CABI

Weed Biological Control

Progress Report 2020

CABI in Switzerland
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www.cabi.org
KNOWLEDGE FOR LIFE

Contents

Cover photos: Cornelia Cloşca collecting *Ceutorhynchus rusticus* on dyer's woad in fall 2019; Oliver Krstić potting yellow toadflax plants galled by the weevil *Rhinusa pilosa*; Madeleine Hiscock placing Vincetoxicum plants infested with *Chrysochus asclepiadeus* in garden bed.

Inside cover: Garden beds at CABI Switzerland

Next page: Patrick Häfliger explaining work to the two summer students Mario Rodriguez and Sandrine Fattore

Notes from the section leader

Hariet L. Hinz

First of all, I hope that you and your families and friends are safe and sound!

I realize that many of you have not seen your labs or offices since several months and are mostly working from home. During these unprecedented times we are fortunate that our operation is located in Switzerland and moreover in a relatively remote area where cases have been amongst the lowest within Switzerland. The local government reacted very fast but at the same time found a good compromise of keeping respective measures as strict as necessary, but at the same time as flexible as possible. All this together allowed us to continue our work, though with a reduced number of staff and students, while still protecting everybody's health and well-being.

As I'm sure is true for many of you and your work, processes and behaviours had to be adapted in a short period of time and official instructions varied and changed nearly daily in the beginning, which was challenging. We were not able to take on any of our planned summer students from North America or other non-European countries, and in addition we greatly reduced the number of students to take on. We were very lucky to quickly find very good 'replacements', mostly from Switzerland, plus two Italian students who started their time in Switzerland with a two-week quarantine period! Project scientists did more of the practical work themselves, since they were not able to conduct any of the planned field trips in April and May. Luckily enough, we have a lot of the candidate agents we work with in rearing at the Centre, which greatly helped to have a nearly 'normal' field season in the end. Despite this you will still often read that certain things could not be conducted or did not work out as planned, because of COVID-19. Unfortunately, also quite a few exchange visits planned for 2020 could not be conducted and of course all conferences were postponed to 2021.

Parallel to the epidemic the extremely sad news reached us that Carol Ellison, our colleague from the CABI UK Centre, passed away. Carol was an extremely experienced and passionate plant pathologist, and a very nice colleague to work with, who will be dearly missed. Carol was involved in the flowering rush project; in the meantime her colleagues Sarah Thomas and Daisuke Kurose took over her responsibilities.

And in the following some project news:

The lesser calamint project for New Zealand was completed last year. After 1½ years of field surveys in southern Europe, 32 insect species, one mite and one fungal pathogen were found. Five of these species were prioritized to have potential as biological control agents, i.e. a leaf beetle, a nepticulid moth, a gall midge, an eriophyid mite, and a rust. A report summarizing the information was prepared and submitted to Landcare New Zealand.

We had planned to send eggs and adults of the syrphid fly *Cheilosia urbana* permitted for invasive **hawkweed control** to Jeff Littlefield (Montana State University) this spring for first releases in the US. However, this was unfortunately not possible due to COVID-19, so releases will have to wait until next year.

Similarly, further surveys on the aquatic invasive *Nymphoides peltata* for the Army Corps of Engineers will most probably have to be postponed to 2021 due to COVID-19 travel restrictions.

We are still waiting for news on release permits for the root weevil *Ceutorhynchus scrobicollis* on **garlic mustard** and the two noctuid moths for common reed control for the U.S. The petition for field release of the eriophyid mite *Aceria angustifoliae* for **Russian olive control**, jointly submitted by Tim Collier (University of Wyoming) and Rosemarie De Clerck-Floate (Agriculture and Agri-Food Canada), was recommended by the USDA, APHIS, Technical Advisory Group (TAG) in May 2020. And finally, the petition for the gall-forming weevil *Ceutorhynchus cardariae* on **hoary cress** was re-submitted to TAG together with Mark Schwarzländer (University of Idaho) in January 2020.

For further details on recent releases and petitions please find the link to a blog **here**.

Apart from that we have started three new smaller initiatives. Two are supported by the British Columbia Ministry of Forests, Lands, Natural Resource Operations & Rural Development. One of these is looking into the feasibility of biological control of the aquatic invasive **parrot's feather** (*Myriophyllum aquaticum*), which has been successfully controlled in South Africa. And the second is to support ongoing studies on a mite for biocontrol of **tree-of-heaven**, mostly conducted by our colleagues at **BBCA**. The third is supported by the Centre for Biological Control (CBC) in South Africa and looks at potential agents for **black locust** (*Robinia pseudoacacia*).

In conclusion, considering the circumstances, we had a successful first half of 2020 and I am pleased what my colleagues were able to achieve despite the restrictions COVID-19 brought with it.

Enjoy reading, and take care!

Hariet L. Hinz
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Ivo Toševski (left) and Oliver Krstić (right) preparing *Rhinusa pilosa* for hand carrying to Switzerland and subsequent shipment to North America

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)

On 21 February 2020, adults of the gall-forming weevil *R. pilosa* on yellow toadflax were hand carried from Serbia to Switzerland for shipment to North America. On 24 February, two shipments were organized, 1050 *R. pilosa* were sent to Rosemarie De Clerck Floate, Agriculture and Agri-Food Canada in Lethbridge, and 1450 adults to Sharlene Sing to the quarantine facility at Montana State University, USA. Both shipments contained four different mitochondrial genetic lineages aligned in four haplotype groups of the weevil.

A total of 14 mitochondrial haplotypes of *R. pilosa* were kept in hibernation in Serbia for rearing in spring 2020. However, severe lockdown measures due to COVID-19 from 15 March to 6 May, interrupted the planned work. To mitigate a complete loss of our rearing colonies, hibernation of the majority of the weevils was abruptly interrupted, and males and females from particular haplotype were set up for copulation. A total of 43 framed cages were planted with yellow toadflax, and weevils released, separately per haplotype. However, the usual careful preparation and selection of females for successful egg laying could not be conducted due to time constraints. This, coupled with an interruption of hibernation resulted in a high mortality of the exposed adults, and only 18 of the 43 cages yielded galls (total of 675 galls of 11 different haplotypes). All galled plants were dug up and planted in pots. In mid August, we are planning to dissect all plants for developed adults.

An additional 23 cages were set up on 1 June, with the still alive weevils that had been left in the overwintering incubator. Of the 120 remaining specimens, all belonging to haplotype A1, 23 females were successfully copulated and set up for rearing. Some females are currently still active, and results of this late rearing will only be available in early September.

On 15 May, a total of 101 *Rhinusa rara* galls were collected from *L. dalmatica* and *L. genistifolia* at two sites in eastern Serbia. The galled plants were individually planted and between 20-24 July, a total of 544 adults of *R. rara* were extracted.

