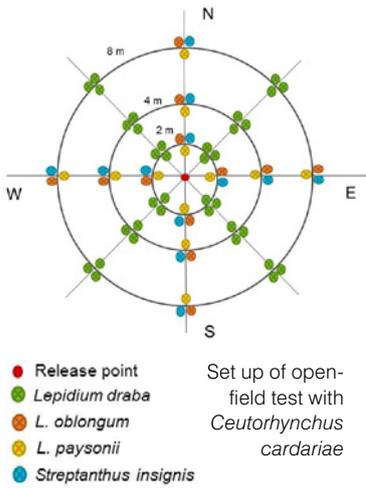
7.2 *Ceutorhynchus turbatus* (Col., Curculionidae)

On 25 May about 1800 adults of the seed-feeding weevil *C. turbatus* were collected in southern Switzerland. On 26 May, we established no-choice oviposition tests with 64 plants; eight *L. draba* control plants and 1-5 replicates of 14 test species. Apart from *L. draba*, eggs were found on four native North American species *Lepidium huberi*, *Lepidium integrifolium*, *Lepidium sordidum* and *Lepidium thurberi*. Larval development tests with these species will be set up in 2018.

In addition we set up a multiple-choice test with two native North American species (*Lepidium oblongum* and *Lepidium nitidum*) that had a relatively high proportion of seeds destroyed under no-choice conditions. Only *L. draba* was found to be attacked in this test confirming the narrow host range of *C. turbatus*.

7.3 *Ceutorhynchus assimilis* (Col., Curculionidae)

Although we decided in 2016 that this root gall former is not specific enough to be further considered as a potential agent for *L. draba*, we regularly checked the 34 test plant species exposed in 2016 for adult emergence in spring 2017 to complete the test series. Fifteen test species supported adult development. Any adults emerging from test species were set up on the same test species to test their survival and fitness.



Philip Weyl collecting data from the open-field test with *Ceutorhynchus cardariae*



Summer student Christian Mathias setting up the multiple-choice cage test with *Ceutorhynchus cardariae*

8 Dyer's Woad (*Isatis tinctoria*)

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8.1 *Ceutorhynchus peyerimhoffi* (Col., Curculionidae)

Between 29 March and 12 June 2017, about 2600 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 woad plants for rearing. From the rearing plants and dyer's woad plants used in the 2017 tests, over 4200 larvae were kept for adult emergence in 2018.

Since *C. peyerimhoffi* was able to develop eggs after feeding on the federally listed *Boecheera hoffmannii*, an additional oogenesis test was set up to see if females can develop eggs after feeding on other test species. Newly emerged weevils that had not fed on dyer's woad were placed directly onto either test species (*B. hoffmannii*, *Sinapis alba*, *Barbarea orthoceras*, *Streptanthus heterophyllus*) or dyer's woad for 14-17 days. Dissection of females is still in progress.

Between 15 April and 29 July, no-choice oviposition tests were established with 55 plants: 15 test species with 1-5 replicates each, and 11 dyer's woad control plants. Apart from dyer's woad, eggs were found on only three test species, the native North American *Cardamine oligosperma*, *Lepidium montanum* and *Boecheera pulchra*. Two of these species were subsequently tested for larval development, but no mature larvae developed on either of the two species.

Due to the few eggs that were recorded on one of the six *B. hoffmannii* exposed in a multiple-choice test in 2016, we set up an open-field test at a site in Italy where *C. peyerimhoffi* occurs naturally, exposing 10 *B. hoffmannii* and 10 *I. tinctoria*. Although adult weevils were seen on *B. hoffmannii*, no eggs were recorded, confirming the narrow host range of *C. peyerimhoffi*.

In addition, we placed naïve adult *C. peyerimhoffi* onto flowering *B. hoffmannii* and tracked the flower development into pods. Although adult weevils are known to feed on flowers, there was no significant difference in the proportion of flowers developing into pods compared to controls (*B. hoffmannii* without weevils).

8.2 *Ceutorhynchus rusticus* (Col., Curculionidae)

A total of 296 plants from 48 test plant species and the control were exposed to *C. rusticus* in no-choice oviposition and development tests. Test plants were regularly checked, and any dying plants were dissected. Three quarters of the dyer's woad control plants supported adult emergence and an even higher number of adults than in 2016 (average of 7.7) emerged per plant. Ten test species, including four new ones, one native to North America (*Hesperidanthus linearifolia*) and three European (*Arabidopsis thaliana*, *Lepidium perfoliatum*, *Nasturtium officinale*) supported limited adult development. We are planning to expose these species in an open-field test in autumn 2017.

