

Weed Biological Control

Progress Report 2018

CABI in Switzerland
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Notes from the section leader

Hariet L. Hinz

I am very pleased to start with some positive news on recent releases and petitions.

In summer 2017, the leaf-feeding noctuid moth *Hypena opulenta* for control of invasive swallow-worts, was finally approved for release in the U.S.! 2017 releases mostly failed, but further efforts are ongoing in 2018 by Lisa Tewksbury and Dick Casagrande at the University of Rhode Island. In the meantime releases are in full swing in Canada with good establishment.

In 2018, approval for release was also finally obtained for the U.S. for the gall forming weevil *Rhinusa pilosa* on Yellow toadflax. Sharlene Sing from the US Forest Service has successfully established a rearing colony in quarantine at Montana State University for future field releases.

On 1 June 2018, the Canadian Food Inspection Agency (CFIA) permitted the release of the root-crown mining weevil *Ceutorhynchus scrobicollis* for garlic mustard control in Canada. This is a big step forward in the biological control project for garlic mustard. *Ceutorhynchus scrobicollis* would be the first agent to be released against an invasive weed in the mustard family. Rob Bourchier from Agriculture and Agri-Food Canada (AAFC) is planning first releases of egg laying females in autumn 2018. In February 2017, the USDA, APHIS, Technical Advisory Group (TAG), recommended release of *C. scrobicollis*. However, the weevil still needs to pass several additional hurdles before being hopefully permitted for field release in the U.S.

In summer 2017, first releases of the root-feeding hoverfly *Cheilosia urbana* for invasive hawkweed control were made by Rosemarie De Clerck-Floate (AAFC, Lethbridge, Alberta). In 2018, more eggs were shipped from Switzerland and transferred onto plants, but results will only become available in 2019. In spring 2016, the USDA, APHIS, Technical Advisory Group (TAG), recommended release of *C. urbana*. However, as with *C. scrobicollis*, the process for actually releasing the agent in the U.S. will still take some time.

Two petitions for field release are close to being submitted by our North American partners to Canadian and U.S. authorities: the eriophyid mite *Aceria angustifoliae* on Russian Olive, and the stem-mining moths *Archanara gemminipuncta* and *A. neurica* on common reed.

In April 2018, data on the gall-forming weevil *Ceutorhynchus cardariae* for hoary cress control was presented to the Technical Advisory Group (TAG) during their meeting in Beltsville, U.S. The plan is to resubmit a petition in winter 2018/19.

We will start one new project in 2018 on lesser calamint (*Calamintha nepeta*) for New Zealand in collaboration with Paul Peterson from Landcare Research. Lesser calamint is an aromatic herb, native to Eurasia. It is not palatable to life stock and is displacing pasture species. Although not yet widespread, lesser calamint is already having negative economic impacts on infested properties. The two-year project will survey for potential biocontrol agents in Europe and collect plant material for DNA analysis to help determine the area of origin of this species in New Zealand.

Both the Swiss and UK Weed Biocontrol Teams recently got involved in CABI's Action on Invasives Programme (AoI; www.cabi.org/projects/project/62665). Besides other things, AoI is developing management strategies for the invasive *Parthenium hysterophorus*, a highly allergenic plant in Pakistan. This programme will be done in collaboration with CABI's Centre in Pakistan, as well as local stakeholders. Although Pakistan has been the source for several weed biocontrol agents, hardly any projects have been implemented in the country. The plan is to introduce the stem-boring weevil *Listronotus setosipennis*, already successfully used in Australia to supplement the accidentally introduced defoliating beetle *Zygogramma bicolorata*.

Finally, the XV International Symposium on Biological Control of Weeds (ISBCW), which takes place from 26-31 August 2018 in Engelberg, Switzerland, is taking shape. We have over 200 participants from 27 countries and 114 institutions giving 81 oral presentations and presenting over 100 posters. So the dwindling numbers in Symposium participants appear to be over. After the ISBCW, about 20 collaborators and colleagues will visit the CABI Switzerland Centre to get an overview of our work and to discuss specific projects. I am looking forward to meeting many friends and colleagues and to an inspiring and successful Symposium with lots of networking, fruitful discussions and also some fun during the various social events!

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Ivo Toševski inspecting toadflax plants with induced galls of *Rhinusa pilosa* Rp-A8 haplotype

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 2114 (88.1%) adults of the stem-gall forming weevil*, R. pilosa,* and 506 (79.9%) *R. rara* survived hibernation. The survival rate of both gall inducers was significantly better than in previous years thanks to improved aestivation and hibernation methods. Adults of *R. pilosa* were set up in outdoor cages with *L. vulgaris* plants separated by their mitochondrial affiliation or their collecting origin for rearing. In addition, mass rearing of some rare haplotypes identified by genotyping in 2017 was carried out. Over 3000 adults of *R. pilosa* were produced. The rearing of seven particular mitochondrial genotypes resulted in 2-1054 adults, respectively. All emerged adults were set up for aestivation separately.

To increase genetic diversity of the laboratory population of *R. pilosa*, 282 galls were collected between 20 and 22 April from 14 different locations in northern and eastern Serbia. Less than 200 *R. pilosa* adults emerged from these galls, but >1800 adults of the inquiline weevil *R. eversmanni*. In addition, a total of 84 *R. pilosa* adults extracted from field collected galls were separated for genotyping to determine corresponding haplotype content for further mass rearing in 2019. After molecular analysis, the adults from selected galls will be fused in batches according to the particular COII gene haplotype and set up separately for aestivation.

