Weed Biological Control

Progress Report 2019

CABI in Switzerland
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www.cabi.org
KNOWLEDGE FOR LIFE

contents

Cover photos: Daisuke Kurose conducting molecular analysis on Himalayan Balsam; Kathrin Altermatt checking for *Aulacidea* galls on hawkweed; Lise Berberat setting up an open-field test with *Melanagromyza albocilia.*

Inside cover: Philip Weyl talking to a student group from the University of Fribourg, Switzerland

Next page: Sharlene Sing (USDA Forest Service Rocky Mountain Research Station) releasing *Rhinusa pilosa* in Montana, USA

Notes from the section leader

Hariet L. Hinz

I hope you will enjoy reading our latest progress report. Most of our projects have advanced already very well in the first half of this year and there are many positive developments.

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

The gall-forming weevil *Rhinusa pilosa* on **Yellow toadflax** was for the first time released in the U.S. in June this year, by Sharlene Sing from the USDA Forest Service Rocky Mountain Research Station, and David Weaver from Montana State University (MSU). This long awaited release comes after a series of successful releases in Canada where *R. pilosa* has established at most sites. Next steps will include monitoring the population growth of the weevil at the Montana release sites and its impact on Yellow toadflax. In addition, local partners will establish insectaries to multiply the agent so that its benefits can be expanded on a broader regional, and eventually, national scale.

A second release is also coming soon; the two stem-mining moths *Archanara gemminipuncta* and *A. neurica* on **common reed** in Canada. The moths were permitted by the Canadian Food Inspection Agency (CDFA) and recommended for release by the USDA, APHIS Technical Advisory Group (TAG) in April 2019. Work is underway by Rob Bourchier from Agriculture and Agri-Food Canada, Lethbridge, implementing the research team's release-monitoring plan that includes both native and invasive Phragmites sites, and to determine the best insect stage for release. In the U.S., a Biological Assessment now needs to be prepared for consultations with the U.S. Fish and Wildlife Service.

For a third agent, the syrphid fly *Cheilosia urbana* petitioned for invasive **hawkweed** control, Jeff Littlefield from MSU received the good news from USDA-APHIS at the beginning of July that the FONSI (Finding of No Significant Impact) has been signed. This means that a permit for field release of the fly will soon be available and we are planning on sending insects to Jeff next spring when egg laying females are available again.

Finally, we are still waiting to hear back from USDA-APHIS and the U.S. Fish and Wildlife Service on our petition for the root-crown mining weevil *Ceutorhynchus scrobicollis* on **garlic mustard**, recommended by TAG in February 2017 and released in Canada in autumn 2018.

A petition for field release of the eriophyid mite *Aceria angustifoliae* for the biological control of **Russian olive** is in preparation and will likely be submitted by our North American collaborators later this summer or in autumn. As is often the case now this will be a joint petition for the U.S. and Canada.

A new project has started this year at CABI Switzerland with support from the Army Corps of Engineers, *Nymphoides peltata* or **yellow floating heart**. Despite its nice name, this plant is listed as noxious in six U.S. states, because of its negative impacts on native species, reduced boating and recreational access, and reduced water flow. Patrick Häfliger is leading the work at CABI.

After Rich Hansen (USDA, APHIS) passed away, the field bindweed project was left without any North American counterpart. Last autumn, John Gaskin (USDA, ARS, Sidney, Montana), stepped in and took over as consortium leader for this project. This is extremely valuable for us, since most of our projects would never see the light of the day without people in the invaded range of the target weed that seek opportunities for funding, send test plant material our way, collect pre-release data, and most importantly, prepare and submit the petitions for field release and test plant lists to the respective regulatory authorities. I would like to take the opportunity to herewith particularly thank all consortium leaders, but also all other people actively involved in consortia for their continuing support and efforts, despite increasingly long time lines before agents can be released!

And just to give you heads up: the Proceedings for the XV International Symposium on Biological Control of Weeds (ISBCW) is close to being completed and will be published soon on **https://www.ibiocontrol.org/proceedings/**. I will send an email around once it's online.

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Oliver Krstić examining mass rearing of *Rhinusa pilosa* haplotypes in frame cages

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 2480 (91%) adults of the stem-gall forming weevil, *R. pilosa,* and 94 (50%) *R. rara* survived hibernation. The survival rate of *R. pilosa* was the same as in the previous year (88%), while survival of *R. rara* was much lower (80%). On 10 January and 15 February 2019, two shipments of *R. pilosa* containing 1159 and 1021 adults were sent to Agriculture and Agri-Food Canada (AAFC, Lethbridge), and to the quarantine facility at Montana State University (USA), respectively. Weevils from both shipments were separated by their mitochondrial affiliation for further propagation and field release experiments.

To increase genetic diversity of the laboratory population of *R. pilosa*, 460 galls were collected between 10 and 20 April, from nine different locations in northern and eastern Serbia. The galls were planted in separate pots to follow adult emergence. From every field collected gall not infested with *R. eversmanni*, samples of *R. pilosa* were taken for molecular analysis to determine the genetic diversity of the weevil within sampled populations. After molecular analysis, adults from the selected galls will be fused in batches according to the particular COII gene haplotype and set up separately for aestivation.

For mass rearing, adults of *R. pilosa* were separated by their mitochondrial affiliation or their collecting origin and set up in four outdoor cages with *L. vulgaris* plants for rearing. A total of 1546 galls were harvested from outdoor cages. In addition, a total of 32 frame cages were set up to propagate rare haplotypes recorded in 2018 which resulted in 764 galls.

For mass rearing of *R. rara,* a total 41 females were set up at the beginning of April in two outdoor cages planted with 60 *L. dalmatica* and *L. genistifolia*, respectively. Unfortunately, similar to 2018, our rearing attempt failed. Probably due to extremely cold and rainy weather during April and May, most plants set up in cages died. Between 3 and 5 May, a total of 302 *R. rara* galls were collected from three locations in eastern Serbia, from which 353 adults emerged.

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

Overwintering survival of stem-boring *Mecinus* spp. was good, especially when adults were left in cages for natural hibernation. Number of adults from a subsequent rearing with *M. heydenii* will be known after dissection of *L. vulgaris* plants set up in six frame cages. Mass rearing of *M. laeviceps* and *M. peterharrisi* failed in 2019, for similar reasons as for *R. rara*. Dissected dead stems of *L. genistifolia* and *L. dalmatica* in one outdoor cage with *M. laeviceps* and three cages with *M. peterharrisi* revealed the presence of several hundred dead L1 and L2 larvae just below the stem surface. It will be necessary to make considerable changes in the rearing methodology for weevils associated with *L. dalmatica* 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, plants of *Nutthalanthus canadenis* were set up on 29 March with ovipositing females of each of the three *Mecinus* species (*M. heydenii:* 11 females, *M. laeviceps:* 13 females, and *M. peterharrisi:* seven females). Plants exposed for oviposition were dissected between 2 and 8 July, revealing development of 31 *M. heydenii* and six *M. laeviceps* on *N. canadensis,* while no development to adult was recorded in plants exposed to *M. peterharrisi*. All *M. heydenii* and *M. laeviceps* were returned onto planted *N. canadensis* to see whether weevils will be able to sustain a population when provided with only this test species.