8.3 *Metaculus* sp. (Aceria, Eriophyidae)

Due to the ongoing difficulties in obtaining research and export permits for Turkey, we surveyed both Serbia and Italy to find new localities of the mite *Metaculus* sp. Despite extensive surveys in Serbia, no mites were recorded. Italy proved to be successful and the mite was found in relatively high numbers at sites in Fonteavignone, L'Aquila Province. The mite was collected in early May 2017 and a quarantine culture has been established at CABI. Unfortunately the populations are not building up as expected. Thus, seed was collected from plants in the L'Aquila region and in autumn 2017 or spring 2018 a fresh culture of the mite will be collected and a rearing will be attempted on plants from the region. Provided successful, we plan on initiating host range tests as well as impact studies with this species.



The impact of the mite, *Metaculus* sp. on *Isatis tinctoria*, recorded in Fonteavignone, L'Aquila Province, Italy. Note the stunted growth and deformed flowers.



Setting up the open-field test in Fonteavignone, L'Aquila Province, Italy, with *Boecheera hoffmannii*

9 Perennial Pepperweed (*Lepidium latifolium*)

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9.1 Field surveys

In May, we conducted a field trip to Kazakhstan with the main goal to collect the eriophyid mite, *Metaculus lepidifolii*, on the PPW site where we had found it in 2016. Unfortunately, this site was destroyed, but we found a new site 500 m further up along the Ili River. Shoots infested with eriophyid mites were collected from this site and imported into the quarantine facility at CABI to establish a rearing colony of *M. lepidifolii* and to conduct host-range tests (see below).

In addition, we planned to investigate whether in the field *M. lepidifolii* also attacks *Lepidium draba* by surveying *L. draba* populations growing sympatrically with *L. latifolium* attacked by *M. lepidifolii*. However, this did not work out as planned since we did not find any *L. draba* growing close to *L. latifolium* attacked by eriophyid mites and on sites where *L. draba* was growing close to *L. latifolium*, no eriophyid mites were found on *L. latifolium*.

9.2 *Metaculus lepidifolii* (Acari, Eriophyidae)

No-choice tests were conducted with *Metaculus lepidifolii* under in the CABI quarantine facility. Mite-infested flowers and shoots were attached to leaves of potted plants of 16 test plant species (1-4 replicates each) and PPW. During six weeks, all plants were checked once per week and the numbers of eriophyid mites were counted. The numbers of mites found on PPW, *L. draba*, *L. eastwoodiae*, *L. paysoni*, *L. virginicum*, *Nasturtium officinale*, *Planodes virginicum*, *Rorippa sinuata* and *Sinapis alba* increased over time, but only relatively low numbers of mites, or no mites were found on the other test plant species. However, more replicates will be needed to confirm that these plant species are not attacked under laboratory conditions. We will discuss and co-ordinate any further work with *M. lepidifolii* with our North American partners.

9.3 *Ceutorhynchus marginellus* (Col., Curculionidae)

We continued to maintain a small rearing colony of this gall-forming weevil in the quarantine facility at CABI. From the 79 adults that were kept in incubators set at 3°C during winter 2016/2017, 35 females and 10 males survived until March 2017. These weevils were transferred onto 22 potted PPW plants to continue our rearing. In total about 300 adults emerged from these plants. The weevils are currently being kept in cylinders and regularly fed with leaves of PPW.



Patch with PPW along the Ili River (Kazakhstan) where *Metaculus lepidifolii* was collected



PPW inoculated with *Metaculus lepidifolii*. Mite-infested material was pinned to leaves of a potted PPW rosette



Sonja Stutz inspecting PPW shoots for eriophyid mites in the field 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 *Chrysochus asclepiadeus* from a tested population originating in Ukraine (UKR) and one population from southern Switzerland (CH). In June, a total of 72 adults of the Ukrainian population emerged from 625 larvae (11.5%) transferred onto *Vincetoxicum nigrum* in 2015. Two beetles have emerged from larvae transferred in 2016 (UKR).

