Weed Biological Control

Progress Report 2021

CABI in Switzerland
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Contents

Cover photos: Nadia Frei dissecting Butomus plants to check rearing success of *Bagous nodulosus;*
Laura Forgione releasing *Cyphocleonus trisulcatus* adults in an open-field test;
Stefan Majnarić setting up oviposition cages for the two noctuid moths on common reed.

Notes from the section leader

This spring continued to be influenced by restrictions related to COVID-19, with a slightly reduced number of students and some insecurity concerning field trips. However, as vaccination advanced in Switzerland, measures were successively reduced and being at work feels a lot more normal now. What hampered and negatively impacted our work nearly more were and are the rather extreme weather conditions with the coldest spring in 30 years, which was followed by very high rainfall from May onwards. For instance, between 21 June and 13 July 2021, more rain has fallen on Switzerland than during the same period for 70 years, leading to inundations in many places. I realize that it was the complete opposite in many parts of the U.S. and Canada with some of the highest temperatures ever recorded in the region, resulting in numerous extensive wildfires! Hopefully politicians will see the writing on the wall.

On a more positive note, I would like to report on some project highlights. Patrick Häfliger and his Team made very good progress in the rearing of the Archanara moths on **common reed**. Since the conventional moth rearing is extremely time consuming, but rearing on artificial diet greatly reduces fitness of moths, they tried a combination of both by starting with the usual rearing on common reed shoots and switching to artificial diet for the later instars and pupation. This seems to have done the trick. This method is less time consuming but still results in satisfactory pupation and fertility of emerging moths.

Similarly, they were able to make progress with the rearing of the weevil *Bagous nodulosus* on **flowering rush**. Under confined rearing conditions, high mortality appears to occur when young larvae leave shoots to find a new home. By keeping first instar larvae on cut leaf pieces and only transferring the larger second instar larvae onto potted flowering rush plants, they were able to greatly improve successful development. This is important since we are planning to ship weevils to the quarantine facility of USDA, ARS in Sidney Montana in 2022 for rearing and future field releases. For this an efficient rearing method, which works under confined conditions needs to be developed.

The fact that we have good rearing colonies of our agents did not only prove extremely advantageous during COVID-19 related travel restrictions, but is also an essential step to facilitate agent multiplication and establishment in the invaded range, and should not be underrated.

In respect to petitions, we are currently preparing a reply to comments of the USDA, APHIS Technical Advisory Group (TAG) for the gall-forming weevil *Ceutorhychus cardariae* on **hoary cress**, and the seed-feeding weevil *Mogulones borraginis* on **houndstongue**. The petition of the eriophyid mite, *Aceria angustifoliae* on **Russian olive** is already further advanced with USDA, APHIS, PPQ currently preparing the Biological Assessment for Fish and Wildlife consultation. We recently received comments on the submitted test plant list for **field bindweed** and will make an effort to obtain the additional test plant species suggested by TAG reviewers.

Three additional petitions should still be submitted in 2021: for the moth *Dichrorampha aeratana* on **oxeye daisy**, the seed-feeding weevil *Ceutorhynchus constrictus* on **garlic mustard**, and *Bagous nodulosus* on **flowering rush**. A big thank you here to our North American partners who invest a lot of time to help us preparing and submitting these petitions and who accompany the regulatory process.

Considering the current economic circumstances our financial situation is thankfully quite stable. All three new projects we started in 2020 will continue to be funded. These are: **parrot's feather** (*Myriophyllum aquaticum*) and **tree-of-heaven** (*Ailanthus altissima*) by British Columbia, and **black locust** (*Robinia pseudoacacia*) by South Africa. We also received additional funding from British Columbia as well Montana for field bindweed.

Finally I would like to take the opportunity to let you know that I will be changing my position within CABI. From 1 October onwards, my colleague Wade Jenner will take over as Centre Director, while I will become responsible for the Invasive Species theme at CABI, which includes projects in several of our overseas Centres. This is an exciting new challenge. However, for the time being I will stay responsible for the 'weed section' at the Centre.

Stay safe and take care!

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Yellow toadflax plant infested with *Mecinus heydenii*

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

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During the 2020 season, a complete lockdown in Serbia, due to COVID-19, severely impacted our work with biocontrol agents associated with toadflaxes.