Unfortunately, rearing of *R. rara* failed in 2018 with only nine induced galls and 34 emerged adults. This is most probably related to very strong temperature fluctuations during April with prolonged cold and rainy weather and absence of the right plant phenostage for oviposition. From 165 field collected galls of *R. rara,* 328 adults emerged.

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

Survival of stem-boring *Mecinus* spp. was good as well, especially when adults were left in cages for natural hibernation. From a subsequent rearing with *M. heydenii*, 1474 adults emerged. Unfortunately, rearing of *M. peterharrisi* and *M. laeviceps* failed in 2018, for similar reasons as for *R. rara*. During dissection, several hundred dead L1 and L2 larvae were found just below the stem surface. It will be necessary to make considerable changes in the rearing methodology for *L. dalmatica* associated weevils to better synchronize plant phenology with weevil oviposition and larval development.

To repeat the survival and fitness experiment of *Mecinus* spp. reared from critical North American (NA) test species, several plants of *Nutthalanthus canadenis* and *Sairocarpus virga* were set up with ovipositing females of each of the three *Mecinus* species. All plants were dissected between 25-27 July, revealing development of 27 *M. heydenii* and 12 *M. peterharrisi* on *N. canadensis,* while no development to adult was recorded in plants exposed to *M. laeviceps*. All *M. heydenii* and *M. peterharrisi* were returned onto *N. canadensis* to see whether weevils will be able to sustain a population when only provided with this test species.

No-choice oviposition and larval development tests were continued with *M. peterharrisi*. A total of 24 plant species and populations, 13 native to NA were tested. Larval development was only recorded on the control *L. dalmatica* ssp. *macedonica* and NA Dalmatian toadflax populations.

Mass rearing of *Rhinusa pilosa* haplotypes in frame cages

2 Houndstongue (*Cynoglossum officinale*)

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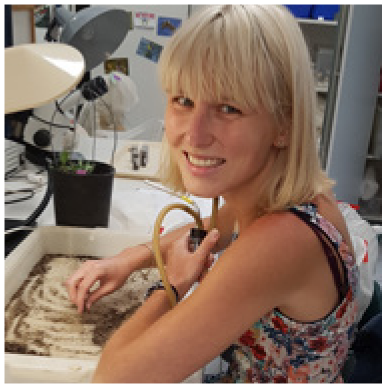
2.1 *Mogulones borraginis* (Col., Curculionidae)

Current work on houndstongue is focused on the seed-feeding weevil *Mogulones borraginis.* It has proven to be the most specific agent acting on houndstongue thus far. CABI Switzerland is maintaining a rearing of the weevil in view of future field releases since *M. borraginis* is extremely rare in the field in Europe and hard to collect.

From 650 *M. borraginis* larvae collected in 2017, 30 adults emerged in October 2017 and 498 in March 2018 and were set up on houndstongue rosettes. On 30 May 2018, 40 pairs of *M. borraginis* were sent to the quarantine facility at the University of Idaho for additional investigations and rearing. At the end of May, about 160 females were retrieved and reset on 55 flowering-seeding houndstongue 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 2700 *M. borraginis* larvae emerged which were separated into cups (25 individuals per cup) and placed in the underground insectary until the adults emerge in autumn 2018 and spring 2019.

In April 2018, Mark Schwarzländer from the University of Idaho (UoI) presented the data collected so far on *M. borraginis* during the USDA APHIS, Technical Advisory Group (TAG) meeting in Beltsville, U.S. This included data on the host finding behaviour of the weevil, collected by several graduate students at UoI. They found that houndstongue was always preferred by female weevils compared to confamilial non-target species. When combining visual and olfactory cues, females responded with indifference to non-target plants or were even repelled by them, including some Federally listed species. His talk was very well received and a petition for field release is in preparation.



Summer student Gaia Besomi searching for overwintered *Mogulones borraginis*

Adult of *Mogulones borraginis*

Tamar Messer collecting *Cheilosia urbana* in southern Germany

Aulacidea pilosellae

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 2017, over 700 galls of *A. pilosellae* ex *P. officinarum* were harvested from rearing and host range tests. From these 75 females emerged in May and June 2018. From 24 May to 23 June, nine species were exposed in no-choice tests, including *P. officinarum* of different origins, four North American (NA) *Hieracium* species, one other NA native and three horticultural species, resulting in a total of 40 plants. Gall harvest is ongoing and so far, galls were only found on *P. officinarum.*

In 2017, over 700 galls of *A. pilosellae* ex *Pilosella* spp. were harvested from plants field collected in Poland and from host range tests. From these 57 females and 80 males emerged in May and June 2018. From 25 May to18 June, four species were exposed in no-choice tests, including *P. caespitosa*, the North American (NA) *Hieracium gracile* and two horticultural species, resulting in a total of 15 plants. Five plants of *H. bolanderi, H. carneum* or *H. scabrum* were exposed together with *P. caespitosa* in single-choice tests. Gall harvest is ongoing and so far, galls were only found on *P. caespitosa.* Final results will be presented in the annual report.

From the plants collected in the Czech Republic and Poland in 2017, about 70% of all emergence consisted of parasitoids (Eurytomidae; preliminary identification). Because of the poor emergence of *A. pilosellae*, we lost the populations from Milkow and Strazne. We are currently maintaining colonies of separate populations from Zweibach, Bouda Jana and Velka Upa.

3.2 *Cheilosia urbana* (Dipt., Syrphidae)

On 6 May 2018, eight female *Cheilosia urbana* were collected in the Black Forest, Germany and shipped the day after to Dr. De Clerck-Floate (Agriculture and Agri-Food Canada, Lethbridge). Eggs were harvested upon reception at AAFC and 150 of these were directly transferred onto hawkweed plants in the field. Results will only become available in spring 2019.