The same rearing methods were used as in previous years. Adults were 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.

10.2 *Euphranta connexa* (Dipt., Tephritidae)

Pupae (N=1355) of the seed feeding fly collected in summer 2016 were overwintered at 5°C. Starting on 4 April 2017, batches of about 80-100 pupae were taken out of cold storage every 2-4 weeks 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 the few mouldy pupae were removed. So far, 136 females and 128 males have emerged with a success rate of over 90%. No-choice oviposition tests are being conducted exposing pods of eight test plant species alternating with *V. hirundinaria* or *V. nigrum* as a control. We observed oviposition behaviour mostly on *V. hirundinaria* (N=55, 95%) and *V. nigrum* (N=17, 88%), but also on *V. rossicum* (N=4, 50%), *Asclepias fasciculare* (N=11, 45%) and *A. rubra* (N=12, 33%). Unfortunately the behaviour was very variable depending on the weather conditions (too hot or too cold) although we were trying to compensate by conducting the tests in a greenhouse, shaded tunnel or outside depending on the weather. Upon dissection we found larvae in only very few exposed *V. hirundinaria* pods. This is probably due to the difficulties in synchronizing females of the right age (10 to 15 days) with pod production and good weather conditions in July. More tests are being planned with fresh females in July and August.

Seed pods of *V. hirundinaria* infested with *E. connexa* have been collected in southern Switzerland on 3 and 4 August. Pupae will be overwintered and emerging flies used for host-specificity tests in 2018.



Chrysochus asclepiadeus in southern Switzerland



Female *Euphranta connexa* ovipositing (photo: A. Leroux)



Ghislaine Cortat setting up host specificity tests with *Euphranta connexa*

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). Since the temperatures in May were much lower than in the years before, the activity of *M. millefolii* was delayed for about three weeks compared to previous years. In total about 120 adults were collected from 11 to 25 June.

Additional no-choice oviposition and development tests were conducted with 12 test plant species. Individual shoots of test and control plants were exposed to females for several days and the shoots are currently being dissected for larvae. So far, no larvae have been found on test plants but on average two larvae have been found on each *Tanacetum vulgare*.

In addition, an experiment was set up to quantify the impact of *M. millefolii* on *T. vulgare*. Plants with one shoot were exposed to 0, 1, 2 or 4 adults for five days and the impact on biomass and number of flower heads will be quantified.

11.2 *Platyptilia ochrodactyla* (Lep., Pterophoridae)

On 13 March, the remaining *T. vulgare* flower heads from 2016 that had been exposed to *Platyptilia ochrodactyla* were dissected and flower heads with larvae were transferred to potted plants of *T. vulgare*, *T. corymbosum*, *T. huronense* and *Chrysanthemum x grandiflorum*. In mid-June, adults emerged from all of the three *Tanacetum* species tested but not from *C. x grandiflorum*.

On 9 and 10 May, about 1000 shoots attacked by *P. ochrodactyla* were collected in western Germany. From these, about 500 mature larvae or pupae were retrieved, but only 80 females and 180 males emerged. No-choice oviposition and development tests were carried out with 11 plant species, with 1-3 replicates each. In addition, a multiple-choice cage test was set up with five *Tanacetum* species. The flower heads of test and control plants will be dissected about two months after the exposure to *P. ochrodactyla*.

11.3 *Chrysolina eurina* (Col., Chrysomelidae)

We continued our investigations on the biology of this leaf feeder. The first larvae from eggs hibernating at ambient temperatures started to emerge in early February 2017 and the first adults emerged in mid-May. Since none of these females started to lay eggs so far, *C. eurina* is likely to have only one generation per year under natural conditions.

We also started to investigate the host-range of *C. eurina*. From 27 February to 5 April, we set up no-choice larval development tests with 17 different test plant species and *T. vulgare* as controls. We transferred five first instar larvae each to Petri dishes containing a leaf of a test plant or of *T. vulgare*. On average 47% of the larvae that were fed with leaves of *T. vulgare* developed to adults, but only 10-15% of the larvae fed with *T. camphoratum*, *T. huronense* or *T. balsamita* developed to adults and 20% of the larvae exposed to *T. parthenium* developed to their third instar. All larvae exposed to any of the other test plant species died before reaching their second instar. These are very promising first results.