1.1. *Rhinusa pilosa* ex *L. vulgaris* and *R. rara* ex *L. genistifolia* (Col., Curculionidae)

In 2021 we continued rearing of the eight remaining genotypes. In the second half of March, 124 females were copulated and on 9 April, three field cages were established for three of the genotypes (A1, A3, B6). Additionally, seven genotypes (A1, A4, A8, A13, A14, B6 and B8) were set up in 50 framed cages planted with yellow toadflax. Unfortunately, the rearing of *R. pilosa* was compromised by an unprecedented long cold period during April and the first half of May with temperatures below 5° C. This situation affected activity of the weevils and resulted in a strong reduction of oviposition and gall formation, not only in our rearing, but also at field sites. Between 10 April and 13 May, we were only able to collect 39 galls during three collection trips. The three field cages and 50 framed cages produced a total number of 347 and 193 galls, respectively. The number of resulting *R. pilosa* adults will be known after gall dissections, which are planned for mid August.

Oliver Krstić dissecting Yellow toadflax plant infested with *Mecinus heydenii*

In contrast to *R. pilosa*, the warm period during March enabled earlier oviposition of *R. rara*, without negative effects on gall development and larval development. On 15 May, a total of 430 *R. rara* galls were collected from *L. dalmatica* and *L. genistifolia* at two sites in eastern Serbia. The galled plants were individually planted between 27-30 June and a total of 1,100 adults of *R. rara* were extracted.

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

Due to the lockdown in 2020, we unfortunately completely lost our rearing colonies of *Mecinus heydenii* and *M. peterharrisi.* In spring 2021, we managed to collect 43 adults of *M. heydenii* (30 females), with which we were able to produce 560 offspring. For *M. laeviceps,* 31 adults (18 females) were collected and 175 offspring produced.

In addition, we continued with survival and fitness trials with the two native North American species *Nuttallianthus canadensis* and *Sairocarpus virga*. Detailed results will be presented in the annual report.

In mid August, a collection trip for *M. peterharrisi* is planned to the Pelagonian plateau in North Macedonia to re-establish the rearing colony.

Jelena Jović, sorting collected Dalmatian toadflax plants infested with galls of *Rhinusa rara*

2 Houndstongue (*Cynoglossum officinale*)

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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 2,400 *M. borraginis* larvae collected in 2020, about 100 adults emerged in October 2020 and an additional 1,000 in March 2021 which were set up on houndstongue rosettes. In mid-June, about 100 females were retrieved and reset on 42 flowering-seeding houndstongue plants. By mid-July, 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. More than 3,000 *M. borraginis* larvae emerged and were separated into cups (30 individuals per cup) and placed in an underground insectary for adult emergence in autumn 2021 and spring 2022.

In May 2021, a total of 300 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.

Mature larvae of *Mogulones borraginis* leaving the fruits of houndstongue and being trapped in a vial for collection

Summer student Clara Fraschini taking down the *Mogulones borraginis* rearing after a successful season

Egg of *Cheilosia urbana* on *Pilosella officinarum*

3 Hawkweeds (*Pilosella* spp.)

Ghislaine Cortat and **Léna Jego**
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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 2020 have been taken out of cold storage regularly since 21 April 2021. Adults emerging from these are being used in host range tests and for rearing.

In 2021, emergence rates of *A. pilosellae* ex *Pilosella* spp. have been variable (25-75%, on average 45%) and the sex ratio was well balanced. We exposed 18 test plant species (12 North American (NA) natives) in no-choice tests.

Emergence rates for *A. pilosellae* ex *P. officinarum* have been very low (max 20%). It was difficult to harvest the galls at the right developmental stage, because of weather fluctuations during the season. A few females emerged from galls harvested in 2019 and kept in cold storage until spring 2021. Seven test plant species (five NA natives) were exposed to this biotype in no-choice tests.

Gall harvest for both species is delayed owing to the wet and cold weather during development. So far, galls were only found on *P. caespitosa* and *P. officinarum.* Final results will be presented in the annual report.

Of the 84 species, subspecies and populations tested with *A. pilosellae* ex *P. officinarum*, and 71 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 test plant together with the natural host (control). Additional choice tests are ongoing with *H. argutum*.