Orange hawkweed (*Pilosella aurantiaca*) in Velka Upa, Czech Republic

4 Russian Knapweed (*Rhaponticum repens*)

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Francesca Marini and Philip Weyl during inoculation of *Rhaponticum repens* plants with *Aceria acroptiloni* at the Institute of Zoology in Tashkent, Uzbekistan

4.1 *Aceria acroptiloni* (Acari, Eriophyoidae)

Iran

In collaboration with Dr Ghorban Ali Asadi, Mashhad University, we continued with host-range testing in Iran. The mite *A. acroptiloni* has been extremely challenging to work with; not only is the species extremely sensitive to transportation, but up until now we have not 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 knowledge of this species and our understanding of the host-range test results.

In spring 2018 we moved the open-field host range test from the experimental farm in Mashhad to Shirvan, where the mite is found naturally. The open-field test includes a limited number of test species and the control. The main emphasis for this year is to obtain long term establishment on the control plants. As in previous years, two methods were used to inoculate the test and control plants. First, mite infested flower buds were cut in two 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, into the experimental plot. Since long term establishment is the goal of the experiment this year, we will not harvest any material. We will observe the plot in spring 2019 to determine whether there has been any establishment.

Uzbekistan

During surveys of the Tashkent and Samarkand regions in June this year, in collaboration with the Uzbekistan Academy of Sciences and the Institute of Zoology, we located a population of *A. acroptiloni* in Samarkand. However, with a lack of support in Samarkand, we have attempted to inoculate plants with this mite at the Institute of Zoology in Tashkent. Since we were late in the season and most flower buds in Samarkand were already dry, the mites were transferred using whole cut plants which were then placed in amongst the already existing plants in Tashkent. We hope that this will allow the mites enough time to leave the galled flowers in search of either overwintering sites or younger flowers which were present on the Russian knapweed plants in the plot.

Kazakhstan

Mite-infested plants were located in Kazakhstan near Lake Bulkhash, however the mite species at the time of collection was unknown. These mites were hand carried to the quarantine facility at CABI in Delémont to establish a colony for potential host range tests. The species is yet to be determined, however it is not *A. acroptiloni* and is likely *A. sobhiani*. This species has undergone limited testing previously which provided ambiguous data. Impact on the plants in the field is impressive. Considering the difficulties we encounter with *A. acroptiloni* we believe that it is worthwhile trying to conduct additional work with *A. sobhiani.*

Dr Ghorban Ali Asadi inspecting the new *Rhaponticum repens* open-field plot in Sirvan, Iran

Summer student Arthur Knecht collecting *Ceutorhynchus constrictus* in Delémont

Ghislaine Cortat setting up tests with *C. constrictus*

5 Garlic Mustard (*Alliaria petiolata*)

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5.1 *Ceutorhynchus constrictus* (Col., Curculionidae)

Between 12 March and 12 April 2018, 130 adults of the seed-feeding weevil *Ceutorhynchus constrictus* emerged from our rearing colony established in 2017. On 7 May, 120 adults were shipped to Dr. Katovitch (University of Minnesota) for rearing and additional host-specificity tests in quarantine. On 8 May, 93 additional adults were collected at field sites in Switzerland. From 14 to 28 May, 26 no-choice oviposition and development tests were conducted, using four North American native test plant species and *A. petiolata* as controls. Three species (*Barbarea orthoceras, Boechera holboellii* and *Braya alpina*) had been accepted for oviposition in previous no-choice tests, but the number of eggs was very low and in the case of *Braya alpina*, one mature larva emerged from a test, but no adult emerged the following year. In order to confirm whether the weevil is able to develop on these plant species, dissections were kept to a minimum prior to larval emergence and damage to pods and seeds as well as oviposition were assessed visually. No oviposition occurred on *Eutrema salsuginea*. These plants were entirely dissected and only 0.4 ± 0.2 of the seeds were damaged by adult feeding. All of the controls were attacked and 49.3 ± 11.7 mature larvae emerged per plant. Oviposition was found on all *Barbarea orthoceras, Boechera holboelli* and three of five *Braya alpina.* Mature larvae emerged only from *Boechera holboelli* (4.3 ± 2.1). Detailed data on the feeding damage to the pods and seeds will be presented in the annual report.

Mating pair of *Ceutorhynchus constrictus*

6 Common Reed (*Phragmites australis*)

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6.1 Rearing of stem-mining moths

We continued maintaining a rearing of the two noctuid moths *Archanara neurica* and *A. geminipuncta.* We tested a different method to facilitate larval rearing by keeping larvae individually in tubes and allowing them to move themselves to a new stem. Although most larvae managed to enter without help into new stems offered at the right time, the method does not seem to make the rearing significantly more efficient.

In March 2018, 800 eggs of *A. neurica* were shipped to Agriculture and Agri-Food Canada (AAFC), Lethbridge, for rearing trials on artificial diet. The McMarron diet turned out to be very successful for rearing *A. neurica.* Provided, it will also work for *A. geminipuncta*, it could significantly reduce time and efforts in future. Additional 500 eggs were shipped to Lethbridge in June 2018, in order to compare hatching rates of eggs obtained from moths reared on diet with moths reared on *Phragmites*. For further tests in 2019, over 1000 eggs of *A. geminipuncta* and several 1000 *A. neurica* eggs will be available.