Set-up of no-choice oviposition and larval development tests with *Microplontus millefolii*



Set-up of no-choice larval development test with *Chrysolina eurina* using cut leaves in Petri dishes



Summer student Coline Braud collecting *Tanacetum vulgare* shoots infested with larvae of *Platyptilia ochrodactyla* at a field site in western Germany

12 Russian Olive (*Elaeagnus angustifolia*)

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12.1 *Aceria angustifoliae* (Acari: Eriophyoidae)

In collaboration with Dr. Ghorban Ali Asadi, the open-field host-range test for *Elaeagnus angustifolia* at the experimental farm of Mashhad University was continued in 2017. As in 2016, the *E. angustifolia* trees showed symptoms of mite attack, in some cases high levels, while none of the test species, including closely related species native to North America, showed any signs.

Since impact data has been challenging to collect, two impact experiments have been initiated in Serbia which can be visited more easily and frequently. Firstly, a controlled experiment using potted plants was setup this spring. These trees have been infested with both species of mites, *Aceria angustifoliae* and *A. eleagnicola* and another set of trees are kept as controls. The second experiment is using naturally occurring populations of *E. angustifolia*, attacked or unattacked by *A. angustifoliae*. We counted the number of flowers and fruits in spring and will assess the number of fruits that develop in autumn. These data from both experiments will become available in winter 2017.

Since CABI was able to establish a pure colony in quarantine, it was not necessary to import more mites this year. Testing in quarantine concentrated on one species, *Shepherdia canadensis*. We had 80% establishment and gall formation on Russian olive with no live mites being recorded on *S. canadensis*. The host range data thus far suggests that this mite has an extremely limited host range. Despite the lack of impact data, the host range data has been sent to our North American collaborators in March 2017, for the preparation of a petition that will be hopefully be completed by the end of winter for submission to TAG.

12.2 *Aceria eleagnicola* (Acari: Eriophyoidae)

In collaboration with Prof. Radmila Petanovic and Dr. Biljana Vidović, University of Belgrade, Serbia, we setup an impact study (coupled with a rearing) of this second mite to study its impact, development and biology. This is still in an early phase, but preliminary results suggest that the set-up has been successful.

12.3 *Anarsia eleagnella* (Lep., Gelechiidae)

The open-field test in Iran, used for *A. angustifoliae* this year doubled as an open field test for *Anarsia eleagnella* since the density of this moth was naturally high in the experimental garden. We were not only able to collect presence/absence data but also density of moth damage on each infested tree in the plot. Larvae were recorded on several Elaeagnaceae including *Hippophae rhamnoides*, *Elaeagnus pungens*, *E. umbellata* and the control, Russian olive, but never on the North American native, *E. commutata*. Non-target attack appears to be sporadic and at a lower density than on Russian olive.

In an attempt to initiate a laboratory culture of *A. eleagnella*, 296 shoots of Russian olive which had signs of larval mining were collected on 24 June and brought into the quarantine facility at CABI Switzerland. Only 11 live larvae were found, of which two were parasitized. From the five females and four males which emerged, limited numbers of eggs have been collected. If this rearing proves to be successful, more in-depth host range studies will be conducted.



Typical *Anarsia eleagnella* damage where the first 5cm or so of the shoot is mined



Anarsia eleagnella female in the CABI quarantine, note the elongated labial pulps of the female that form a horn



Russian olive open-field host range plot in the experimental garden, Mashhad, Iran

13 Oxeye daisy (*Leucanthemum vulgare*)

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13.1 *Dichrorampha aeratana* (Lep., Tortricidae)

On 10 February a field trip was conducted to southern Switzerland to collect oxeye daisy roots infested with hibernating larvae of *Dichrorampha aeratana* in order to increase our rearing colony. Adults started emerging in mid-April and from 24 April to 1 June, additional no-choice larval development tests were set up with 17 different test plant species and varieties, 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 and an open-field test with four species that had supported development under no-choice conditions (*Ismelia carinata*, *Matricaria chamomilla*, *M. discoidea* and *M. occidentalis*) and *Leucanthemum vulgare* as controls. Between 28 April and 23 May, nine females were released into each of four field cages containing three plants of each species and between 10 and 21 May, 25 females were released in the open-field test containing 16 plants of each species. All plants will be dissected for larvae in autumn.