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

Ivo Toševski planting toadflax plants with induced galls of *Rhinusa pilosa* from mass rearing cage

2 Houndstongue (*Cynoglossum officinale*)

Cornelia Cloşca, **Philip Weyl** and **Alissa Cereghetti**
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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.

Adult of *Mogulones borraginis*

From over 3600 *M. borraginis* larvae collected in 2018, about 500 adults emerged in October 2018 and an additional 2000 in March 2019 which were set up on houndstongue rosettes. At the beginning of June, about 130 females were retrieved and reset on 41 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 2400 *M. borraginis* larvae emerged and were separated into cups (30 individuals per cup) and placed in an underground insectary until the adults emerge in autumn 2019 and spring 2020.

On two occasions (December 2018 and May 2019), Mark Schwarzländer from the University of Idaho (UoI) hand carried a total of 550 adults of *M. borraginis* to the quarantine facility at the University of Idaho. These adults were used to collect data on the host finding behaviour of the weevil, 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.

Mature larvae of *Mogulones borraginis* leaving the fruits

Summer student Alissa Cereghetti searching for *Mogulones borraginis*

3 Hawkweeds (*Pilosella* spp.)

Ghislaine Cortat, **Kathrin Altermatt** and **Guillaume Kuhn**

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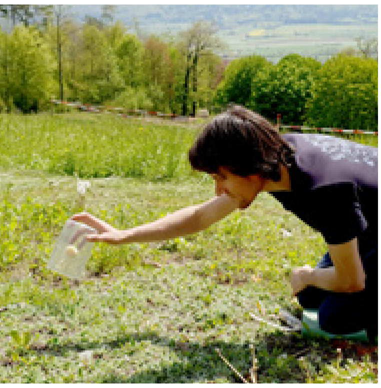

Summer student Guillaume Kuhn collecting *Cheilosia urbana* at CABI Switzerland

3.1 *Aulacidea pilosellae* (Hym., Cynipidae)

Two biotypes of this gall wasp are being investigated. The first biotype is *A. pilosellae* ex *Pilosella* spp. pooled from wasps from the northern range (eastern Germany, Poland and the Czech Republic) and the second biotype is *A. pilosellae* ex *Pilosella officinarum* of which we have been testing a population pooled from wasps collected in the southern range (Switzerland and southern Germany). Studies conducted at AAFC showed that the second biotype carries Wolbachia, which is thought to be responsible for the biotype differentiation.

In 2018, about 300 galls of *A. pilosellae* ex *P. officinarum* were harvested from rearing and host range tests. From these 162 females emerged in May and June 2019. From 17 May to 17 June, ten species were exposed in no-choice tests, including *P. officinarum* of different origins, two North American (NA) *Hieracium* species, one other NA native and six other species including crops and ornamentals, resulting in a total of 65 plants. Gall harvest is ongoing. Beside *P. officinarum* (100% attacked, 28 ± 4 galls per plant), we so far only found one gall on one of two *H. bolanderi* and up to three small galls per plant on three of seven *H. horridum.* The galls will be overwintered for emergence in 2020 and the attacked *Hieracium* species will be exposed in choice tests in 2020. On 22 May, the NA *H. argutum* which sustained limited attack under no- and single-choice conditions, was exposed together with *P. officinarum* in three field cages. Plants will be checked for galls in the coming weeks.

Adult of *Aulacidea pilosella*

In 2018, about 500 galls of *A. pilosellae* ex *Pilosella* spp. were harvested from host range tests. From these 121 females and 302 males emerged in June 2019. From 3 to 14 June, ten species were exposed in no-choice tests, including *P. caespitosa*, the NA *H. horridum*, two other NA natives and six other species including crops and ornamentals, resulting in a total of 59 plants. Gall harvest is ongoing. So far, galls were only found on *P. caespitosa.* Final results will be presented in the annual report.

From the separate populations collected in the Czech Republic and Germany, we were only able to maintain a colony from Velka Upa. This is owed to a low number of galls and a sex ratio in favour of males (Velka Upa 68% and Zweibach 93%).

3.2 *Cheilosia urbana* (Dipt., Syrphidae)

Between 24 April and 7 May 2019, 13 females of the syrphid fly *C. urbana* were collected in the CABI garden. The hoverflies were stored in an incubator at 10°C, with 12h light and shipped on 13 May to Dr. De Clerck-Floate (Agriculture and Agri-Food Canada, Lethbridge). Eggs were harvested upon reception at AAFC and 100 of these were used for larval transfers onto potted plants for later field releases.

Choice-test with *Aulacidea pilosellae* ex *Pilosella officinarum* in a field cage at CABI Switzerland protected against rain with a plastic cover.

4 Russian Knapweed (*Rhaponticum repens)*

Philip Weyl, **Cornelia Cloşca (CABI)**, **Francesca Marini** and **Massimo Cristofaro (BBCA)**
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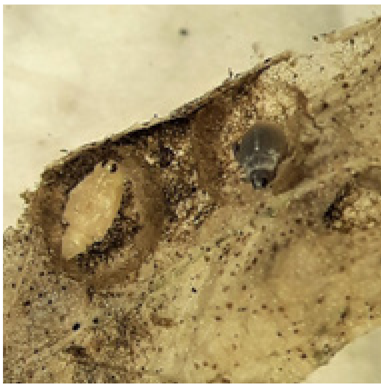
Typical leaf damage of the jumping weevil, *Pseudorchestes distans*

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 main emphasis for this move was to obtain long term establishment on Russian knapweed (controls). As in previous years, two methods were used to inoculate the test and control plants in 2018. 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. In 2019, no further inoculations were made to determine if the mite was able to establish on the control plants. The plot was surveyed in late June at the peak of mite development and impact. Unfortunately, to date, no establishment was observed on our experimental plants. Despite the high impact of this mite on the reproductive output of *R. repens* we have decided to suspend any additional work with this species.



A pupa (left) and pre-emerged adult (right) of the jumping weevil, *P. distans* in pupal chambers constructed within the leaf mine

4.2 *Pseudorchestes distans* (Coleoptera, Curculionidae)

Kazakhstan

During surveys in 2018 of the Ile River Valley between Almaty and Lake Balkhash in south west Kazakhstan, a jumping weevil, *Pseudorchestes distans* was collected. In early May and again in June 2019 we were able to re-collect 112 and 175 adults respectively, which were hand carried to CABI's quarantine facility in Switzerland.

We have initiated a rearing of this weevil and are in the process of studying the biology. Under laboratory conditions (26˚C, 16:8 light:dark), the development of this weevil is extremely fast at 21 days from egg to adult. The females are highly fecund, laying roughly 5 eggs per day for at least six weeks into the parenchymatic tissue on the edge of the leaf. After 6-7 days the eggs hatch and the larvae mine the leaf. There are three larval instars, after which the weevil pupates within a pupal chamber constructed inside the leaf mine created by the larvae. The effect of the larval mining usually kills a large portion the leaf. The adults emerge and feed voraciously on *R. repens.* At this stage it is unclear how many generations the weevil has per year. Initially we expected two generations, however, under laboratory conditions we may be able to obtain more. The combined effect of larval mining and adult feeding can have a significant effect on plant productivity and we have noted plant mortality with high densities of the weevil. We plan to initiate host range tests with this species in spring 2020.