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

Rearing of *Mecinus laeviceps* and *M. peterharrisi* had to be cancelled because of COVID-19 measures. To sustain a *M. heydenii* population, some 150 adults were released on 15 March on a yellow toadflax population established at the Institute of Plant Protection and Environment in Serbia. These plants will be dissected for adults in mid August. The adults of *M. laeviceps* and *M. peterharrisi* will be collected from field populations to re-establish a laboratory rearing.

Ivo Toševski collecting yellow toadflax plants infested with *Rhinusa pilosa*

2 Houndstongue (*Cynoglossum Officinale*)

Cornelia Cloşca, **Philip Weyl** and **Hariet L. Hinz**

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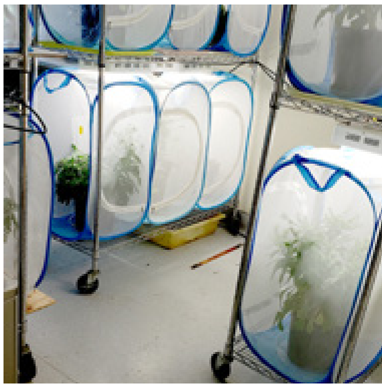
Current work on houndstongue is focused on the seed-feeding weevil *Mogulones borraginis.* It has proven to be the most specific agent acting on houndstongue thus far. CABI Switzerland is maintaining a rearing of the weevil in view of future field releases since *M. borraginis* is extremely rare in the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

From over 2400 *M. borraginis* larvae collected in 2019, about 100 adults emerged in October 2019 and an additional 1000 in March 2020 which were set up on houndstongue rosettes. At the beginning of June, about 100 females were retrieved and reset on 39 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 more than 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 2020 and spring 2021.

On two occasions (January and June 2020), at total of 500 adults of *M. borraginis* were sent to the quarantine facility at the University of Idaho (UoI) run by Mark Schwarzländer. Currently work has focussed on developing the most effective way to rear *M. borraginis* under quarantine conditions to maximise space and number of adults. Between 15 June and 24 July over 1200 mature larvae were collected from these plants. Currently, all fruits are being dissected to analyze the impact of the weevil and the relative productivity of the three rearing setups tested.



Different cage setups to determine the most efficient rearing methods for *Mogulones borraginis* in the quarantine facility at University of Idaho

Waiting for mature larvae of *Mogulones borraginis* to leave the fruits

PhD student from University of Idaho, Anjila Thapa, developing rearing methods of *Mogulones borraginis* suitable for quarantine conditions

3 Hawkweeds (*Pilosella* spp.)

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Gall of *Aulacidea pilosellae* with emergence hole

3.1 *Aulacidea pilosellae* (Hym., Cynipidae)

Two biotypes of this gall wasp are being investigated. The first biotype is *A. pilosellae* ex *Pilosella* spp. which we have been collecting in the northern range (eastern Germany, Poland and the Czech Republic) and we are currently testing a population from Poland. 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 Agriculture and Agri-Food Canada in Lethbridge showed that the second biotype carries Wolbachia, which is thought to be responsible for the biotype differentiation. Batches of galls harvested from lab infested plants in 2019 have been taken out of cold storage regularly since 30 April. Adults emerging from these are being used in host range tests and for rearing.

In 2020, emergence rates of *A. pilosellae* ex *Pilosella* spp. have generally been good (on average above 50%) and the sex ratio was well balanced. We exposed 14 test plant species (10 North American (NA) natives) and five *Pilosella* species in no-choice tests, and conducted single-choice tests with two native *Hieracium* species previously attacked under no-choice conditions.

Adult of *Aulacidea pilosellae*

Emergence rates for *A. pilosellae* ex *P. officinarum* have been very variable. This is probably because temperatures fluctuated during development in 2019, which led to the harvest of galls at different developmental stages, and even the emergence of some adults during 2019. Five test plant species (two NA natives) were exposed to this biotype in no-choice tests.

Gall harvest for both species is ongoing. So far, galls were only found on *P. caespitosa* and *P. officinarum.* Final results will be presented in the annual report.

Of the 80 species, subspecies and populations tested with *A. pilosellae* ex *P. officinarum*, and 55 with *A. pilosellae* ex *Pilosella* spp., galls were only found on the genus *Pilosella* and *Hieracium. Hieracium argutum* was the only test plant attacked when exposed to *A. pilosellae* ex *P. officinarum* in choice tests exposing a plant test together with the natural host (control). In 2020, plants of this NA native test plant were not in good condition and additional choice tests could therefore not be conducted.

3.2 *Cheilosia urbana* (Dipt., Syrphidae)

In 2020, the syrphid fly *C. urbana* was observed in the CABI garden from 15 April onwards. We were not able to collect a large enough number of adult flies due to unsuitable weather conditions at a critical time for potential shipment. Moreover, the fly is short lived when in transit and eggs hatch after only a few days. The risk of a shipment being delayed during the COVID-19 disruptions was too risky and access to laboratory was restricted for our collaborators in Canada and the USA. We therefore decided to postpone shipments to for field releases until 2021.

Summer student Madeleine Hiscock counting galls of *Aulacidea pilosellae* on plants in a single-choice test

4 Russian Knapweed (*Rhaponticum repens*)

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4.1 *Pseudorchestes sericeus* (Syn: *P. distans*) (Coleoptera, Curculionidae)

During surveys in 2019 of the Ile River Valley in south western Kazakhstan, the jumping weevil, *Pseudorchestes sericeus* was collected. We have initiated a rearing of this weevil in CABI's quarantine facility in Switzerland and are in the process of studying its biology and host range.

Under laboratory conditions (25˚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 on average of 3.3 eggs per day for at least eight weeks into the parenchymatic tissue 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. The effect of the larval mining usually kills a large portion the leaf. The adults emerge and feed voraciously on Russian knapweed, especially during the pre-oviposition period of 10-14 days. The combined effect of larval mining and adult feeding can have a substantial effect on plant productivity and we have noted plant mortality in quarantine with high densities of the weevil.