From 1 to 5 May Dr. Andrew McConnachie from the Department of Primary Industries in New South Wales, Australia visited us and hand-carried 1387 *D. aeratana* eggs from our rearing colony to Australia in order to establish a rearing colony in their quarantine facility and to study the potential of *D. aeratana* as biological control agent for oxeye daisy in Australia.

13.2 *Oxya nebulosa* (Dipt., Tephritidae)

From 5 to 9 June several field sites where the root-galling tephritid fly *Oxya nebulosa* had been found in 2016 were visited in the Czech Republic and a total of 663 galls were collected. From these galls, 120 females and 130 males of *Oxya nebulosa* as well as 105 parasitoids emerged so far and host-range tests are ongoing. Females are individually kept in plastic cylinders together with one or two males and provided with a *Leucanthemum* flower and a pollen-honey mixture as a food source and a small oxeye daisy rosette inserted in a moist florist sponge for oviposition. As soon as females start to lay eggs (about five days after emergence), they are individually placed on test and control plants for 5-10 days. Since in 2016, only very few galls had been found in control plants, oxeye daisies of various origins and ages are this time being used as control plants. Some plants will be dissected at regular intervals to investigate larval and gall development. All plants will be checked for galls in September. Plants without galls will be dissected, while plants with galls will be kept for adult emergence in spring 2018.



Set-up of multiple-choice cage test with *Dichrorampha aeratana*



Oxya nebulosa are kept in plastic cylinders containing a *Leucanthemum* flower and an oxeye daisy rosette for mating



Summer student Fabrizio Valinotto collecting oxeye daisy with galls of *Oxya nebulosa* in the Czech Republic

14 Field Bindweed (*Convolvulus arvensis*)

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14.1 *Melanagromyza albocilia* (Dip., Agromyzidae)

Since it has proven difficult to conduct tests with this stem-mining fly under confined conditions, we established an open-field test in southern Germany, where the fly occurs naturally. On 20 June, 10 plants each of *Convolvulus arvensis*, *Ipomoea batatas* and the North American natives *Calystegia macrostegia*, *Cal. soldanella* and *Con. equitans* were planted into the soil in as many blocks as possible at the edge of a maize field. Plants were watered once a week. Plants that were not fit or dying were harvested on 13 July and the rest on 19 July. About 25 wild *Con. arvensis* plants growing close to the exposed test plants were also harvested to determine the attack rate in the field. So far, we found attack (feeding, mines, larvae or pupae) on more than half of the wild plants and on two of five control plants, but not on test plants. These are very encouraging preliminary results. Dissections are ongoing and detailed results will be presented in the Annual Project Report.

14.2 *Tinthia brosisiformis* (Lep., Sesiidae)

Ivo Toševski, our scientist located in Serbia, has been intensively looking for *T. brosisiformis* in the field using three different techniques:

1. Installing pheromone traps to attract clearwing moth males using commercial pheromone lures produced for the apple borer clear-wing moth *Synanthedon myopaeformis*;
2. Sweep-netting *Con. arvensis* populations, and
3. Active searching of *T. brosisiformis* on flowering plants of *Sambucus ebulus* (elderberry), since Ivo knew from his previous experience with this species that adults like to feed on this plant.

Ten sites were visited several times during 25 June and 25 July but not one single *T. brosisiformis* could be collected. In addition, only three specimens of *S. myopaeformis* were attracted by the pheromones, while this pest is usually much more common. This indicates that weather conditions this spring and early summer might have negatively affected normal development and/or phenology of clearwing moths. However, after a last effort at the beginning of August to find the moths, we received the great news from Ivo that he had finally been able to collect three females and two males of *T. brosisiformis* in eastern Serbia. More news to follow in the project Annual Report.



Female of *Melanagromyza albocilia*



Male of *Tinthia brosisiformis* collected on field bindweed in Serbia



Open-field experiment with *Melanagromyza albocilia* in a corn field in southern Germany



Adult of *Phytoliriomyza ornata*



Butomus plant damaged by larval feeding of *Phytoliriomyza ornata*

15 Flowering Rush (*Butomus umbellatus*)

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15.1 *Bagous nodulosus* (Col.: Curculionidae)

On several field trips to Slovakia, Germany and Serbia, about 240 adults of *B. nodulosus* were collected. When visiting new sites in Eastern Germany, we found the weevil on five out of six sites, confirming again that the species is more common than usually assumed.