Larva of *Archanara neurica* ready to be transferred into a *Phragmites* stem

6.2 Larval dispersal experiments with *Archanara neurica*

In order to test larval dispersal of *A. neurica*, we setup a small field experiment. Two old stem bases of *Phragmites* with 20 eggs of *A. neurica* each were placed end of April in the soil at three sites near Delémont. The plan was to check the sites after two weeks and record the distances of infested shoots from the release point. Unfortunately, no larva was able to infest surrounding stems. A heavy cold snap must have killed them. The same happened with a larval choice experiment setup in our common garden with potted plants of native and invasive *Phragmites*. These experiments will have to be repeated in 2019.

Old stems with eggs of *A. neurica* placed in a field site to test larval dispersal

Summer student Ayaka Gütlin collecting common reed for noctuid moth rearing

Single-choice test with *Physaria fendleri* (left) and *Lepidium draba* (right)

7 Whitetop or Hoary Cress (*Lepidium draba*)

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7.1 *Ceutorhynchus cardariae* (Col., Curculionidae)

Of the 1500 adults of the gall-forming weevil, *Ceutorhynchus cardariae* that overwintered at CABI, 55% survived and were used for additional tests in spring 2018.

We continued conducting host-specificity tests as recommended by the USDA Technical Advisory Group (TAG). During no-choice development tests we were able to increase the number of replicates for four species or subspecies. Only one individual of the native North American (NA) *Lepidium oxycarpum,* developed a single gall and a limited number of adults emerged. A single adult emerged from *Physaria fendleri* in 2017, however no galls developed. To confirm this result we increased the number of no-choice replicates and conducted a single-choice test. None of the additional 10 no-choice test replicates, or the five single-choice replicates were attacked. This suggests that the initial result was possibly due to contamination.

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 24 May, over 1800 adults of the seed-feeding weevil *C. turbatus* were collected in southern Switzerland. On 25 May, we established no-choice oviposition tests with 64 plants; six *L. draba* control plants and 1-5 replicates of 12 test species. Apart from *L. draba,* eggs were found on one native North American species *Lepidium eastwoodiae.*

Following the oviposition tests, we also set up a development test with four NA test plant species that had been accepted for oviposition in the past. *Braya alpina* supported larval development only to second instar larva, while mature larvae emerged from *Lepidium huberi.* We intend to perform additional tests with this species to determine whether it is accepted under choice conditions in 2019.

In addition we set up a multiple-choice test with two native North American species (*Cardamine oligosperma* and *Lepidium densiflorum*) that had a relatively high proportion of seeds destroyed by adult feeding only, under no-choice conditions. Only *L. draba* was attacked in this test.

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 still had ongoing survival tests to complete. We regularly checked the eight test plant species exposed in 2017 for adult emergence that were set up on the same test species to test their survival and fitness. *Ceutorhynchus assimilis* was only able to complete an F2 generation on *L. draba* and no F2 adults emerged from any of the test species.

Cornelia Cloşca collecting *Ceutorhynchus turbatus* in southern Switzerland

8 Dyer's Woad (*Isatis tinctoria*)

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8.1 *Ceutorhynchus peyerimhoffi* (Col., Curculionidae)

Between 6 April and 8 June 2017, approximately 3600 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 2018 tests, over 4300 larvae emerged and were kept for adult emergence in 2019.

Between 9 and 21 May, no-choice oviposition tests were established with 18 plants from three test species with 1-5 replicates each, and seven dyer's woad control plants. Apart from dyer's woad, eggs were found on one test species, the federally listed North American, *Sibara filifolia*, however, the pods are extremely small and unlikely to support the development of mature larvae. Provided we can grow more reproducing *S. filifolia,* we will conduct additional tests with this species in 2019. Between 9 and 14 May we setup development tests with two species that had been accepted for egg laying in previous tests, *Erysimum cheiri* and *Boechera pulchra.* However, the latter did not develop viable seeds so the test was not valid. No mature larvae developed on *E. cheiri*.

Since *C. peyerimhoffi* was able to develop eggs after feeding on the federally listed *Boechera hoffmannii*, an additional oogenesis test was set up to increase the number of replicates and variations of the time spent on plants. Newly emerged weevils that had not fed on dyer's woad were placed directly onto either *B. hoffmannii* or dyers woad for 10 days. Plants were kept for development and dissected after emergence. No larvae developed this year on any of the eight *B. hoffmannii* plants exposed, while we had mature larvae emerge from controls. Dissection of females for egg development is still in progress.

Summer student Gaia Besomi inspecting test plants that have been exposed to *Ceutorhynchus peyerimhoffi*

8.2 *Ceutorhynchus rusticus* (Col., Curculionidae)

A total of 240 plants from 42 test plant species and the control were exposed to *C. rusticus* in no-choice oviposition and development tests between autumn 2017 and spring 2018. Test plants were regularly checked and any dying plants were dissected. Only half of the dyer's woad control plants supported adult emergence, and to a lesser degree than in 2017 (an average of 1.3 adults per plant emerged, in comparison to 7.7 adults per plant in 2017). Of the 42 test plant species, we recorded typical *C. rusticus* mining in 21 test species, while only two test species (*Descurainia pinnata* and *Streptanthus coulteri*, both native to NA) supported limited adult development. Due to the low adult emergence in dyer's woad this year we will repeat the no-choice development tests with all species where typical mining was recorded.

8.3 *Metaculus* sp. (Aceria, Eriophyidae)

On 19 April, a small colony of the mite was collected from the Kaiserstuhl area in south-western Germany, and transported to CABI Switzerland. Initial rearing methods, as well as possible host range testing protocols have been investigated this summer and host range testing will begin in autumn this year. Unfortunately we could not locate a large enough population in the field to conduct an impact experiment. We will conduct additional surveys in autumn.