3.2 *Cheilosia urbana* (Dipt., Syrphidae)

In 2021, the sun appeared only on very rare days during the usual flying period of the syrphid *C. urbana.* The first individual was observed in the CABI garden on 22 April. Females were collected between 27 April and 9 May, and kept in an incubator at about 10-15°C until they were shipped to Dr Littlefield, Montana State University. Eggs were collected from the storage containers on 12 May (N=30) and 16 May (N=170) and placed in two small vials with a piece of paper towel to keep them in place. The eggs and 9 females were sent to Montana on 18 May. There were delays during the transport and unfortunately the females died without laying new eggs. A few larvae hatched from the eggs collected prior to the shipment, but died before arrival and no larvae hatched from the remaining eggs.

No-choice tests established with both biotypes of *Aulacidea pilosellae* at CABI

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 host range.

The host range testing this spring and summer has been successful and so far we have been able to screen 17 test plant species, with several more to be tested in the coming months. Of these, only two species in the tribe Cardueae (same as Russian knapweed), *Cynara scolymus* (globe artichoke) and *Cynara cardunculus* (cultivated for its edible leaf stems) were accepted for oviposition, and adults developed on both, but always to a much lower degree than on the control, with a relatively high level of larval mortality. In subsequent choice tests we found a clear preference for Russian knapweed with about 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 feeding and oviposition, thus, an open-field test will be necessary to fully understand the risk that this weevil might pose to these crop species.

4.2 Additional surveys

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

The larval mining commonly seen on Russian knapweed leaves (above) compared to those commonly seen on non-target plants, in this case artichoke (below)

Adult female *Pseudorchestes sericeus* from the quarantine culture maintained at CABI.

C. constrictus emerging from overwintering containers

5 Garlic Mustard (*Alliaria petiolata*)

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Between 25 February and 28 April 2021, 1,305 adults of the seed-feeding weevil *Ceutorhynchus constrictus* emerged from our rearing colony established in 2020. Because of changes in the quarantine use and restricted access to the lab at the University of Minnesota, no adults were shipped to Dr. Katovitch (UMN) this spring. After testing for oviposition, fertile females were used at CABI for rearing and host range tests with the native North American (NA) *Descurainia nelsonnii*. We had planned to conduct tests with three additional NA species, unfortunately either these did not flower at all in 2021, were not synchronised with the weevil oviposition period or died due to the constantly bad weather conditions in spring.

In previous no-choice tests, *D. nelsonnii* was accepted for oviposition, but only few eggs were found and no mature larva emerged. 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 of two *D. nelsonnii,* but without the typical window feeding marks on the pods. Mature larvae only emerged from garlic mustard.

In previous tests, *C. constrictus* was able to complete development on the native NA *Boechera holboellii.* An experiment to study whether females can develop ovaries when fed exclusively with this test plant was conducted in 2020 and repeated in 2021. In March 2021, 60 females and 30 males were placed in cages containing rosettes and flowering plants of *B. holboelli.* In May, only eight live females and eight males were retrieved and female dissections are ongoing. Ovariole development will be compared with females placed on *A. petiolata* plants during the same period.

In June and July, about 450 mature larvae of *C. constrictus* were harvested from garlic mustard for adult emergence in spring 2022.

Between April and July, adult *C. scrobicollis* emerged from rearing plants infested with field collected adults in October 2020. So far about 140 adults emerged and units of two females and one male are kept on potted rosettes for aestivation. Weevils will either be shipped to AAFC for additional field releases in autumn and/or kept at CABI for rearing.

Cage exposing *Boechera holboellii* to *Ceutorhynchus constrictus* for an oogenesis study

6 Common Reed (*Phragmites australis*)

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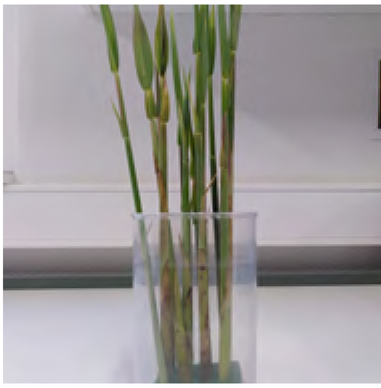
6.1 *Archanara neurica* and *Lenisa (Archanara) geminipuncta* (Lep: Noctuidae)

In 2021, we aimed at producing as many pupae of both moth species as possible to support further field releases in Canada. We focused on our conventional rearing method using cut stem sections, because fitness of *A. neurica* exclusively reared on artificial diet is greatly reduced, and because all *L. geminipuncta* we tried to rear on diet in 2020 died before pupation. About 750 larvae of *A. neurica* and 3500 larvae of *L. geminipuncta* were individually transferred into cut stem sections of *P. australis*. This resulted in 250 pupae of *A. neurica* and about 750 pupae of *L. geminipuncta*.