Philip Weyl collecting *Pseudorchestes distans* in the Ile River valley, Kazakhstan

Mating pair of *Ceutorhynchus constrictus*

Single-choice tests with *C. constrictus*

5 Garlic Mustard (*Alliaria petiolata*)

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5.1 *Ceutorhynchus constrictus* (Col., Curculionidae)

Between 14 March and 15 April 2019, 395 adults of the seed-feeding weevil *Ceutorhynchus constrictus* emerged from our rearing colony established in 2018. On 7 May, 160 adults were shipped to Dr. Katovitch (University of Minnesota) for rearing and additional host-specificity tests in quarantine. The remaining adults were used in host range tests at CABI. From 20 May to 12 June, 14 no-choice oviposition tests were conducted, using three native North America test plant species and *A. petiolata* as controls. Four of five controls were attacked and no eggs were found in the test plants. *Barbarea orthoceras* and *Boechera holboellii* had been accepted for oviposition in previous no-choice tests. In 2019n, five single-choice tests were conducted with each of these two species, offering one shoot of the test plant together with *A. petiolata* in a gauze-bag to two females and one male. After two weeks, the plants were dissected for eggs and the feeding damage was recorded. All controls were attacked. No eggs were found on *B. holboellii* and only one exploratory feeding hole was found. Eggs were only found on one of the *B. orthoceras* which constituted 11% of the total number of eggs laid. Seed damage on *B. orthoceras* was negligible. So far 284 mature larvae emerged from *A. petiolata.* Adult weevils that emerged in spring 2019 from larval development in *B. holboellii* in 2018, were placed onto a potted *B. holboellii. S*urvival was low and the one remaining female did not lay eggs.

Summer student Kathrin Altermatt harvesting mature larvae of *Ceutorhynchus constrictus*

6 Common Reed (*Phragmites australis*)

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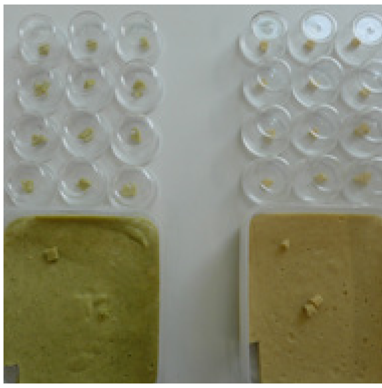
At the beginning of 2019, we received the good news that the two noctuid moths *Archanara geminipuncta* and *A. neurica* have been approved for release in Canada and that the USDA, APHIS Technical Advisory Group (TAG) recommended release for the USA. Recent taxonomic publications apparently now use the genus name *Lenisa geminipuncta* instead of *Archanara geminipuncta*. We will adapt the names accordingly, as soon as we have more evidence confirming this decision.



Artificial diet for moth rearing with (left) and without (right) addition of fresh *Phragmites*

6.1 Rearing of *Archanara geminipuncta* and *A. neurica* (Lep., Noctuidae)

We continued maintaining a rearing of the two noctuid moths. In addition to the established rearing on *Phragmites* stem sections, we also started rearing on the McMarron diet that was successfully tested with *A. neurica* by the group of Rob Bourchier at Agriculture and Agri-Food Canada (AAFC), Lethbridge in 2018. Several 1000 eggs of *A. neurica* were obtained from adults reared on this artificial diet and much less work needed to be invested compared to the usual rearing on *Phragmites* stems. However, rearing of *A. geminipuncta* on diet is more difficult. Although adding fresh *Phragmites* to the diet greatly helped getting young larvae established, older larvae seemed to have problems molting and died. More trials will be needed, before *A. geminipuncta* can also be reared on a time saving diet. Nevertheless, several 1000 eggs will be available of this species too due to very good success of the conventional rearing in 2019.

In March 2019, 700 eggs of *A. neurica* and 300 of *A. geminipuncta* were shipped to Agriculture and Agri-Food Canada (AAFC), Lethbridge, for additional rearing trials on artificial diet and for preparation of field releases.

Summer student Teodor Trifonov working on the conventional *Archanara* rearing using stem sections of *Phragmites*

6.2 Larval dispersal experiments with *Archanara neurica*

In order to test larval dispersal of *A. neurica*, we repeated a small field experiment that failed in 2018 because of a cold snap. Four old stem bases of *Phragmites* with 40 eggs of *A. neurica* each were placed at the end of April in the soil at six plots at a field site near the Centre. We selected plots that were mown during winter and removed all old stems to make sure that no overwintering *Archanara* eggs of the natural population could be present. The plan was to check the sites after several weeks and record the distances of infested shoots from the release point. Unfortunately, a cold snap heavier than the year before killed not only the eggs/larvae, but also many *Phragmites* shoots. Since we still had stored some eggs in a fridge, we were nevertheless able to setup the same experiment again at three plots, where not too many *Phragmites* stems had died. At one of these plots, we found six damaged stems after six weeks, all within a 1m distance of the egg release point. Three of the stems were damaged by first instar larvae, and three by third instar larvae. Thus, larvae did not manage to move far away from the egg release point. However, it will be necessary to repeat this experiment under more controlled conditions at the natural hatching time of eggs, to measure larval dispersal capacity. The eggs suffer when stored too long in the cold and the few hatching larvae may not have been the fittest.

Summer student Mariel Guala setting up a larval dispersal experiment with *Archanara neurica*.

7 Whitetop or Hoary Cress (*Lepidium draba*)

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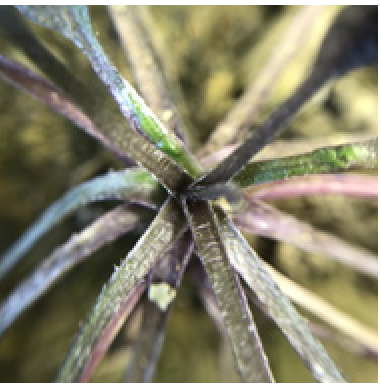

Reduced gall formation of *Ceutorhynchus cardariae* on *Lepidium draba* growing in serpentine soil at the University of Idaho

7.1 *Ceutorhynchus cardariae* (Col., Curculionidae)

Of the 1000 adults of the gall-forming weevil, *Ceutorhynchus cardariae* that overwintered at CABI, 90.4% survived. The rearing continues very well and we have over 3000 adults currently in summer aestivation.

This spring we conducted a few additional host-specificity tests. Of ten replicates established with the native North American (NA) *Descurainia incana*, two formed galls but neither plant supported adult development. *Cardamine flexuosa* remained completely unattacked. Since one plant of the NA native, *Lepidium oxycarpum* supported adult development under no-choice conditions in 2018, we conducted a single-choice test this spring. Only one very small gall was recorded on a single plant of *L. oxycarpum.* No adult emergence was recorded and upon dissection a dead first instar larvae was found. Two additional open-field tests were performed this spring with NA native test plants that had supported adult development under multiple-choice conditions. The data collection is in progress and will be presented in the annual report.