Surveying for *Metaculus* sp. in the Rhine Valley, Germany

Potential larva of *Lasiosina deviata* in a stem of PPW

9 Perennial Pepperweed (*Lepidium latifolium*)

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9.1 Field surveys

Two field trips were conducted to Kazakhstan, one in early April and one in mid-May. The goal of the first field trip was to re-visit known sites and look for herbivores that might have been missed during earlier surveys conducted in May. The goal of the second field trip was to re-visit sites where potential biological control agents were found during the first field trip. During both field trips we aimed to identify collection sites for the stem-mining fly *Lasiosina deviata*.

In April, we collected PPW from seven sites and dissected their roots and shoots. In addition, we dissected old stems of PPW for pupae of *Lasiosina deviata* whenever they were still present. At one of the sites we found an adult weevil in the root crown of PPW. The weevil will be sent to a taxonomist for identification, but it is likely to be the oligophagous *Lixus myagri*. At another site, we found a Diptera larva (probably a syrphid) feeding in the centre of a rosette. In addition, we found a few empty Diptera pupae in the old stems of PPW at another site.

The last two sites were re-visited in May and five Diptera larvae were found in the new stems of PPW at one of the sites. In June, one adult fly emerged. We believe it is *Lasiosina deviata,* but sent it to a taxomonist for verification. In addition, two larvae were transferred to 96% EtOH for molecular identification.

In June, opportunistic surveys were conducted in Uzbekistan. Stems of PPW were dissected at two sites but no herbivores were found.

9.2 *Ceutorhynchus marginellus* (Col., Curculionidae)

We continued to maintain a 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 2017/2018, 46 females and 31 males survived until March 2017. These weevils were transferred onto 38 potted PPW plants to continue our rearing. In total about 730 adults emerged from these plants. The weevils are currently being kept in cylinders and regularly fed with leaves of PPW.

Massimo Cristofaro (BBCA) collecting PPW in Kazakhstan

10 Swallow-Worts (*Vincetoxicum* spp.)

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Chrysochus asclepiadeus

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 from Ukraine (UKR). In June 2018, a total of 109 adults (14.5%) emerged from 750 larvae transferred onto *Vincetoxicum nigrum* in 2015. Seven beetles have emerged from larvae transferred in 2017 (UKR).

On 22 June, about 350 adults were field collected at sites in Franche Comté, France. Since the beetle had attacked one native North American (NA) *Asclepias* species in previous multiple-choice cage tests, we set up an open-field test in the CABI garden to investigate its host choice behaviour under close to natural conditions. *Vincetoxicum hirundinaria* was used as a control. Exposed native NA non-targets included: *Asclepias incarnata*, which had supported complete larval development of the French population of the beetle (data provided by EBCL-USDA-ARS); *A. syriaca,* which supported oviposition and limited larval development, and *Apocynum cannabinum*. Larvae of the Ukrainian *C. asclepiadeus* population (tested at CABI) were able to develop to adult on *A. cannabinum*, and it was used as surrogate for *A. speciosa* and *A. tuberosa,* which were not available.

On a circular plot of 24m in diameter, 36 potted plants of *V. hirundinaria* and 12 potted plants of each of the three test species were arranged in eight lines (four perpendicular lines with *V. hirundinaria* only and four lines with the three test species) radiating out from a central release point, at distances of 2.5 m, 5 m and 10 m. On 26 June, 72 pairs of *C. asclepiadeus* were released at the centre of the plot. Each plant was checked for adults 1-2 times per week for a month after release and the percentage of defoliation was estimated. Presence of adults and feeding was only recorded from *V. hirundinaria*, indicating a very narrow host range of the beetle under natural field conditions. The plants will be covered and kept in a garden bed to be dissected for larvae in spring 2019.

Euphranta connexa laying eggs in a swallow wort pod

10.2 *Euphranta connexa* (Dipt., Tephritidae)

Pupae (N=1725) of the seed feeding fly collected in summer 2017 were overwintered at 5°C. Starting beginning of May 2018, batches of about 130-240 pupae were taken out of cold storage every 2-3 weeks and placed into an incubator at 10°C for a week, then stored in the lab for adult emergence. The containers were regularly checked for emergence and mouldy pupae removed. So far, 138 females and 146 males have emerged (emergence success rate of > 90%). No-choice oviposition tests and egg transfer tests are being conducted and results will be presented in the annual report.

Summer students Janisse Deluigi and Arthur Knecht releasing *Chrysochus asclepiadeus* in the open-field test

Tanacetum vulgare plant heavily defoliated by *Chrysolina eurina* larvae

Garden chrysanthemum exposed to females of *Microplontus millefolii*

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). From the 22 larvae and pupae that were kept at 5 °C for overwintering nine adults emerged, unfortunately they were all dead at the time they were discovered.

A total of 105 adults were collected in the field from 4 to 17 June. Additional no-choice oviposition and development tests were conducted with ten test plant species. Individual shoots of test and control plants were exposed to females for several days and the plants are currently being dissected.

In addition, an experiment was set up to quantify the impact of *M. millefolii* on *T. vulgare*. Seventeen plants with one shoot were exposed to three females for five days while twenty plants were used as controls. The impact on biomass and number of flower heads will be quantified.

11.2 *Platyptilia ochrodactyla* (Lep., Pterophoridae)

From March to May 2018, no-choice larval development tests were conducted with larvae of *Platyptilia ochrodactyla* originating from *T. vulgare* flower heads that had been collected in Germany in October 2017. Approximately five larvae each were transferred onto 16 test plant species as well as on *T. vulgare*. Adults emerged from three test plant species outside the genus *Tanacetum* and from *T. vulgare* and pupae were found on two additional test plant species.