We have now nearly completed no-choice oviposition tests with *B. nodulosus*. We hope to obtain the last missing test plant species before spring 2018. In 2017, we found for the first time eggs on a test plant, *Baldellia ranunculooides*. However, this was only in one replicate and we were not able to reproduce this result in additional tests setup with this plant species. No eggs were found on any of the 39 other species tested.

We also repeated an impact experiment, releasing five pairs of *B. nodulosus* onto each of eight potted *Butomus* plants covered with gauze bags. After six weeks, the length of each leaf was measured and feeding damage recorded. Preliminary analysis show that the sum of leaf lengths of plants exposed to weevils was 45% shorter than on control plants. Results of analysis of biomass are expected by early August and will be presented in the Annual Project Report.

15.2 *Bagous validus* (Col.: Curculionidae)

Between May and July 2017, we visited the site in eastern Slovakia with the largest population of *B. validus* four times, checked *Butomus* plants for adults and dug up plants for dissection. We found several eggs of *B. validus* in the leaves, but still no larvae. The dissection of the last sample is in progress

15.3 *Phytoliriomyza ornata* (Dipt.: Agromyzidae)

From about 30 overwintering pupae of this agromyzid fly, 17 adults emerged in 2017, and we were able to setup seven potted plants with one female and 1-2 males each. Flies were moved after 3-4 days to a new plant, where they died after about a week. Up to 11 pupae successfully developed per plant, and several plants started wilting after 2-3 weeks. Thus, contrary to our earlier assumption, this fly can have a strong impact on *Butomus* and should be further investigated as a potential agent.

15.4 *Doassansia niesslii* (Fungi, Doassansiaceae)

Studies on the susceptibility of different genotypes of *B. umbellatus* from North America to this white smut have shown that populations from Wisconsin, Montana and South Dakota (genotype 1) are not susceptible to the currently studied strain from Bremen, North Germany, while the population tested from Bouchie Lake, Canada (genotype 2) proved to be highly susceptible. Results of host specificity tests have shown that *Sagittaria graminea*, *Alisma plantago-aquatica* and *Carex obnupta* are immune to infection by the pathogen. Provided the availability of sufficient funding, additional strains of *D. niesslii* will be tested over the next two years to identify strains that are virulent on all genotypes invasive in North America.



Channel with *Butomus umbellatus* in Eastern Germany

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

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Japanese knotweed (*Fallopia japonica*), giant knotweed (*F. sachalinensis*) and their hybrid *F. x bohemica* have become serious invasive weeds in North America.

16.1 *Aphalara itadori* (Hemiptera, Psyllidae)

In 2016/17 we began assessing the performance of the hybrid between the *A. itadori* strain ex Kyushu with a preference for *F. japonica* and *F. x bohemica*, and a strain ex Hokkaido which favours giant knotweed. Both parental strains are currently under consideration as biocontrol agents in North America and prior to the release of either strain it is fundamental to assess the relative performance and potential non-target effects of the hybrid resulting from the crossing of these strains. The performance of the hybrid has been tested on Japanese knotweed in comparison with two stocks of the Kyushu strain: old stock kept under lab conditions for >100 generations and new stock collected in 2015. Two different temperature and humidity treatments (key variables in *A. itadori* development) have been assessed in order to evaluate the relative performance of the three psyllid populations under stress conditions (i.e. high temperature and low humidity). Preliminary results suggest differences in performance between strains and across treatments. Further host specificity tests for the hybrid will now be conducted against *Fallopia ciliinodis* and *Fagopyrum esculentum*, two non-target plant species native to North America which have been identified as marginal hosts for both parental strains.