In collaboration with Mark Schwarzlaender, University of Idaho, we are studying the influence of serpentine soils on host selection and performance of *C. cardariae.* There is evidence to suggest that host selection and performance of insects may differ when plants adapted to serpentinic soils are grown on generic potting soil. These experiments are ongoing and detailed data will be presented in the annual report.

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

7.2 *Ceutorhynchus turbatus* (Col., Curculionidae)

On 30 May, over 1300 adults of the seed-feeding weevil *C. turbatus* were collected in southern Switzerland. On 31 May, we established no-choice oviposition and development tests with 40 plants; nine *L. draba* control plants and 1-5 replicates of eight test species. Apart from *L. draba,* eggs were not found in any of the native NA test species.

We were also able to set up a development test with two native NA test plant species (*Lepidium integrifolium* and *L. thurberi*) and two European species (*Lepidium campestre* and *L. heterophyllum*) that had been accepted for oviposition in the past. Only *Lepidium campestre,* which is very closely related to *L. draba*, supported larval development.

The mature larvae that emerged from *Lepidium huberi* in 2018, developed into adults and emerged in May 2019. These adults were set up again on *L. huberi*, to determine whether they can develop eggs and sustain a population feeding only on *L huberi*. No oviposition or larval development was observed on these plants suggesting that *L. huberi* cannot sustain a population of *C. turbatus*. We also conducted a single-choice test with *L. huberi.* During this test, *L. huberi* remained completely unattacked, while there was oviposition in over 50% of the fruits for the control plants.

Summer student Alissa Cereghetti collecting *Ceutorhynchus* turbatus in southern Switzerland

8 Dyer's Woad (*Isatis tinctoria*)

Philip Weyl, **Hariet L. Hinz**, **Cornelia Cloşca**, **Alissa Cereghetti (CABI)** and **Francesca Marini (BBCA)**.

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8.1 *Ceutorhynchus peyerimhoffi* (Col., Curculionidae)

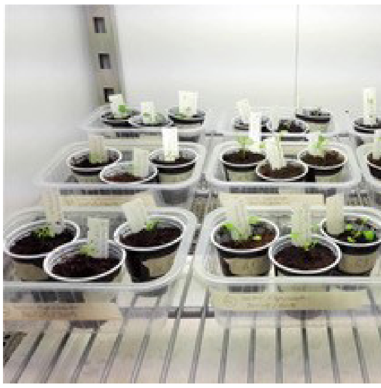
Between 12 April and 15 June 2019, approximately 2000 adults of the seed-feeding weevil *Ceutorhynchus peyerimhoffi* emerged from our rearing colony. These adults were used in tests or transferred onto flowering dyer's woad plants for rearing. From the rearing plants and dyer's woad plants used in tests during 2019, over 1400 larvae were collected and were kept for adult emergence in 2020.

Between 29 April and 20 May, no-choice oviposition tests were established with 19 plants from four test species with 1-5 replicates each, and five dyer's woad control plants. Apart from dyer's woad, eggs were not found on any test species which were exposed. We were also able to setup development tests with three species that had been accepted for egg laying in previous years, *Sibara filifolia, Streptanthus lasiophyllus* and *Boechera pulchra.* No larvae emerged from any of the test plants. Dissections for larvae remaining in the fruits are in progress, and the data will be presented in the annual project report.

Typical symptoms of the eriophyid mite *Metaculus* sp. in the field in early spring

8.2 *Ceutorhynchus rusticus* (Col., Curculionidae)

A total of 250 plants, from 46 test plant species and the control were exposed to *C. rusticus* in no-choice oviposition and development tests between autumn 2018 and spring 2019. Test plants were regularly checked and any dying plants were dissected. Adult emergence was much better in 2019 than in 2018, with 77% of the dyer's wood control plants supporting adult emergence and an average of 4.3 adults emerging per plant (vs. 1.3 adults/plant in 2018). Of the 46 test plant species, we recorded typical *C. rusticus* mining in 18 test species, while only four test species (*Lepidium lasiocarpum, Stanleya albescens, Streptanthus flavescens* and *Streptanthus tortuosus*, all native to NA) supported limited adult development.



Host range testing of *Metaculus* sp. under controlled laboratory conditions at BBCA

8.3 *Metaculus* sp. (Aceria, Eriophyidae)

From the colony that was collected from the Kaiserstuhl area in south-western Germany and in the Abruzzo region of Italy in autumn 2018, we were able to initiate host range tests at CABI Switzerland and BBCA, Italy in spring and summer 2019. Preliminary host range tests suggest that under laboratory conditions, this mite is able to reproduce and induce symptoms on several Brassicaceae species. However, there is a discrepancy in host range depending on the test location, with a much tighter host range under more natural outdoor conditions compared to laboratory conditions. In collaboration with BBCA, we are trying to untangle these observed differences.

Summer student Alissa Cereghetti checking test plants for *Ceutorhynchus rusticus* emergence

Damage caused by the stem-mining fly *Lasiosina deviata*

9 Perennial Pepperweed (*Lepidium latifolium*)

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9.1 *Lasiosina deviata* (Dip., Chloropidae)

From 24 to 30 May, we conducted a field trip to Kazakhstan to collect the stem-mining fly *Lasiosina deviata*. We surveyed several sites of perennial pepperweed (PPW) in the vicinity of the site where *L. deviata* had been found in 2018.

We collected a total of 150 stems showing symptoms that could potentially be attributed to *L. deviata*. We dissected a few of them and since we observed that most of the *L. deviata* were still in the larval stage, we inserted the stems in moist florist sponge to keep them fresh. On 11 June, we dissected all stems and removed the pupae. Approximately 50 pupae and three larvae were found. They were placed on moist filter paper in small Petri dishes. From 21 June to 3 July a total of four females and five males emerged from them. They were kept in plastic cylinders together with shoots of PPW inserted in a piece of moist florist sponge. We regularly checked the shoots for eggs, but did not find any. On 5 July we therefore decided to put the surviving 3 females and 2 males together with a potted plant in a large cage. We regularly exchanged the plant and checked it for eggs, but so far no eggs were found. The plants will be dissected for larvae in August.

Gall and exit hole produced by the weevil *Ceutorhynchus marginellus*

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 423 adults that were kept in incubators set at 3°C during winter 2018/2019, 167 females and 119 males survived until March 2019. These weevils were transferred onto 39 potted PPW plants to continue our rearing. More than 1000 adults emerged from these plants. A subset of the weevils is currently being kept in cylinders and regularly fed with leaves of PPW or on potted plants.

Philip Weyl looking for PPW stems infested with *Lasiosina deviata* 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 French population. In July 2018, twenty *V. rossicum* were each infested with 25 larvae. Adult emergence is ongoing and so far, we obtained 39 females and 21 males. Eggs will be harvested regularly for larval transfer tests and to continue the rearing.