On 13 June, an open-field test with four test plant species was set up on a field site with a large population of *P. ochrodactyla* in Germany. The plants were left on the field site for four weeks and are currently being dissected for larvae.

11.3 *Chrysolina eurina* (Col., Chrysomelidae)

From 7 March to 25 April 2018, we set up no-choice larval development tests with 35 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 36% of the larvae that were fed with leaves of *T. vulgare* developed to adults, but only one adult (2.8%) emerged from the larvae fed with *T. parthenium* and one adult (2.0%) from the larvae fed with *Artemisia californica*. In addition, 6% of the larvae exposed to *M. chamomilla* developed to their third instar. All larvae exposed to any of the other test plant species died before reaching their second or third instar.

In addition, we conducted no-choice larval development tests with potted plants. For this we transferred ten first instar larvae each to potted plants of seven *Tanacetum* species (including *T. vulgare*), as well as on *Artemisia californica*. On average 70% of the larvae transferred to *T. vulgare* developed to adults and between 20-52% of the larvae transferred onto *T. balsamita*, *T. camphoratum* and *T. huronense* developed to adults. No adults emerged from any of the other plants tested.

We also evaluated the multiple-choice cage test that was set up in October 2017 using four non-target species in the genus *Tanacetum* as well as *T. vulgare* as controls. Larvae or adults were found on all plants and detailed results will be presented in the annual report.

Our gardener, Lise Berberat setting up an open-field test with *Platyptilia ochrodactyla* in Germany

12 Russian Olive (*Elaeagnus angustifolia*)

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Characteristic symptoms of *Aceria eleagnicola* recorded in Uzbekistan

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 2018. As in 2017, all *E. angustifolia* trees showed symptoms of mite attack, in some cases at high levels, while none of the test species, including closely related species native to North America, showed any symptoms.

Since impact data has been challenging to collect, two impact experiments have been initiated in Serbia, which can be visited more easily and frequently. The first is a controlled experiment using potted plants, which was setup in 2017. These trees have established populations of 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 have selected branches where the mite is present or absent and will compare the number of fruits produced late in summer before they ripen and possibly be removed by birds. These data will become available in winter 2018.

The possibility of exporting *Aceria angustifoliae* from Iran, for release in the USA, seems to be unlikely in the foreseeable future. We therefore decided to conduct host-range tests with the population of *A. angustifoliae* located in Serbia on a limited number of species within the Elaeagnaceae family, since export from Serbia is possible. Provided there are no significant differences in host range between populations of this mite we propose the petition to focus on the Serbian population, rather than the original Iranian population.

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 species to study its impact, development and biology. We have had successful establishment of the mite in an experimental garden, however, this is still at an early stage. During surveys in Uzbekistan in June this year, in collaboration with the Uzbekistan Academy of Sciences and the Institute of Zoology, we located substantial populations of this mite, however, it appears that impact on the reproductive output is limited.

12.2 *Anarsia eleagnella* (Lep., Gelechiidae)

Host range testing of *Anarsia eleagnella* under laboratory conditions progressed well this year. Since oviposition behaviour of females is not selective under caged quarantine conditions, testing proceeded with larval no-choice tests, as well as investigating its developmental biology. We were able to complete testing with two species, *Shepherdia rotundifolia* and *Prunus persica* (peach). Neither species supported development of the larvae to adult, while we got up to 80% survival on the controls.

We plan to collect additional data in late summer from the open-field test at the experimental farm of Mashhad University, as we did in 2017. Hopefully the NA natives, *Elaeagnus commutata* and *Shepherdia argenta,* will be of a more comparable size to the controls by then. It appears from previous tests that plant size plays a role in host selection, with taller trees being more attractive.

Dr. Ghorban Ali Asadi inspecting test plants in the Russian olive open-field plot for symptoms of *Aceria angustifoliae*

Oxeye daisy rosette infested with larva of *Dichrorampha consortana* (see circle)

Mating pair of *Oxyna nebulosa*

13 Oxeye daisy (*Leucanthemum vulgare*)

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13.1 *Dichrorampha aeratana* (Lep., Tortricidae)

Adults of the root-mining moth *Dichrorampha aeratana* started emerging in mid-April. From 4 to 23 May, additional no-choice larval development tests were set up with twelve different test plant species (eight for Australia, four for North America), with 2-10 replicates each. Five first instar larvae were transferred onto each of the plants. All plants will be dissected for larvae in autumn.

To delay adult emergence of *D. aeratana* and to enable a shipment of eggs to the quarantine facility at Agriculture and Agri-Food Canada (AAFC) in Lethbridge in July, a subset of the oxeye daisy plants that had been infested with *D. aeratana* in 2017 were kept in an incubator set at 2°C from 8 March onwards. From 17 June onwards, the temperatures in the incubator were steadily increased and on 3 July all plants were removed from the incubator and kept at ambient temperatures. From 20 to 28 July a total of 11 females and 13 males emerged. A total of 1077 eggs were shipped to Rosemarie De Clerck-Floate at AAFC on 30 July where the emerging larvae will be used to test *Hulteniella integrifolia*, a test plant species which we were not able to grow at CABI.

13.2 *Dichrorampha consortana* (Lep., Tortricidae)

From 14 to 19 May we visited five field sites in Norway with recent records of the stem-miner *Dichrorampha consortana*. At that time, the oxeye daisy plants were still in the rosette stage, but plants infested with *D. consortana* were easily recognizable by their curled leaves. Plants with larvae were collected and a total of 30 males and 30 females emerged between 11 June and 7 July. Adults were kept in plastic cylinders and provided with water and leaves of oxeye daisy for oviposition. The larvae started to emerge about eight days after the eggs were laid. First instar larvae were transferred onto potted oxeye daisies to study the biology of *D. consortana* and to establish a rearing colony. In addition, a few no-choice larval development tests were set up with five Shasta daisy varieties.