16.2 *Mycosphaerella polygoni-cuspidati* (Fungi, Mycosphaerellaceae)

We have continued comparative virulence assessments of this leaf-spot pathogen using two distinct strains from different locations on the Japanese island of Kyushu. Tests followed a standardized methodology using mycelial broth for inoculations and incubating surface sterilized leaf pieces with typical leaf-spot symptoms to induce the potential formation of spermogonia (the reproductive bodies of the first life cycle stage) and the production of spermatia (the sex gametes). Both strains caused severe leaf necrosis on *F. sachalinensis* and *F. bohemica bohemica* and developed spermogonia with oozing spermatia on the respective necrotic lesions. These disease symptoms were, however, always less severe compared to those recorded on Japanese knotweed. Susceptibility assessments were also carried out for critical non-target species i.e. *Fallopia ciliinodis*, *F. scandens*, selected Canadian varieties of buckwheat (*Fagopyrum esculentum*) and *Polygonum glaucum*, a rare North American species. Necrotic leaf-spotting and the formation of spermogonia was recorded for *F. scandens*, but oozing of spermatia was not observed indicating that the spermogonia did not fully mature. *Fallopia ciliinodis* as well as all buckwheat varieties showed unspecific chlorosis with no development of spermogonia indicating that these species are not susceptible to the pathogen. On *P. glaucum* one of the pathogen strains caused the development of characteristic leaf-spot symptoms with subsequent formation of spermogonia and spermatial oozing. For the second strain, known to be less virulent, only few immature spermogonia were observed on the most susceptible older leaf stage of this non-target species. The ability of *M. polygoni-cuspidati* to form the first stage of its life cycle on *P. glaucum* precludes it from being recommended as a classical agent against Japanese knotweed in the UK and North America.

However, due to its genetic properties requiring two different mating types for reproduction and spread *M. polygoni-cuspidati* is considered to have potential as a mycoherbicide based exclusively on a single mating type. This would allow for targeted application of the pathogen in the field without the risk of establishment and spread to non-target species. The idea has been protected by a patent held in the name of the Secretary of State for Environment, Food and Rural Affairs, UK and research has commenced in the UK for proof of concept which will be supported by funds from British Columbia.



Disease symptoms caused by the *Mycosphaerella* leaf-spot on Japanese knotweed, 3 weeks after mycelial spray application



Chanida Fung, PhD student at Reading University, assesses psyllid development on Japanese knotweed

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

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17.1 *Puccinia komarovii* var. *glanduliferae* (Fungus, Pucciniaceae)

Since 2014, this rust has been released at selected sites across England and Wales. Limited spread and reduced pustule size, in comparison to infection in both the native range and under glasshouse conditions, suggested that a number of different biotypes of Himalayan balsam are present in the UK. In order to confirm these observations, plants from 18 release sites were screened under controlled conditions at CABI for infection and revealed significant variation in the susceptibility of individual populations of Himalayan balsam to the rust. This suggests that Himalayan balsam has been introduced into the UK a number of times and from different locations in the native range. A molecular study is underway to investigate this further.

In 2016, the rust was released at fewer sites close to the CABI Centre in the UK in order to investigate the effect of a number of biotic and abiotic factors on the level of rust infection and spread in the field. High levels of infection of the rust were observed at three release sites during the summer of 2016 and subsequent monitoring for seedling infection during spring 2017 confirmed that the rust had successfully overwintered at two of these sites. The progression of the rust at these sites throughout the 2017 growing season will be monitored.

Since not all UK populations of Himalayan balsam are susceptible to the rust isolate from India, a new isolate of the rust from Pakistan (held in CABI's liquid nitrogen facilities) was selected for virulence testing against Indian rust-resistant weed biotypes. The host-specificity of this strain was also assessed by testing a number of closely related *Impatiens* species and confirmed that it has the same level of specificity as the Indian strain. Permission to release the Pakistan strain from quarantine was approved by Defra in January 2017. An Administrative Trial Permit (ATP) issued by the Chemical Regulation Directorate (UK Health and Safety Executive) has enabled applications of the rust to be inundative, i.e. applied in the field as a spray diluted with water plus surfactant. Using results from the biotype experiments, the most virulent rust strain for each population has been applied at field sites during June 2017. Two subsequent rust releases will be made during the 2017 growing season.

For Canada, a test plant list containing 51 species native to North America was compiled by Dr Alec McClay and to date 45 species have been tested with no non-target impacts recorded to date. In 2017, research will continue to evaluate the host specificity of the rust against the remaining non-target species; assuming they can be sourced. Assessment of the virulence of the two rust strains towards Canadian populations of Himalayan balsam will also be made.



Field application of the rust using spores mixed with water and a surfactant



Himalayan balsam seedlings naturally infected in the field after successful overwintering, showing symptoms of the aecial stage of the rust *Puccinia komarovii* var. *glanduliferae*



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