Adult of *Chrysochus asclepiadeus*

Plants exposed to adult *C. asclepiadeus* in an open-field test in summer 2018 were searched for larvae in April−May 2019. 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*. No larvae were found on *Asclepias incarnata, A. syriaca* or *Apocynum cannabinum.* A total of 41 larvae were found around the roots of *V. hirundinaria.* So far five adults emerged from these plants. Results confirm the narrow host range of *C. asclepiadeus* under natural field conditions.

10.2 *Euphranta connexa* (Dipt., Tephritidae)

Pupae (N=844) of the seed feeding fly collected in summer 2018 were overwintered at 5°C. Starting beginning of May 2019, batches of about 100-180 pupae were taken out of cold storage every 2-4 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, 82 females and 118 males have emerged. No-choice and single-choice oviposition tests, and egg transfer tests are being conducted and results will be presented in the annual report.

Mating pair of *Euphranta connexa*

Summer student Guillaume Kuhn harvesting newly emerged *Chrysochus asclepiadeus* from a rearing plant

11 Common Tansy (*Tanacetum vulgare*)

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Summer student Tessa Bokla preparing stems infested with *Platyptilia ochrodactyla* in Germany

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 4 to 17 June a total of 39 females and 41 males were collected in the field. Additional no-choice oviposition and development tests were conducted with four 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 open-field test was set up. Seven potted plants each of the two native North American *Tanacetum camphoratum* and *T. huronense* as well as of *T. vulgare* were exposed on a meadow and a total of 25 females were released. The plants are currently being dissected. In addition, stems infested with *M. millefolii* were collected in the field and the emerging adults will be shipped to Rosemarie De Clerck-Floate at Agriculture and Agri-Food Canada (AAFC) in Lethbridge. Detailed results will be presented in the annual report.

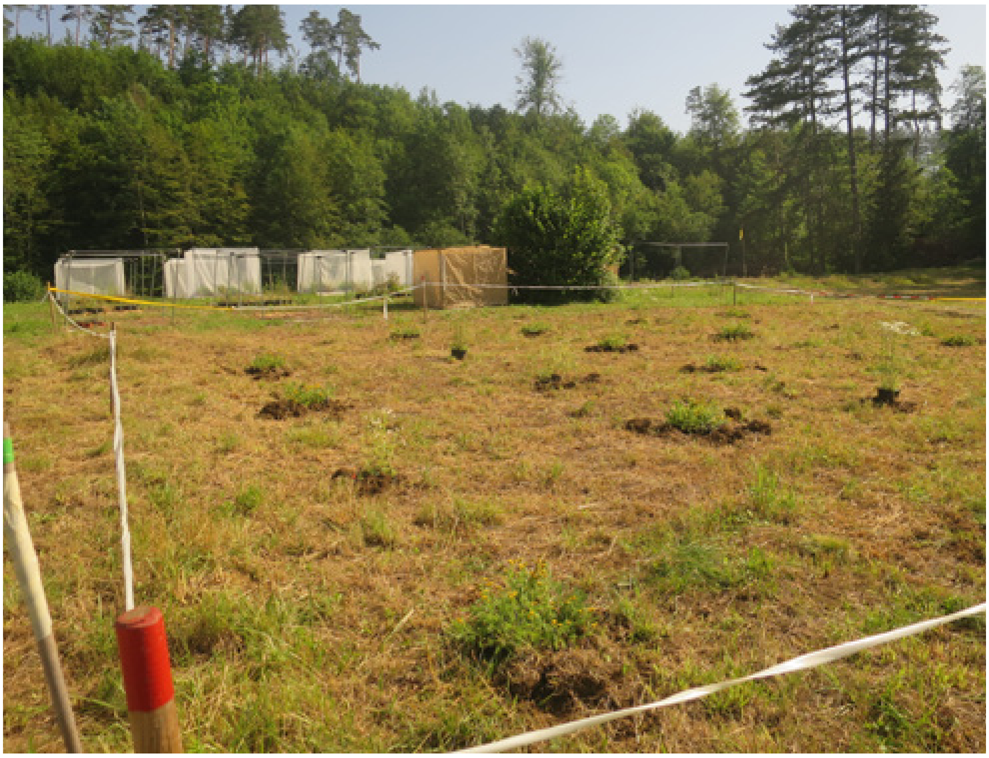
11.2 *Platyptilia ochrodactyla* (Lep., Pterophoridae)

On 14 and 15 May, we collected about 1100 shoots attacked by *P. ochrodactyla* in western Germany. From 13 June to 1 July, a total of 96 females and 159 males emerged. An open-field test was conducted with flowering *T. camphoratum*, *T. huronense*, *T. parthenium* and *T. corymbosum*. A total of 77 females and 100 males were released between 25 June and 1 July. Since no flowering *T. vulgare* were available in June, they were separately exposed at the same place and another 10 females and 50 males were released at the end of July. The flower heads of test and control plants will be dissected about two months after the exposure to *P. ochrodactyla*.

Set up to retrieve larvae of *Microplontus millefolii* from stems of *Tanacetum vulgare* collected in the field in St Petersburg

11.3 *Chrysolina eurina* (Col., Chrysomelidae)

In spring 2019, only very few larvae emerged from the overwintering eggs we had collected from beetles reared on *T. vulgare* (emergence rate 3%), *T. balsamita* (4%), *T. camphoratum* (30%) and *T. huronense* (6%). The emerging first instar larvae were transferred onto leaves of the same plant species as their parents had been reared from. Unfortunately, the survival rate was extremely low, even for larvae fed with *T. vulgare* and only very few adults emerged from *T. vulgare* and *T. huronense*.



Open-field test set up with *Platyptilia ochrodactyla*

12 Russian Olive (*Elaeagnus angustifolia*)

Philip Weyl, **Cornelia Cloşca (CABI)**, **Francesca Marini** and **Massimo Cristofaro (BBCA)**
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12.1 *Aceria angustifoliae* (Acari: Eriophyoidae)

The host range testing for the eriophyid mite, *Aceria angustifoliae* is now completed and the petition for release is in the process of being prepared. With the possibility of exporting *A. angustifoliae* from Iran, for release in the USA, unlikely in the foreseeable future, we conducted additional host-range tests with the population of *A. angustifoliae* located in Serbia. The tests were conducted on a limited number of species within the Elaeagnaceae family. The results from the no-choice laboratory trials (both from the Iranian and Serbian populations) as well as the open-field tests in Iran suggest that this is a highly specialized herbivore and the risk to non-target plants in North America is negligible. In addition, the impact study revealed significant reductions in fruit set, which will likely translate to a reduction in long distance dispersal in the invaded range. We believe that this is an effective biological control agent that will significantly add to the management efforts of Russian olive in North America.

The symptoms of the eriophyid mite collected in Kazakhstan, which are unlike the symptoms of *Aceria angustifoliae* and *A. eleagnicola*

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. The impact of this species appears to be limited and by the end of the 2019 season a decision will be made whether we continue to invest time in this species as a potential biological control agent.