13.3 *Oxyna nebulosa* (Dipt., Tephritidae)

In May, more than 450 galls were harvested from the oxeye daisy plants that had been exposed to adults of *Oxyna nebulosa* in 2017. Between 28 May and 10 July 2018, 242 females and 174 males emerged from these galls.

Egg-laying females were individually placed onto test and control plants for approximately ten days. No-choice oviposition and larval development tests were set up with 31 different test plant species and varieties, with 1-18 replicates each. All plants will be checked for galls in autumn. Plants without galls will be dissected, while plants with galls will be kept for adult emergence in spring 2019.

In addition, we set up an impact experiment where 18 potted oxeye daisy plants were exposed to two females of *O. nebulosa* each and another set of 18 plants were used as controls. The impact on above and below ground biomass as well as on the number of flower heads will be quantified in 2019.

Summer student Tamar Messer collecting oxeye daisy infested with *Dichrorampha consortana* in Norway

14 Field Bindweed (*Convolvulus arvensis*)

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14.1 *Melanagromyza albocilia* (Dip., Agromyzidae)

The open-field test conducted in southern Germany in 2017 proved successful and we used the same design to expose additional test plant species in 2018.

On 25 June, 10 plants each of *Convolvulus arvensis*, *Calystegia purpurata, Calystegia sepium* and the North American natives *Calystegia longipes* and *Dichondra argentea* were planted into the soil in blocks at the edge of a maize field. Plants were watered once a week. All plants were alive upon harvest on 24 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, the level of attack (feeding, mines, larvae or pupae) on the wild plants has been relatively high. Dissections are ongoing and detailed results will be presented in the Annual Project Report.

Ghislaine Cortat harvesting plants from the open-field test

14.2 *Tinthia brosiformis* (Lep., Sesiidae)

In 2017, Ivo Toševski, our scientist located in Serbia, field collected over 80 plants infested by larvae of *T. brosiformis.* These were planted in the soil and covered by a gauze cage. A first female emerged on 13 July 2018, and we are expecting some more emergences in the next few weeks. Eggs from the rearing or field collections will be sent to CABI in Delémont mid-August for preliminary tests.

In order to study the relative taxonomic positions of *T. brosiformis* and *T. tineiformis,* 12 specimens were collected from several sites in Greece, along the western Ionian coast and Peloponnesus and in northern Greece close to Macedonia. Molecular analysis will clarify the species status of *T. brosiformis* and *T. tineiformis* and any genetic differences between the central European (Serbia) and southern European (Greece) populations.

Morphological differences in *T. brosiformis* and *T. tineiformis*

Open-field test with *Melanagromyza albocilia* in southern Germany

15 Flowering Rush (*Butomus umbellatus*)

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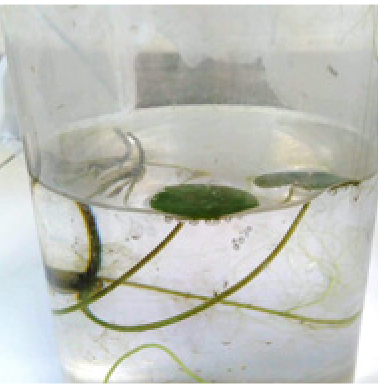
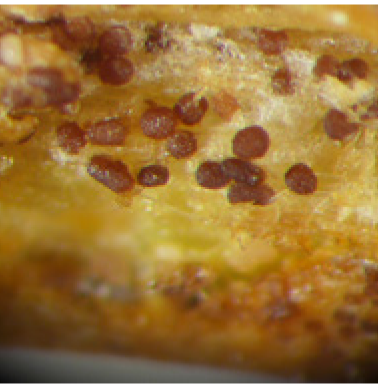


Cylinder setup for no-choice larval development test of *Bagous nodulosus*

15.1 *Bagous nodulosus* (Col.: Curculionidae)

During field trips in May and June 2018, we collected 250 weevils from field sites in Germany, Slovakia, Hungary and Serbia to continue host-specificity tests. In addition, we collected 200 weevils from potted flowering rush plants growing in our artificial pond.

At the end of April, we established another impact experiment. Three pairs of *B. nodulosus* were placed onto each of 17 potted *Butomus* (genotype 1, South Dakota) covered with gauze bags. Plants were placed between 30 "trap plants" in an artificial pool. Seventeen control plants were established in the same way. Weevils and gauze bags were removed after five weeks, and after 9-11 weeks, plants were dissected for larvae and pupae and above and below ground biomass harvested. Data are still being analysed. However, we regularly found larvae, pupae and freshly emerged weevils on trap plants, indicating that larvae of *B. nodulosus* can actively move between plants. This has implications for our host-specificity testing in that we should also consider larval host choice.



Spore balls of the white smut *Doassansia niesslii*

Sequential no-choice oviposition tests with *B. nodulosus* were basically completed in May-July 2018. Only one species, *Blyxa aubertii*, is still missing. Eggs were only found on one of the 44 species tested, *Baldellia ranunculoides*, which is considered as introduced in North America. Weevils laid eggs on all genotypes of flowering rush currently growing at the centre (genotype 1-5).

We also started developing a method for larval no-choice and choice tests and already successfully tested 15 plant species. While larvae usually developed to L2 on controls, we found no larva surviving on test plants so far.