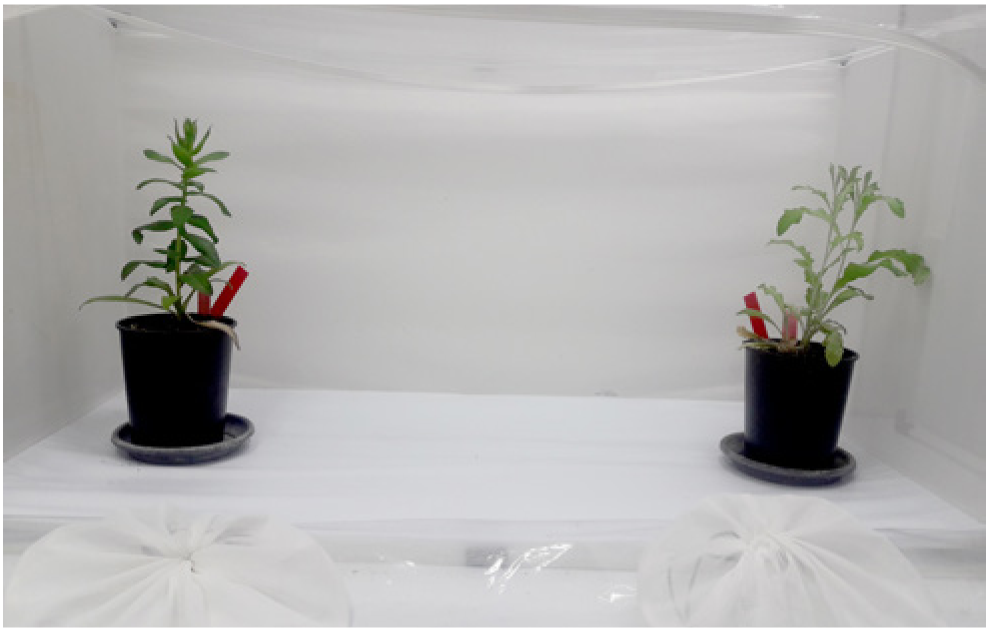
The host range testing this spring and summer has been successful and so far we have been able to screen 23 test plant species. Of these, only four species in the tribe Cardueae (same as Russian knapweed), were accepted for oviposition and adults developed on three, but always to a lower degree than on the control. In subsequent choice tests we found a clear preference for Russian knapweed with 5-10 times more larvae on the control than on the test plants. It is not unusual in small cages, under quarantine conditions to have some non-target attack, thus, an open-field test will be necessary to fully understand the risk that this weevil might pose to the attacked non-target species. The host range testing is ongoing, but, these initial results are very encouraging.

4.2 Additional surveys

Unfortunately, due to COVID-19 travel restrictions, all field surveys for additional agents have been put on hold.

Clip cages used in the no-choice test with the weevil to confine individual, egg laying females to a plant, in this case, *Centaurea americana*

Adult of *Pseudorchestes sericeus*



Choice-test setup in quarantine to investigate the oviposition behaviour of *Pseudorchestes sericeus* females when offered Russian knapweed (right) and the North American native *Centaurea americana* (left), simultaneously

5 Garlic Mustard (*Alliaria petiolata*)

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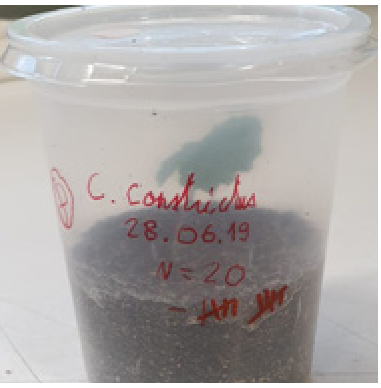
Mating pair of *Ceutorhynchus constrictus*

Between 18 February and 23 March 2020, 172 adults of the seed-feeding weevil *Ceutorhynchus constrictus* emerged from our rearing colony established in 2019. Because of COVID-19 disruptions (shipment delays and restricted access to the lab at the University of Minnesota), no adults were shipped to Dr. Katovitch (UMN) in spring 2020. After testing for oviposition, all fertile females were used in host range tests with native North American (NA) plants at CABI instead.

In previous no-choice tests, the native NA species *Braya alpina* and *Descurainia nelsonnii* were accepted for oviposition. However, only one mature larva emerged from *B. alpina.* In order to assess whether the weevil can complete development to adult on this species, we infested more plants. Oviposition was only found on one *B. alpina*, but on none of the *D. nelsonnii.* The infested *B. alpina* was kept for larval emergence. This species was also exposed in single-choice tests together with the control garlic mustard*.* Feeding and oviposition were only found on garlic mustard except for limited feeding on one *B. alpina*, which might have been contamination by *Ceutorhynchus contractus.*



Containers for overwintering and emergence of *C. constrictus*

Barbarea orthoceras was accepted for oviposition in previous no-choice tests and once in a single-choice test. Although no mature larvae emerged, the percentage of seeds damaged was relatively high (22%), and we therefore decided to expose the plant together with garlic mustard in an open-field test. During the period of exposure, adult *C. constrictus* were only observed on garlic mustard, and about 60% of the garlic mustard pods were attacked (feeding or oviposition holes) vs 1.4% for *B. orthoceras*. Two eggs were found in *B. orthoceras* pods. However, since their appearance looked suspicious and other weevil species were observed on the plants, the eggs were placed in ethanol and will be sent for molecular analyses to confirm species identity.

In June and July, over 1500 mature larvae of *C. constrictus* were harvested from garlic mustard for adult emergence in spring 2021.

Summer student Anouchka Perret-Gentil preparing the open-field test with *Ceutorhynchus constrictus* in the CABI garden

6 Common Reed (*Phragmites australis*)

Patrick Häfliger, **Cornelia Cloşca**, **Sandrine Fattore**, **Mario Rodriguez**, **Martina Nardelli**, and **Hariet L. Hinz**

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Field release of the two noctuid moths, *Archanara geminipuncta* and *A. neurica,* was permitted for Canada in spring 2019, and first pupae of *A. neurica* reared on artificial diet were released in summer 2019 by Rob Bourchier (Agriculture and Agri-Food Canada, Lethbridge) in a field cage in southern Ontario. Overwintering survival of eggs and larval dispersal will be monitored in 2020. Due to the restrictions related to COVID-19, eggs shipped to Lethbridge in February 2020 could not be used for further testing of rearing on artificial diet. They were used instead to test release methods. Unfortunately, no larvae seem to have hatched, most probably because eggs had to be kept too long in the cold to synchronize hatch with the late onset of spring in Canada. We are now planning to ship eggs to Canada in fall 2020, so that eggs can be placed onto plants before the onset of winter. We hope that this will increase adaptation to local climatic conditions, and that larval hatch will be synchronized with the emergence of common reed stems in spring 2021.

Successful pupation of *A. geminipuncta* transferred as third instar larva onto artificial diet

Since our first rearing trial of *A. neurica* on a McMorran diet worked quite well in 2019, we set up most larvae of *A. neurica* hatching in spring 2020 on diet. However, rearing success was reduced by 50% compared to 2019, probably because larvae hatching from eggs obtained from adults reared on diet were less fit. Nevertheless, 42 adults emerged and we were able to obtain about 1700 eggs that should suffice to rebuild a larger colony in 2021.