12.3 *Anarsia eleagnella* (Lep., Gelechiidae)

Host range testing with *Anarsia eleagnella* under laboratory conditions using larval no-choice tests, has revealed that this species is able to complete its development on several Elaeagnaceae species including native North American (NA) species.

We plan to collect additional data in late summer from the open-field test at the experimental farm of Mashhad University. 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.

12.4 Additional surveys

In June we surveyed the Ile River valley with the objective of collecting a stem boring weevil, *Temnocerus eleagni* which has been collected in the region in the past. Unfortunately, this was not successful and no adults were collected. However, we were able to collect an eriophyid mite which is causing a deformation of the new stems, reducing stem length and most likely affecting flower and fruit production. The symptoms are unlike any eriophyid mite recorded on Russian olive so far which suggests that this is potentially a new species. We have sent samples of these mites to our colleagues Dr. Biljana Vidovic and Prof. Radmila Petanovic at the University of Belgrade for identification. If this proves to be a new species we plan to initiate biology and host range testing in summer 2020.

Francesca Marini from BBCA using the beating method to collect herbivores from Russian olive in Kazakhstan in spring 2019

13 Oxeye daisy (*Leucanthemum vulgare*)

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Galls of *Oxyna nebulosa* on oxeye daisy

13.1 *Dichrorampha aeratana* (Lep., Tortricidae)

On 13 May, we shipped a total of 393 eggs of *D. aeratana* to Dr De Clerck-Floate at Agriculture and Agri-Food Canada (AAFC) in Lethbridge for rearing.

From 3 to 22 May, we set up additional no-choice larval development tests with seven test plant species that are either native or horticultural plants in Australia. Five first instar larvae were transferred onto each of the plants. All plants will be dissected for larvae in autumn.

In addition, we set up an open-field test with *Cotula cotuloides*, *Mauranthemum paludosum* and *Tanacetum parthenium*, plant species that had supported larval development in previous tests. A total of thirty females of *D. aeratana* were released between 14 and 24 May and the plants will be dissected in autumn.

13.2 *Dichrorampha consortana* (Lep., Tortricidae)

Only a total of ten females and 23 males emerged between 24 May and 14 June from the 53 oxeye daisy plants that had been infested in summer 2018 with 5-10 larvae each. One female and one male also emerged from one of the 23 Shasta daisy plants that had been infested in summer 2018. We conducted no-choice larval development tests with 13 species and varieties, but due to the low number of available larvae, only a total of 39 plants could be set up.

Cyphocleonus trisulcatus feeding on a leaf of oxeye daisy

13.3 *Oxyna nebulosa* (Dipt., Tephritidae)

In May and June, we harvested more than 1000 galls from the oxeye daisy plants that had been exposed to adults of *Oxyna nebulosa* in 2018. On 13 May, 177 of these galls were shipped to Dr De Clerck-Floate at AAFC. From the remaining galls, more than 800 adults emerged between mid-June and mid-July.

We set up no-choice oviposition and larval development tests with 15 different test plant species and varieties. For this, two egg-laying females each were placed onto test and control plants for approximately one week. 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 2020.

In addition, we evaluated the impact experiment that was set up in 2018. We found that the number of flower heads and the above ground biomass of plants exposed to two females of *O. nebulosa* for one week in 2018 were reduced by 60% compared to non-infested control plants.

13.4 *Cyphocleonus trisulcatus* (Col., Curculionidae)

In June and July, we set up no-choice oviposition and larval development tests with 14 test plant species that are either native or horticultural plants in Australia. For this, two egg-laying females each were placed onto test and control plants for approximately one week. The plants will be dissected for larvae in August and September.

Summer student Vito Lionetti releasing *Dichrorampha aeratana* in the open-field test

14 Field Bindweed (*Convolvulus arvensis*)

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14.1 *Melanagromyza albocilia* (Dip., Agromyzidae)

The open-field tests conducted in southern Germany in 2017 and 2018 proved successful and we used the same design to expose additional test plant species in 2019.

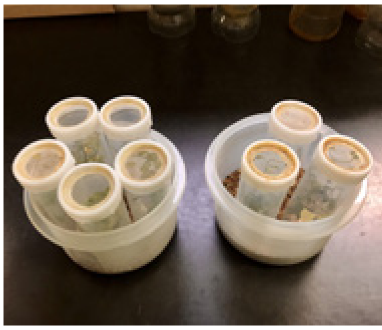
On 13 June, ten plants each of *Convolvulus arvensis* and the North American (NA) natives *Calystegia occidentalis, Calystegia stebbinsii,* and six *Calystegia malacophylla* and *Ipomoea lindheimeri* were planted into the soil in ten blocks at the edge of a field. In 2019, the field was planted for the first time with oat instead of maize. So instead of planting the blocks in the first row of maize, the test plants had to be placed at the edge of the crop. Plants were watered once a week. Plants that were found dead or dying during the last watering session beginning of July, were harvested and dissected in the lab. The remaining plants were harvested on 9 July, just before the oat harvest. About 20 wild *Con. arvensis* plants growing close to the exposed test plants were also harvested to determine the attack rate at the edge of the field. Dissections are ongoing and detailed results will be presented in the annual project report.

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

14.2 *Microsphecia brosiformis* (Lep., Sesiidae)

In 2018 eggs sent by Ivo Toševski, our scientist located in Serbia, were used mid-August for preliminary tests with this sesiid moth. The plants received two eggs each and in October 2018, the soil and roots were searched for larvae. One larva was found in one of four *Convolvulus arvensis* and *Calystegia soldanella*. The plants were stored in an unheated greenhouse overwinter. So far, no adult has emerged.

Between 15 July and 2 August 2019, 15 females of *M. brosiformis* were colleby visiting over 30 different locations in eastern Serbia. All females were set up for oviposition in plastic vials (114 x 44 mm) with fresh stem cuts of bindweed. Obtained eggs will be used for screening tests under no-choice conditions with two *Convolvulus* and six *Calystegia* plant species native to North America. The remaining eggs will be used to develop rearing methods under controlled conditions to establish a laboratory colony of this clear-wing moth.



Females of *Microsphecia brosiformis* set up for oviposition

Ivo Toševski collecting adults of *Microphecia brosiformis* in Serbia

15 Flowering Rush (*Butomus umbellatus*)

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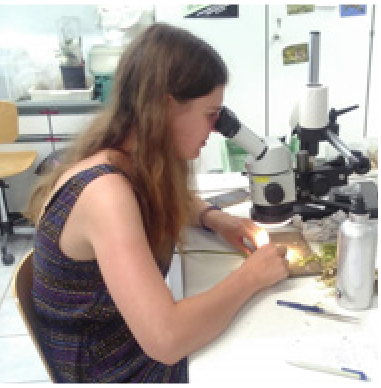


Summer student Pia Stettler dissecting flowering rush plants

15.1 *Bagous nodulosus* (Col.: Curculionidae)

About 180 weevils were collected during field trips to Slovakia and Hungary in May and June to continue host-specificity tests and carry out another impact experiment. Around 70 weevils, fewer than in 2018, were collected from potted flowering rush plants growing in our artificial pond. In early July 2019, we identified three additional sites with *B. nodulosus* during a survey through the Netherlands.