15.2 *Phytoliriomyza ornata* (Dipt.: Agromyzidae)

To establish a rearing, study the biology of the fly and develop methods for host-specificity tests, we infested 71 flowering rush plants by exposing them to individual fly pairs between 3 days and 3 weeks. We used various setups, i.e. plants covered by plastic cylinders, gauze bags, submerged and not submerged plants, without food or providing honey or honey and pollen. After 2-6 weeks, plants were dissected for pupae. So far, we found 93 pupae on 33 plants. Although the species can have two generations a year, we found many more overwintering pupae than emerging pupae (distinguishable by their colour). Since eggs of *P. ornata* are extremely difficult to detect even on control plants, we will have to establish development tests using potted plants.

15.3 *Doassansia niesslii* (Fungi, Doassansiaceae)

Research on this white smut has continued in 2018 to fully elucidate its life history. Experiments have recently been set-up to investigate whether the asexual pycnidiospores are able to infect plants growing completely underwater; results are pending.

Spores liberated from the pycnidia will grow as white slimy colonies of sporidia, in culture, and cause severe infection when brushed on to plants. Experiments are underway to investigate spray application to plants, since there is the potential to develop the fungus as a mycoherbicide.

The isolate we are currently working with was only able to successfully infect genotype 2, but not the most common genotype 1. Plants of genotype 4 (from Maine, Ohio and New York States) and genotype 5 (from Minnesota State), are currently being grown and will be tested. We also established further host-specificity tests with four species; results are pending.

Summer student Aylin Kerim searching for *Bagous nodulosus* in our artificial pond

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

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Mycelial broth of
M. polygoni-cuspidati

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 the UK, field releases continue using the newer psyllid stock ex Kyushu with a preference for *F. japonica*, with some overwintering observed, but with limited long-term field establishment. Various approaches continue to be trialled to improve survival and reproduction in the field, including plant cutting to provide various plant growth stages for the psyllid, sleeving of psyllids to limit immediate dispersal, encourage mating and exclude predators, use of humid riparian sites for releases, use of newer and outdoor-overwintered stock, use of new release sites across a wider geographic range, and release of overwintering morph adults to improve survival into spring.

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

Mycosphaerella polygoni-cuspidati has currently been ruled out as a classical biocontrol agent for Japanese knotweed due to its ability to form the first stage of its life cycle on critical native non-target species. However, due to its unique biology and genetic properties requiring two different mating types for reproduction in the field the pathogen is considered to have potential as a mycoherbicide. The idea has been protected by a patent held in the name of the Secretary of State for Environment, Food and Rural Affairs, UK. Funds from British Columbia are currently supporting the proof-of-concept research.

We have commenced studies to quantify the impact of *M. polygoni-cuspidati* on Japanese knotweed plants when applied as macerated mycelial broth of different concentrations. Preliminary results indicate that higher mycelial concentrations (1 x 105 - 1 x 107 fragments/ml) result in more pronounced disease symptoms compared to lower concentrations (1 x 104 fragments/ml). We have also established that the age of the leafspot culture used for mycelial broth production plays a critical role, with cultures maintained on agar longer than 6 months exhibiting lower virulence. We could show that freeze drying extends mycelial viability of the pathogen and that the addition of the lyoprotectants sorbitol and inositol enhances fragment survival and preserves their infectivity. Post-inoculation ambient humidity has been shown to be another important factor for disease symptom expression on Japanese knotweed.

Disease symptoms caused by the *Mycosphaerella* leaf-spot on Japanese knotweed, 2 weeks after mycelial spray application, concentration 1 x 105 fragments/ml

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

Experiment set up in the CABI UK garden to obtain base-line data on the impact of the rust on Himalayan balsam

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

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

Since 2014, an isolate of this rust collected in India, has been released at selected sites across England and Wales. However, there is significant variation in the susceptibility of individual populations of Himalayan balsam to the rust, which could neither be explained by unfavourable environmental conditions nor a sub-optimal release strategy alone. Therefore, a new isolate of the rust from Pakistan was selected and a number of closely related *Impatiens* species tested to confirm that it has the same level of specify as the Indian strain. Permission to release the Pakistan strain from quarantine was approved in January 2017. An Administrative Trial Permit has enabled applications of the rust spores to be applied as a suspension in water, rather than infected plants being place in field populations. Seedlings from all plant populations where the 2017 and 2018 rust releases were planned were tested for susceptibility to the two rust strains, before release. Some populations have shown to be resistant to both strains.

During summer 2017, high levels of leaf infection by the rust were observed at 13 release sites, but subsequent monitoring for seedling infection during spring 2018 found successful overwintering at only four of these sites. This unexpectedly low successes rate is under investigation.

For Canada, host-range testing has been completed for 45 out of the 51 species native to North America with no non-target impacts recorded to date. The remaining six species have proven more difficult to source and although seeds were collected for two of these species, they have proven difficult to germinate. As such we are working with our collaborators to source these two species as field collected seedlings. Six Canadian populations of Himalayan balsam have also been assessed for their susceptibility towards the two rust strains. All populations were found to be resistant to the strain from Pakistan (the strain which infects more of the UK populations) whilst the strain from India was found to infect only some of the Canadian populations. In addition, the level of infection was lower than that observed on Himalayan Balsam from the UK. It is likely that there is a different biotype of Himalayan balsam in Canada and an additional strain of the rust may be required to have an adequate impact on this genotype.

A molecular study that included samples of Himalayan balsam from the native and introduced range in the UK and Ireland was undertaken, based on six chloroplast DNA sequences. Phylogenetic analyses concluded that the plant has been introduced to the UK at least three times from the native range. The results also identified where surveys in 2018 to collect additional strains of the rust need to focus in the native range. During 2018, additional molecular work will be conducted to include samples from Canada.

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