Since we were not successful in the past to rear *A. geminipuncta* on artificial diet, we continued our conventional rearing on stem sections of common reed. We obtained over 300 pupae were able to produce over 11000 eggs that will be available to be shipped to the U.S. and to Canada for further rearing trials and releases, respectively.

Summer student Sandrine Fattore setting up oviposition cages for *A. geminipuncta*

In 2019, we were able to show that addition of fresh tissue of common reed, collected mainly above the growing point, greatly improved larval survival of *A. geminipuncta* when reared on artificial diet. In 2020, we therefore transferred over 1200 freshly hatched larvae on artificial diet with the addition of different amounts of common reed (100g/litre and 160g/litre). We also transferred about 100 larvae first reared on reed stems until the third instar to plain McMorran diet and diets with addition of common reed. Many larvae survived and developed up to four weeks on diet, but none survived for longer than six weeks. Only larvae first reared on reed stems and then transferred to diet did quite well and a few even managed to pupate and emerge as adults. We will continue our efforts to try and find a successful method to rear *A. geminipuncta* on artificial diet.

Summer student Martina Nardelli collecting young *Phragmites australis* stems for *Archanara* rearing

Philip Weyl collecting *Ceutorhynchus turbatus* in southern Switzerland

7 Whitetop or Hoary Cress (*Lepidium draba*)

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7.1 *Ceutorhynchus cardariae* (Col., Curculionidae)

In January 2020, the petition for this gall-forming weevil was finally resubmitted together with Mark Schwarzländer (University of Idaho) to the USDA, APHIS Technical Advisory Group (TAG), after completing additional tests, especially with native North American species in the same family as the target weed, hoary cress.

Despite this, we conducted an additional open-field test with two native North American (NA) *Caulanthus* species this spring. These two species had been attacked under field conditions previously and are therefore considered particularly critical. We used a triangular design developed by Mark Schwarzlaender to test whether attack by *C. cardariae* differs if test species are exposed in pure or mixed stands (together with hoary cress). Slightly higher attack occurred on the non-targets when exposed together with hoary cress than in pure stands. Overall, attack on *Caulanthus* species was 10-fold lower than on hoary cress.

We also continue maintaining a rearing of the weevil at the Centre. Of the 1000 adults that overwintered at CABI, about 80% survived. These were set up on potted plants, from which about 1200 *C. cardariae* emerged.

7.2 *Ceutorhynchus turbatus* (Col., Curculionidae)

On 20 May, over 1300 adults of the seed-feeding weevil *C. turbatus* were collected in southern Switzerland. On 21 May, we established no-choice oviposition and development tests with 27 plants; four *L. draba* control plants and 1-5 replicates of nine 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 *Braya alpina*) that had been accepted for oviposition in the past. None of the test plants supported larval development of the weevil, while an average of 3.25 larvae emerged from hoary cress control plants. Obtaining good pod quality in potted control plants remains a challenge and is likely linked to the low number of larvae on the control plants this year.

Triangular design of the open-field test set up with *Ceutorhynchus cardariae* in spring 2020 in the CABI garden

8 Dyer's Woad (*Isatis tinctoria*)

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

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8.1 *Ceutorhynchus peyerimhoffi* (Col., Curculionidae)

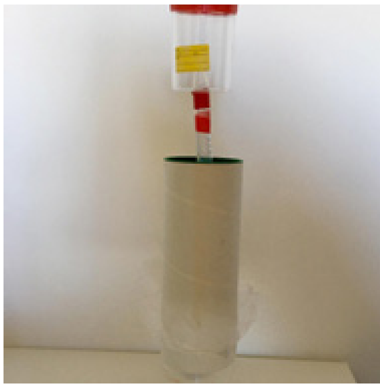
Over 800 adults of the seed-feeding weevil *Ceutorhynchus peyerimhoffi* emerged from our rearing colony this spring. These adults were used in tests or transferred onto flowering dyer's woad plants for rearing. From these, over 3000 larvae were collected and were kept for adult emergence in 2021.

With the host range testing for this weevil coming to an end and the petition for field release being prepared we used this year to complete longevity and fecundity studies with *C. peyerimhoffi*. Between 8 April and 27 May the fecundity of 20 female weevils was tracked. The number the eggs per female is staggering with an average of over 5 eggs per day for the five-week period (average of roughly 200 eggs per female during her lifetime). Of the 20 females, only three died prior to the end of the experiment, while the remaining females survived until 15 June, when there were no more flowering dyers woad plants. Had more flowering plants been available the females were expected to have lived even longer. It appears that longevity and egg laying is linked with plant phenostage, as long as flowers and pods are available the females continue to oviposit.

A typical dyer's wood site site in the Abruzzo mountains where *Ceutorhynchus peyerimhoffi* occurs naturally

We were also able to collect some information on parasitism in the field. Plants with developed pods were collected in the Abruzzo mountains in Italy where *C. peyerimhoffi* occurs naturally and kept for either weevil larvae or parasitoid emergence. No weevil larvae emerged, however, several parasitoids emerged which have been sent for identification. The lack of weevil larvae is interesting, however, it could be that we were too late and all healthy larvae had already exited the pods prior to collection, while parasitoids remained in the pods and emerged later.

In June we sent the remaining egg laying adults of *C. peyerimhoffi* to Mark Schwarzländer at the University of Idaho which were used to collect data on the host finding behaviour of the weevil. We were extremely lucky that this shipment went smoothly and only took 4 days to arrive, but the process was time consuming to organize due to limited flights available. Results so far suggest that naïve females of *C. peyerimhoffi* are either repelled by or reacted with indifference to the two native NA test plant species offered.



The collection method for parasitoids emerging from dyer's wood pods attacked by *C. peyerimhoffi*. Seed pods are placed into a cardboard cylinder with two collection tubes, one at the bottom for weevil larvae and one at the top for emerging parasitoids

8.2 *Ceutorhynchus rusticus* (Col., Curculionidae)

A total of 70 plants, from 15 test plant species and the control were exposed to *C. rusticus* in no-choice oviposition and development tests in autumn 2019. Test plants were regularly checked and any dying plants were dissected. Adult emergence was successful in 2020, with about 65% of the dyer's woad control plants supporting adult emergence with an average of 4.8 adults per plant. Of the 15 test plant species, we recorded typical *C. rusticus* mining in three test species in the genus *Streptanthus,* however, none were able to support adult development.