In mid-May, we established another impact experiment. In order to be able to apply three clearly separated treatments (5 pairs of adults, 60 larvae, controls), we set up 24, 50l containers covered with gauze bags and filled with water containing three pots of three different populations of *Butomus* (Montana (genotype 1, triploid), Minnesota (genotype 5, diploid), Slovakia (triploid)). In August, all plants will be dissected for larvae and pupae and biomass will be recorded from leaves, rhizomes and fine roots.

We also tried completing sequential no-choice oviposition tests and continuing larval no-choice tests. However, we made only little progress so far, because the rate of ovipositing females was for some reason lower this year.

Carol Ellison sampling for *Doassansia niesslii* in France

15.2 *Bagous validus* (Col.: Curculionidae)

We had already given up working with this weevil, because we were never able to find larvae in the field. However, we kept some remaining adults on potted flowering rush plants covered with gauze bags. In spring 2019, we found over 20 weevils on a plant, onto which five adults had been released in June 2018. This means, the weevils must have developed on the plant. The weevils were setup on new plants in May 2019 and we finally found in July a few first instar larvae mining just underneath the surface of rhizomes. A few eggs were found in leaf bases but also in the rhizomes. Additional plants will be checked in August to identify the exact feeding niche and potential impact of larger larvae.

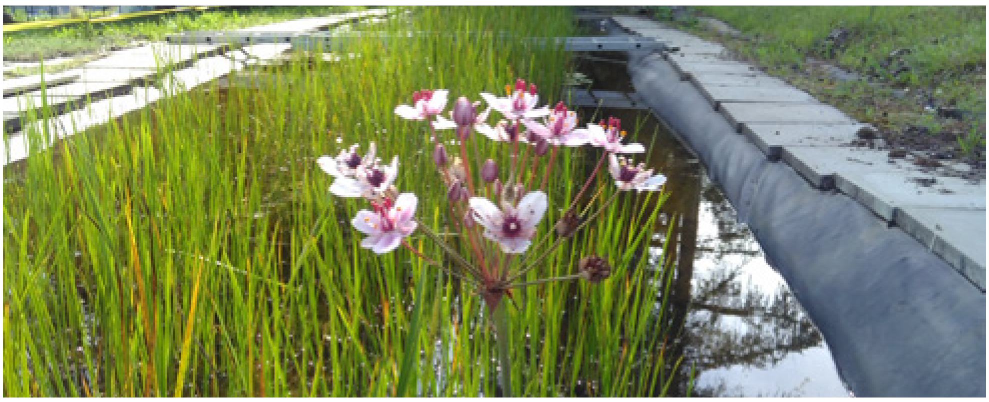
15.3 *Phytoliriomyza ornata* (Dipt.: Agromyzidae)

From 192 overwintered pupae obtained from rearing and collected in 2018, 125 flies emerged between 27 April and 18 May. From 25 pairs setup on potted plants for rearing, we obtained only 110 pupae, wherefrom 50 where transparent and emerging the same year. The low rearing success is probably due to the exceptional long cold period in May. We also setup 27 pairs on 11 test plant species for no-choice development tests. So far, no signs of development were found on test plants.

15.4 *Doassansia niesslii* (Fungi, Doassansiaceae)

Good progress has been made this year concerning the elucidation of the life cycle of the white smut. The asexual sporidia produced in culture are able to infect plants growing completely underwater. In addition, spray application of the sporidia to aerial plant parts produces severe infection and plant death. These results open the opportunity to develop the pathogen as a mycoherbicide.

The white smut isolate we are currently working with is only able to successfully infect genotype 2 (from British Columbia, Canada). However, two new isolates have now been collected and are being tested against the most common genotype 1 (from South Dakota, Wisconsin and Montana States), plants of genotype 4 (from Maine, Ohio and New York States) and genotype 5 (from Minnesota State); results are pending. We also established further host-specificity tests with eight plant species; no symptoms developed on any of the test plants.



Artificial pond for rearing of *Butomus umbellatus*

16 Yellow floating heart (*Nymphoides peltata*)

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In 2019, we started work on *Nymphoides peltata*, yellow floating heart, with support from the Army Corps of Engineers. *Nymphoides peltata* is an introduced floating-leaved plant native to Eurasia and has been present in the U.S. since 1882. It occurs in at least 27 states in all regions of the U.S. and is listed as noxious in six states. While quite abundant in Europe, it is not clear whether the plant is truly native or naturalized. For instance, although the species is protected in many parts of Europe, it is considered invasive in Hungarian fish ponds. In contrast, it is widely accepted that Asia is part of the native range. Impacts from dense infestations come from competition with beneficial species for nutrients, shading of submersed species, reduction of dissolved oxygen and reduced habitat quality for aquatic animals, reduced boating and recreational access, and reduced water flow.

Flowering *Nymphoides peltata*

In order to clarify the origin of *N. peltata* in the U.S., DNA samples from Europe and Asia are being collected. We already sampled sites in Switzerland, Hungary, Germany, the Netherlands and Kazakhstan this spring, and will send samples to the U.S. for molecular characterisation. In addition, we contacted collaborators throughout Europe and western Asia to help with the sampling of DNA material.

No monophagous herbivores were found on *N. peltata* in the European literature. First dissections of a Swiss and two Dutch population only revealed a few Chironomid fly larvae and a few holes in petioles and rhizomes so far.

Plants growing in a dry channel in the Netherlands

Site of *Nymphoides peltata* in Oldenstadt, Germany

The rust *Puccinia menthae* on lesser calamint in southern France

Lepidopteran larva found in the roots of lesser calamint in southern France

17 Lesser Calamint (*Calamintha nepeta,* Syn.: *Clinopodium nepeta*)

Sonja Stutz and **Mariel Guala**

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Lesser calamint is an aromatic perennial herb that has been introduced to New Zealand from Europe. Currently present on the east coast of the North Island, lesser calamint is considered an emerging weed; it is affecting desirable pasture species and having negative economic impacts. Since no effective control methods are currently available, CABI Switzerland was asked in 2018 to conduct surveys for potential biological control agents and collect plant material for DNA analysis to help clarify the area of origin of lesser calamint in New Zealand.

17.1 Field surveys in southern France

From 7 to 10 May, we conducted a field trip to southern France. During this trip, we surveyed five sites that we had already visited in September 2018 and three newly identified sites. At two of the sites, we found lepidopteran larvae feeding in the roots which are likely the olygophagous sesiid moth *Chamaesphecia aerifrons*. In addition, we observed empty mines in the roots collected from several other sites that were most likely caused by the same species. In addition, we found leaf mines most likely caused by the oligophagous buprestid *Stachys menthae* and by the narrowly oligophagous nepticulid moth *Trifurcula saturejae* at several sites. Furthermore, we detected two plants infested with the rust *Puccinia menthae* at one site. *Puccinia menthae* is reported to infest a wide range of plants in the family Lamiaceae. However, more host specific strains might exist.