Philip Weyl and Cornelia Cloşca setting up the fecundity and longevity test for *Ceutorhynchus peyerimhoffi*

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

9 Perennial Pepperweed (*Lepidium latifolium*)

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9.1 *Ceutorhynchus marginellus* (Col., Curculionidae)

We continued to maintain a rearing colony of the gall-forming weevil *Ceutorhynchus marginellus* in the quarantine facility at CABI. From the 223 adults that were kept in incubators set at 2–3°C during winter 2019/2020, 68 females and 39 males survived until March 2020. These weevils were transferred onto 41 potted perennial pepperweed plants to continue our rearing. More than 1000 weevils emerged from these plants. They are currently being kept in cylinders and regularly fed with leaves of perennial pepperweed or were placed onto potted perennial pepperweed plants.

9.2 *Lasiosina deviata* (Dip., Chloropidae)

A field trip was planned to collect perennial pepperweed stems infested with the fly *Lasiosina deviata* in Kazakhstan in May 2020. However, the trip could not be conducted due to COVID-19 travel restrictions, and therefore no work could be conducted with this species.

Summer student Martina Nardelli maintaining the rearing of *Ceutorhynchus marginellus* in the CABI quarantine

10 Swallow-Worts (*Vincetoxicum* spp.)

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


Adult of *Chrysochus asclepiadeus*

The root-mining beetle *Chrysochus asclepiadeus* can develop and emerge as adult after one to three years. We are currently maintaining a rearing colony originating from a French population. In 2020, adults only emerged from plants infested in 2018 (mostly *Vincetoxicum rossicum*). These produced few eggs and mortality was relatively high. In July 2020, we therefore field collected additional adults in Franche-Comté, France. Laid eggs will be harvested regularly and will be used to continue no-choice larval transfer tests.

Plants exposed to adult *C. asclepiadeus* in an open-field test in summer 2018 were searched for larvae in spring 2019. In 2019 and 2020, 39% of larvae found on the control plants (*V. hirundinaria*) successfully developed into adults. None of the exposed native North Amercian (NA) non-targets (*Asclepias incarnata*, *A. syriaca,* and *Apocynum cannabinum*) were attacked.

All plants used in no-choice larval transfer tests in 2019 were searched for larvae in spring 2020. Of the ten test plant species (eight NA natives) and *V. hirundinaria*, and *V. nigrum* as controls, larvae were found on about 60% of the controls and a single larva was found on the native NA *Asclepias syriaca.* However, *A. syriaca* was not attacked under open field conditions during the test set up in 2018.


Eggs of *C. asclepiadeus*

Results so far confirm the narrow host range of *C. asclepiadeus* under field conditions.

10.2 *Euphranta connexa* (Dipt., Tephritidae)

About 750 pupae of the seed feeding fly collected in summer 2019 were overwintered at CABI. Starting beginning of May 2020, batches of about 80-160 pupae were taken out of cold storage every few weeks and first placed into an incubator at 10°C, and then stored in the lab for adult emergence. The containers are regularly checked and mouldy pupae removed. So far, the adult emergence rate has been quite high and no parasitoids emerged. Due to limited funding, we will only be able to conduct a limited number of tests this year. However, we are still planning to collect infested pods in August for potential additional host range tests in 2021.


Summer students Martina Nardelli and Madeleine Hiscock field collecting *Chrysochus asclepiadeus* in France

11 Common Tansy (*Tanacetum vulgare*)

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Tanacetum camphoratum plant exposed in the open-field test with *Microplontus millefolii*

11.1 *Microplontus millefolii* (Col., Curculionidae)

Work on the shoot-mining weevil *Microplontus millefolii* is being conducted in Russia by Dr Margarita Dolgovskaya and her team (Russian Academy of Sciences, Zoological Institute, St Petersburg). In June, a total of 75 females and 45 males were collected in the field. In addition, 11 females and ten males emerged from a rearing plot set up in 2019.

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 open-field test was set up with the two native North American (NA) species, *Tanacetum camphoratum* and *T. huronense*. Sixteen potted plants of each of the two NA natives as well as of *T. vulgare* were exposed on a meadow and a total of 49 females and 47 males were released. The plants are currently being dissected. Detailed results will be presented in the annual report. In addition, stems infested with *M. millefolii* were collected in the field to try and set up a rearing colony.

Adult of the leaf-feeding beetle *Chrysolina eurina*

11.2 *Chrysolina eurina* (Col., Chrysomelidae)

In spring 2020, we evaluated the single-choice cage test that had been set up in October 2019 with the two native NA species *Tanacetum camphoratum* and *T. huronense* and *T. vulgare* as control. On average two and 21 times more larvae or newly developed adults were found on *T. vulgare* than on *T. camphoratum* and *T. huronense*, respectively. These results show that this leaf feeding beetle prefers *T. vulgare* over the native *Tanacetum* species, but indicate that especially *T. camphoratum* may be attacked should *C. eurina* be released as a biocontrol agent in North America.

Set up of open-field test conducted with *Microplontus millefolii* in Russia

12 Russian Olive (*Elaeagnus angustifolia*)

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12.1 *Aceria angustifoliae* (Acari: Eriophyoidae)

Studies with the mite, *Aceria angustifoliae* suggest that this species is extremely specific and appears to have a significant impact on the reproductive output of Russian olive under natural field conditions. The petition for field release was submitted to both the U.S. and Canada in November 2019. In May 2020, both the USDA, APHIS Technical Advisory Group (TAG) and the Canadian Biological Control Review Committee recommended release of the mite. However, the Canadian Food Inspection Agency did not approve its field release, mainly based on concerns over the lack of experimental impact data. We are in the process of reviewing the comments of the reviewers and plan to submit additional information to address the points raised and hopefully fill in any gaps which may sway the original decision.

Deformed leaves and flowers of Russian olive showing the potential impact of the eriophyid mite *Aceria angustifoliae*

12.2 Additional surveys

In October 2019 we collected seeds of Russian olive from five sites along the Ile river and one site in the upper catchment of the Karatal River to the west of the Ile river, in Kazakhstan. The seeds were maintained at winter temperatures in an incubator over the winter and removed in early April 2020 for the emergence of any seed attacking insects. Unfortunately, no insects emerged and a total of 300 seeds per site were subsequently dissected. None of the seeds had any signs of insect life or damage. This suggests that either October may have been too late for collection and any damaged seeds would have dropped to the ground or that this region of Kazakhstan is depauperate of seed attacking insects. Unfortunately, due to COVID-19 travel restrictions, all field surveys for additional agents have been put on hold.

Philip Weyl (CABI) and Francesca Marini (BBCA) collecting seeds from Russian olive in Kazakhstan in search of seed attacking insects

Adult of *Dichrorampha aeratana* on oxeye daisy

Adult of *Cyphocleonus trisulcatus* on oxeye daisy

13 Oxeye daisy (*Leucanthemum vulgare*)

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13.1 *Dichrorampha aeratana* (Lep., Tortricidae)

In May, we set up additional no-choice larval development tests with test plant species that are either native or of value as horticultural plants in Australia. All plants will be dissected for larvae in autumn. In addition, we set up a multiple-choice cage test with the Australian native *Cotula cotuloides*, the ornamental *Mauranthemum paludosum* and the medicinal herb *Tanacetum parthenium*, plant species that had supported larval development in previous tests. The plants were exposed to egg-laying females in May or June and will be dissected for larvae in autumn. We also sent eggs of this root-mining moth to Dr Andrew McConnachie at the New South Wales Department of Primary Industries, Australia in May. Since the shipment took 12 days to arrive, all eggs developed to larvae during transit. Nevertheless, 20 % these larvae survived and could be transferred onto plants.

13.2 *Dichrorampha consortana* (Lep., Tortricidae)

A field trip that was planned to re-collect larvae of *D. consortana* in Norway in mid-May had to be postponed to 2021 due to COVID-19 travel restrictions. As a consequence, we could not continue with the host-range testing with this species. However, two females and two males emerged from the four rearing plants that had been set up in 2019. Both females laid eggs and the emerging larvae were transferred to *L. vulgare* and *L. ircutianum* plants. The plants will be dissected at regular intervals to follow the larval development.

13.3 *Oxyna nebulosa* (Dipt., Tephritidae)

In May and June, we harvested galls from the oxeye daisy and Shasta daisy plants that had been exposed to adults of the root-galling tephritid fly *Oxyna nebulosa* in 2019. Between early June and mid-July adults emerged from the galls and were set up in no-choice oviposition and larval development tests with several test plant species and varieties of importance to North America (NA). 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 2021. In addition, we set up a multiple-choice cage test with the NA native *Achillea borealis*, the ornamental *A. ptarmica* and with *Tanacetum vulgare.* The plants were exposed to egg-laying females in late June and will be dissected for larvae in autumn.

13.4 *Cyphocleonus trisulcatus* (Col., Curculionidae)

In May and June, we set up no-choice oviposition and larval development tests with 21 test plant species and varieties that are either native or of horticultural value in Australia. The test plants are currently being dissected for larvae while the control plants are being kept for adult emergence. So far, larvae were only found on *Leucanthemum* x *superbum* (Shasta daisy), *Achillea ptarmica* and *Tanacetum parthenium*, three species on which larvae had already been found in previous years. In May, we sent adults of this root-mining weevil to Dr Andrew McConnachie in Australia, which surprisingly arrived all alive despite the long transit (see above).

Martina Nardelli preparing the multiple-choice cage test with *Dichrorampha aeratana*

14 Field Bindweed (*Convolvulus arvensis*)

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14.1 *Melanagromyza albocilia* (Dip., Agromyzidae)

The shoot and root mining fly *Melanagromyza albocilia* shows promise as a potential biological control agent for field bindweed. However, our attempts at conducting host range tests under confined and semi-natural conditions yielded low success. We are therefore conducting tests under natural conditions at a site where the fly occurs naturally. Open-field tests have been successfully conducted in southern Germany since 2017. Just in time to set up another open-field test, the border to Germany re-opened in mid-June! Six test plant species (five North American (NA) natives and one ornamental) and *Convolvulus arvensis* as the control were planted into the soil in ten blocks at the edge of a maize field. Plants were watered once a week and fitness was recorded. The plants were harvested after four weeks. Wild *C. arvensis* plants growing close to the exposed test plants were also harvested to determine the natural attack rate at the edge of the field. Dissections are ongoing and detailed results will be presented in the annual project report.

One block of plants in the open-field test with *Melanagromyza albocilia* in southern Germany

14.2 *Microsphecia brosiformis* (Lep., Sesiidae)

Larvae of this root-mining sesiid moth have been shown to be very destructive and we therefore put much hope in it as a potential biocontrol agent.

Most of the work is currently being conducted by our colleague and sesiid specialist Ivo Toševski in Serbia. Since the species is hard to collect in the field, Ivo tried to establish a rearing colony in 2019, by transferring eggs onto specially prepared field bindweed plants under common garden conditions. The rearing was successful, however, so far mostly males (n=15) and only three females emerged and only one of the females laid eggs. We are hoping that more females will emerge during August.

Potted plants of eight test species (seven NA natives) and *C. arvensis* were similarly infested with eggs in 2019. All plants were dissected in June 2020. None of the test species were found to be attacked, while nine out of ten field bindweed roots were mined and six larvae were found. This confirms, so far, the very narrow host range of this species. Egg transfer tests are currently being continued.

Calystegia soldanella onto which two eggs of *Microsphecia brosiformis* were transferred to test larval development

Quentin Donzé (our new garden technician) preparing the open-field test with *Melanagromyza albocilia* in Germany

Setup of oviposition tests with *Bagous nodulosus*

15 Flowering Rush (*Butomus umbellatus*)

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15.1 *Bagous nodulosus* (Col.: Curculionidae)

Despite not being able to conduct any field trips outside of Switzerland this spring due to COVID-19 restrictions, we are well on schedule with most of our planned work. About 30 *Bagous nodulosus* females that overwintered at CABI were set up for oviposition in mid-April and we obtained enough larvae to nearly complete the no-choice larval establishment tests. Some mining and larval development was found on one additional species, the European *Alisma plantago-aquatica*. However, no attack on *Alisma* was found in single-choice tests and no further development was found in longer lasting larval development tests with this species.

Since we experienced in the past very high larval mortality while rearing the weevil on potted plants, we also tested a method using cut leaf sections in Petri dishes. This allowed us to double successful development from 3% to 6%, but there is still room for improvement.

Setup for rearing, host-specificity tests and impact experiment with the agromyzid fly *P. ornata*