Immature stages were reared to adult or placed in ethanol and will be sent to specialists for morphological and/or molecular identification. We also collected leaf samples for DNA analyses from all new sites.

17.2 Field surveys in southern Spain

From 13 to 18 June, we conducted a field trip to southern Spain where we surveyed a total of seven sites. In Spain, the plants were more difficult to find and they are most likely a different (sub-) species than in southern France. We found leaf mines most likely caused by *T. saturejae* at all of the sites. In addition, we found leaf mines most likely caused by *S. menthae* at two sites, root mines (potentially caused by *C. aerifrons*) at three sites and scale insects (potentially of the family *Margarodidae*) at three sites. Immature stages were reared to adult or placed in ethanol and will be sent to specialists for morphological and/or molecular identification. We collected leaf samples for DNA analyses from all sites.

17.3 Opportunistic surveys in Italy, Greece and France

Opportunistic surveys were conducted by Massimo Cristofaro, Francesca di Cristina and Michèle Guedj from BBCA. Leaf samples for DNA analyses were collected from ten sites in Italy, four sites in Greece and three sites in southern France. In Greece damage by a stem-mining insect was observed at one site, which may be caused by *Pempeliella sororiella*, a pyralid moth with an unknown host range that has previously also been found in southern France. In addition, a large number of a small leaf-beetles (probably of the subfamily Cryptocephalinae) was found at one site in Sicily. Some of the beetles were preserved in ethanol for morphological and/or molecular identification.

Summer student Mariel Guala inspecting lesser calamint in southern France

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

Marion Seier, **Daisuke Kurose**, **Corin Pratt**, **Kate Constantine**, **Kate Pollard**, **Sarah Thomas** and **Nikolai Thom**

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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.

Severe leaf curling damage on Japanese knotweed in Niigata Prefecture in the northern regions of Japan

18.1 *Aphalara itadori* (Hemiptera, Psyllidae)

A survey was conducted to collect fresh stock of the Kyushu strain as well as new strains of psyllids in the central and northern part of Japan in June 2019. In the first part of the survey many adults were collected at Mt. Aso, Kyushu Island. In the central part of Japan, adults were collected at Haruna-san, Gunma Prefecture (Gunma strain) where psyllids had been observed inflicting severe damage to knotweed in 2006 but this impact was not observed this time. However, extensive and severe leaf curling damage attributable to psyllid nymphs was found in two populations in Murakami, Niigata Prefecture, in the northern regions of Japan (Honshu North strain). This collection area has a much better climatic match to the invaded range. All strains of psyllids and the curled leaves with nymphs were brought back to the Egham quarantine facility to establish cultures and remove predators and parasitoids. The next step will be to carry out host-range tests with the Honshu North strain against five prioritised closely related species of particular relevance to Canada. Once host-specificity has been confirmed, the performance of these strains will be compared under controlled conditions.

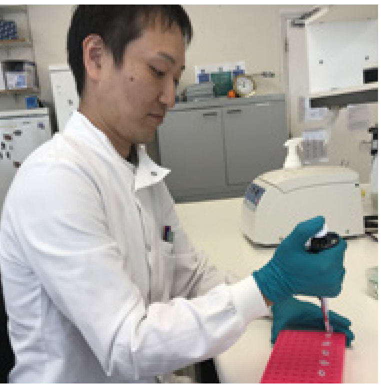
18.2 *Mycosphaerella polygoni-cuspidati* (Fungi, Mycosphaerellaceae)

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.

Disease severity caused by the *Mycosphaerella polygoni-cuspidati* on Japanese knotweed, 3 weeks after mycelial spray application;
A) concentration 1 x 105 fragments/ml,
B) 1x107 fragments/ml.

Continued studies to quantify the impact of *M. polygoni-cuspidati* on Japanese knotweed plants when applied as macerated mycelial broth of different concentrations confirmed previous results. Faster and more pronounced disease development was recorded for higher mycelial concentrations of 1 x 107 fragments/ml derived from leafspot cultures less than 6 months old. Post-inoculation ambient humidity was confirmed to be an important factor for disease symptom expression on Japanese knotweed. Inoculation with *M. polygoni-cuspidati* tend to stimulate the production of new shoots linked with an increased length of and number of leaves on these shoots in treated Japanese knotweed plants. This suggests that the pathogen interferes with shoot apical dominance and thereby activates the growth of rhizome buds. If approved by the respective UK authorities limited experimental field trials using a single mating-type isolate of the pathogen will be conducted in the grounds of CABI in Egham during summer 2019. In parallel, collaboration with private industry is pursued in order to take this mycoherbicide initiative further.

Daisuke Kurose during field surveys in Japan to collect the psyllid *Aphalara itadori*.



Daisuke Kurose undertaking molecular analysis at CABI UK

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

Carol Ellison, **Sonal Varia**, **Daisuke Kurose** and **Kate Pollard**
Email: **c.ellison@cabi.org**

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

A strain of the rust was approved for release in the UK in 2014 and initial field results were highly variable; infection levels on Himalayan balsam were much lower than anticipated at a number of sites and at some sites the plant populations appeared to be resistant to the pathogen. In response to this, an isolate from Pakistan (held in CABI's liquid nitrogen facilities) was selected for virulence testing against Indian rust-resistant weed biotypes. It became clear that the two strains of rust infect a different range of UK populations of Himalayan balsam. The host-range of the Pakistani strain was confirmed using a number of closely related *Impatiens* species and permission to release this strain from quarantine was approved by Defra in January 2017. Using results from biotype testing, the appropriate rust strain for each population was released at each field site. However, some populations are resistant to both rust strains.

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. In July, two important native *Impatiens* species, *Impatiens aurella* and *Impatiens ecornuta* were imported into CABI's quarantine facilities for assessment. The full six replicates of each species have been inoculated using both rust strains and plants are being closely monitored for any symptom development. Six Canadian populations of Himalayan balsam from BC have also been assessed for their susceptibility to 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). The strain from India was found to infect some of the Canadian populations, but these populations were only weakly susceptible. Additional strains of the rust are required to have an adequate impact on Canadian populations. Rust pathogens can demonstrate intraspecies specificity so that for biocontrol to be successful, it is essential that plant genotypes are matched to the most virulent rust isolates; broadly, from areas in native range from where the original seed were collected.

A molecular study that included samples of Himalayan balsam from the native and introduced range in the UK and Ireland was undertaken in 2017. Phylogenetic analyses of six chloroplast DNA sequences concluded that the plant has been introduced into the UK on at least three separate occasions, from different locations within the native range. The results have also identified where surveys to collect additional strains of the rust for resistant UK weed populations need to focus in the native range. Samples from seven Canadian populations have also been incorporated into this study. UK populations can be placed into 10 haplotypes and these into three main groups. The results from the Canadian samples suggest that there are two haplotypes present in Canada; five of the populations belong to haplotype E and two to haplotype J. These are the two most common haplotypes present in the UK, suggesting that the Canadian populations originated from here. Surveys are underway in the native range to collect new rust strains and the Canadian populations will be screened with the strains, as they become available.

The rust fungus *Puccinia komarovii var. glanduliferae*

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