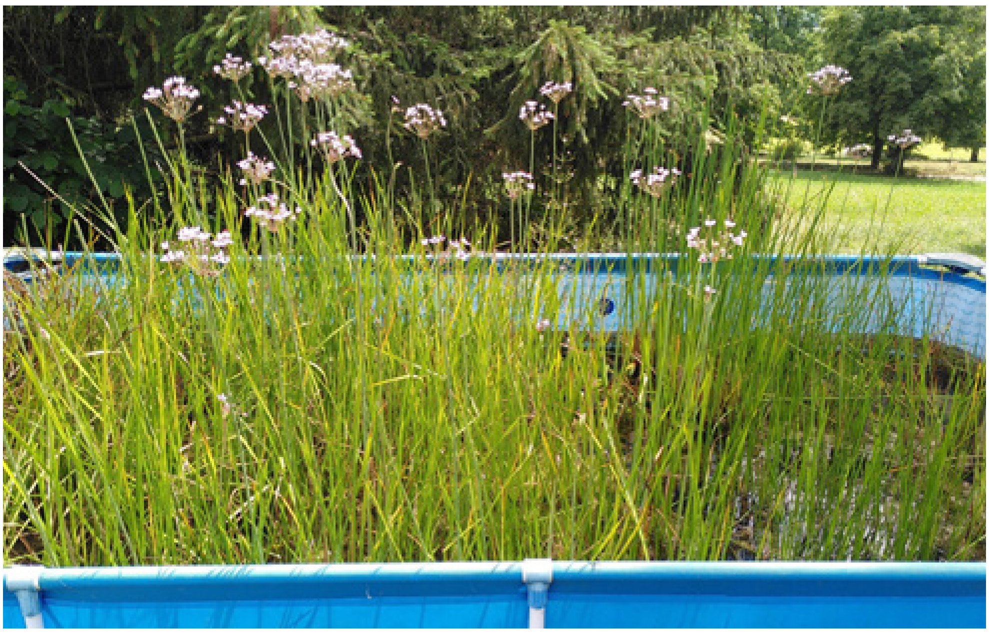
15.2 *Phytoliriomyza ornata* (Dipt.: Agromyzidae)

In order to use emerging adults of this agromyzid fly most efficiently, we decided to combine host-specificity tests with a rearing and a preliminary impact experiment. We exposed 45 flowering rush plants and 42 test plants (14 species) to pairs of newly emerged flies. No larvae or pupae were found so far on test plants, while an average of nine pupae per plant were found on flowering rush. This is an improvement to 2019, where only 3.5 pupae were found per plant.

Our small impact experiment (including 5 exposed and 5 control plants) indicated a biomass reduction of only 10% for attacked plants. However, we expect that impact can potentially be much higher and we plan for 2021 to release more than one female per plant and to include the second generation of *P. ornata*.

15.3 *Doassansia niesslii* (Fungi, Doassansiaceae)

Work has continued to develop and refine the methods to obtain and maintain mycoparasite-free cultures of smut isolates for future experiments and host range screening. Since the most common genotype 1 of flowering rush in North America has so far proven resistant or only slightly susceptible towards the smut isolates tested, preparations are underway to locate flowering rush herbaria samples from Europe and Asia, for inclusion in molecular analysis conducted by John Gaskin (USDA, ARS). The aim is to determine the origin of genotype 1 in Eurasia to enable more targeted future surveys for new strains of *D. niesslii*.



Pool with flowering rush plants at CABI Switzerland

16 Tree of Heaven (*Ailanthus altissima*)

Massimo Cristofaro, **Francesca Marini (BBCA, Italy)**, **Philip Weyl** and **Hariet L. Hinz**

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Ailanthus altissima, commonly known as tree of heaven, is a rapidly growing, deciduous tree native to China, which was introduced on various continents as an ornamental and has subsequently become invasive in Europe and North America. Tree of heaven is a prolific seed producer but can also reproduce vegetatively by sprouting. In China it is used for its wood and as a food plant for a type of silkworm. In spring 2020, CABI Switzerland received some start-up funding from British Columbia to support the ongoing work of *Ailanthus* biocontrol by BBCA and start developing a test plant list for Canada.

Drying and necrosis of the apical parts of the stems observed on heavily infested plants (photo: Panzarino Onofrio)

In 2016, the eriophyid mite *Aculus mosoniensis* was discovered in Europe by BBCA, and has since been found in Hungary, Austria, Slovakia, Slovenia, Italy, Serbia, Montenegro, Croatia, Greece and Macedonia. BBCA started conducting host-specificity tests with *A. mosoniensis* in 2019, exposing eight non-target species. Results were very promising in that none of the test plants were infested by the mite, whereas tree of heaven plants exposed as controls were severely damaged due to the presence of thousands of mites. It is expected that the mite could be used as an inundative biocontrol agent in Europe and as a classical agent in North America.

In 2020, host-specificity tests were continued exposing six plant species of economic interest, and the control, tree of heaven. Leaves of tree of heaven infested with the mite were fixed to newly developing stems of each plant. Thus far the control trees are just beginning to show symptoms. Detailed results will be available by the end of summer.

Method for inoculation of mites

The feeding and galling of this mite impacts young shoots and plants of tree of heaven, inducing vascular necrosis and even death, especially after repeated infestation and on young trees. To quantify the impact of the mite, an experiment has been initiated this summer by BBCA in Rome. So far five trees have been infested with 15 mites each, while five other trees are being kept free of mite attack using an acaricide. As in the host range test, the infested trees are just starting to show signs of mite attack, such as narrow, deformed and twisted leaves, with edges folded or rolled upward lengthwise. It is planned to run this experiment for at least 2 years to also capture the impact of repeated infestations by the mite. However, we are hoping to already find a measurable impact by the end of this summer.

Master student Erica Profeta recording symptoms of *Aculus mosoniensis* attack during host-specificity tests

Adult leaf beetles (*Lysathia* sp.) attacking the emergent shoots of parrot's feather

17 Parrot's Feather (*Myriophyllum aquaticum*)

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Parrot's feather (*Myriophyllum aquaticum*) is an aquatic macrophyte native to South America. It has been spread to many parts of the world, most likely through the aquatic plant trade. It is a popular aquatic garden species and intentional planting has spread this species into natural water bodies. British Columbia (BC) on Canadas west coast is no exception and parrot's feather is considered problematic around Vancouver, Vancouver Island and Frasers Valley. The aim of this project is to determine the feasibility for the biological control of parrot's feather in this area.

Despite much of BC being extremely cold, the region around Vancouver, Vancouver Island and Frasers Valley has a relatively warm climate influenced by the ocean. This may work to our advantage and allow biological control agents to establish and control parrot's feather. Currently there are two potential agents which originate from Argentina at our disposal.

The defoliating chrysomelid beetle *Lysathia* sp. already released into South Africa is effective and has brought parrot's feather under control in much of the country. However, *Lysathia* sp. requires relatively warm temperatures and may not be entirely suitable for BC. Less is known about the second potential agent, the stem-mining weevil *Listronotus marginicollis.* It has been collected around Buenos Aires, Argentina, but it is unclear if it also occurs in cooler sites in Argentina or elsewhere in South America. Some initial host range testing has been done for South Africa. In addition to thermal tolerances, host specificity will be crucial, since North America has many native *Myriophyllum* species.

The next steps include developing a climate matching model together with David Ensing, Agriculture and Agri-Food Canada, to identify the climatically most suitable agents or agent populations for use in BC. This will allow us to further develop a potential biocontrol project for parrot's feather in BC.

Waterbody infested with parrot's feather in British Columbia (Photo: https://bcinvasives.ca/invasive-species/identify/invasive-plants/parrots-feather)

18 Black Locust (*Robinia pseudoacacia*)

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Robinia pseudoacacia, commonly known as black locust, is a medium-sized hardwood deciduous tree. It is endemic to a few small areas of the United States, but has been widely planted as an ornamental and as a wood tree. It has naturalized in temperate North America, Europe, Southern Africa and Asia and is considered an invasive species in some areas. It is a category 1 invader in South Africa, and since mechanical and chemical control have thus far proven unsuccessful at controlling this species, considerable effort and resources have been channeled into biological control. In South Africa the biocontrol project is led by Grant Martin from the Centre for Biological Control (CBC), Rhodes University.

Black locust was introduced to Europe in the 17th century and is now naturally found in most of the continent, from southern Italy to southern Norway. Since its introduction many of its natural enemies native to North America have found their way over and many cause considerable damage to the trees. Surveys for natural enemies occurring in southern Germany and Switzerland were initiated by CABI in July this year. Thus far, surveys have proven successful and four potential biological control agents have been identified: the sawfly, *Nematus tibialis*, two lepidopteran leaf miners, *Phyllonorycter robiniella* (syn. *Macrosaccus robiniella*) and *Parectopa robiniella* and the leaf rolling midge, *Obolodiplosis robiniae*.

A first shipment of the leaf rolling midge has already been sent to the CBC quarantine facility at Rhodes University for further testing. Future research in Europe will focus on understanding the biology of these species, levels of parasitism and predation as well as surveying for non-target attack on species in the same family as *Robinia pseudoacacia* (Fabaceae).

Galls or leaf rolls of the midge *Obolodiplosis robiniae;* each gall may contain several larvae

Damage of the leafminer *Parectopa robiniella* collected in southern Germany

Adult of the leaf rolling midge *Obolodiplosis robiniae* found in Switzerland

Set-up of experimental field trial at CABI, UK with Japanese knotweed plants inoculated with *Mycosphaerella polygoni-cuspidati*

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

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Japanese knotweed (*Fallopia japonica*), giant knotweed (*F. sachalinensis)* and their hybrid *F. x bohemica* have become serious invasive weeds in North America. Two natural enemies from the native range, the psyllid *Aphalara itadori* and the leaf-spot pathogen *Mycosphaerella polygoni-cuspidati* have been prioritized as classical biological control agents following extensive research, both in the field and under greenhouse conditions.

19.1 *Aphalara itadori* (Hemiptera, Psyllidae)

This psyllid has been released in the UK, Canada and most recently the USA but establishment has yet to be achieved. A survey was conducted in June 2019 to collect fresh stock of the Kyushu strain as well as new strains of psyllids in the central and northern part of Japan. 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 CABI UK quarantine facility to establish cultures and remove predators and parasitoids.

In order to complete host range testing with the Murakami line, no-choice and multiple-choice oviposition tests were set up with *Fallopia baldschuanica*, *Muehlenbeckia axillaris* and *Muehlenbeckia complexa*. None of these are native to North America, but *F. baldschuanica* and *M. axillaris* had supported adult development of the original *A. itadori* line. In no-choice oviposition test, some eggs were laid on all three species and adults developed, but numbers were far lower than on *F. japonica*. The follow-up multiple-choice oviposition tests are currently being assessed.

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

The fungal pathogen *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 UK and US native non-target species. However, due to its unique biology and genetic properties requiring two different mating types for reproduction, and consequently establishment and spread in the field, use of a single mating type isolate of the pathogen is considered to have potential as a mycoherbicide. Protecting this idea, a European patent held in the name of the Secretary of State for Environment, Food and Rural Affairs, UK, has been granted while patent applications in the US and Canada are pending. Funds from British Columbia have previously supported the proof-of-concept research.

Based on funding secured from the UK and the Netherlands, research is ongoing to improve the application and infection of the pathogen under field conditions, and its shelf-life as a future product.

Set-up of host range tests with the Murakami line of the psyllid. Left: no-choice oviposition tests; right: multiple-choice oviposition tests

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

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

A Himalayan balsam leaf heavily infected with the rust *Puccinia komarovii* var. *glanduliferae* in the field

The rust fungus *Puccinia komarovii* var. *glanduliferae* was first approved for release in England and Wales in 2014 and to date, two strains of the rust have been released; one from India and one from Pakistan. In order to determine which strain to release, the susceptibility of a Himalayan balsam population is determined through inoculating plants with the respective rust strains under controlled conditions. The strain which is found to be most virulent and pathogenic (producing large uredinia with lots of spores) is then selected for release into the field. At fully susceptible field sites, the rust is performing well; high levels of leaf infection have been observed throughout the summer and subsequent seedling infection recorded in the spring the following year. Nevertheless, populations which are resistant to infection by the two rust strains exist. For Canada, six populations from BC were assessed for their susceptibility to the two rust strains, all of which were resistant to the strain from Pakistan. In addition, a few populations were weakly susceptible (producing fewer, smaller uredinia, with less spores) to the strain from India, with the rest being resistant. For biological control of Himalayan balsam to be successful in the UK and Canada, additional rust strains which are infective towards these resistant population need to be identified.

To address this, a molecular study, based on the phylogenetic analysis of six chloroplast regions, was conducted and found that Himalayan balsam in the British Isles fall into three groups. This confirmed the hypothesis that the species was introduced into the British Isles on more than one occasion, from multiple locations within the native range. The study also identified key regions in the native range to survey for additional rust strains which are more likely to be fully compatible with UK populations that are currently showing resistance. It is essential that plant populations are matched to the most virulent rust strains; broadly, from areas in the native range from where the original seeds were collected. A preliminary molecular study using leaves from seven Canadian populations was undertaken; these populations were assembled into two of the three groups previously identified during the analysis of samples from the British Isles. The study has since been extended to include a further ten Canadian populations and analysis of the sequence data is underway.

Host-range testing has been completed for 47 of the 51 species of importance to North America. With no non-target impacts recorded on any of the species. This corroborates with UK assessments that the rust is highly host specific, completing its life cycle on Himalayan balsam only. The four outstanding species to be tested for Canada have been much harder to source. Given the high level of host-specificity of the rust, it is likely that these will be removed from the test plant list.

In January 2020, permission to export an additional strain of the rust from Karchon, Uttarakhand, India was finally granted. The rust has been propagated in CABI's quarantine facilities and its virulence towards both Canadian and UK Himalayan balsam is currently being assessed. Surveys for the rust in Kashmir, a region prioritised based on the molecular analysis, are also being conducted by an in-country collaborator. However, collections are hampered by political unrest and COVID-19 restrictions.

Kate Pollard and student Jacob Horner assessing Himalayan balsam leaves for the rust fungus

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