Since our conventional rearing method is very time consuming, we transferred about 1,300 larvae of *L. geminipuncta* as fourth and fifth instar larvae onto artificial diet. This was quite successful with about 65% pupation (35% without deformities). In addition, emerging moths readily laid eggs comparable to moths originating from our conventional rearing. Combining rearing on stems for early instars and rearing on artificial diet for late instars appears to be a good compromise to increase rearing efficiency, while retaining fitness.

A total of three shipments were sent to Ottawa, Canada in 2021 for additional rearing attempts on artificial diet and field releases. In March and April, 300 eggs of *A. neurica* and 2,000 eggs of *L. geminipuncta* were sent; in July over 270 pupae of *L. geminipuncta* were provided. Due to restrictions related to COVID-19, monitoring of establishment success of earlier releases of *A. neurica* was not possible for our Canadian partners. Hopefully this will be possible in spring 2022, by searching for dead shoot tips.

In 2021, special care was taken to produce parasitoid free eggs. Stems offered for oviposition were kept for 2 days in a freezer at -18°C, in order to kill any eggs potentially already laid and parasitized in the field. In addition, oviposition cages were set up in our basement to prevent parasitism of newly laid eggs. So far, we were able to produce 10,000 eggs of *A. neurica*. We expect to obtain more eggs of *L. geminipuncta* until August 2021. Most of them will be sent to Canada for further releases in 2022.



Setup of stems for larval transfer of first instar larvae of *L. geminipuncta*.

Setup for release of moth pupae at a field site in Canada (photo: Michael McTavish)

Summer student Nadia Frei collecting young *Phragmites australis* stems at a field site close to CABI

Single-choice cage test where *Lepidium integrifolium* (left) and the control, *Lepidium draba* (right) were exposed to *C. turbatus*

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 resubmitted together with Mark Schwarzländer (University of Idaho) to the USDA, APHIS Technical Advisory Group (TAG). On 12 August 2020, TAG provided us with a summary of the reviews and concluded that at this point in time, the group cannot recommend the field release of the weevil. Following additional discussions with the chair of TAG, USDA-APHIS and US Fish and Wildlife Service personnel in December 2020, we agreed on a strategy to collect and provide additional data for *C. cardariae*.

In spring 2021, we conducted an additional open-field test with two native North American (NA) species, *Lepidium acutidens* and *Stanleya pinnata*. These two species had been attacked under multiple-choice conditions previously, however, were not yet tested under open-field conditions. We used our typical circular design in order for the data to be comparable to previous test results. The level of attack on the control was good, with an average of 7 galls recorded per plant, while the test plants were completely unattacked.

In addition to this, we setup an impact study with *Caulanthus anceps,* a NA native plant that had relatively high levels of attack during no-choice tests. Plants which were exposed to the weevils showed relatively high levels of attack with an average of about 6 galls per plant. However, seed output was not different to unattacked control plants with approximately 1g of seeds produced per plant, irrespective of treatment.

We also continue maintaining a rearing of the weevil at the Centre. Of the 1,000 adults that overwintered at CABI, about 80% survived. These were set up on potted plants, from which about 1,200 *C. cardariae* emerged in 2021.

7.2 *Ceutorhynchus turbatus* (Col., Curculionidae)

On 9 June, about 400 adults of the seed-feeding weevil *C. turbatus* were collected in southern Switzerland. On 10 June, we established no-choice oviposition tests with nine plants; two *L. draba* control plants and six replicates of the T&E *Streptanthus glandulosus* ssp. *Niger*, and one replicate with *Physaria chambersi*. Apart from *L. draba,* no eggs were found in any of the native NA test species.

Development tests were set up with two native NA test plant species (*Lepidium integrifolium* and *Stanleya pinnata*) that had been accepted for oviposition in the past. None of the test plants supported larval development of the weevil, while an average of 12.7 larvae emerged from hoary cress control plants. We were also able to set up a single-choice cage test with *L. integrifolium,* where none of the non-target plants were attacked while there was an average of over 70 larvae per control plant.

Philip Weyl collecting *Ceutorhynchus turbatus* in southern Switzerland

8 Dyer's Wood (*Isatis tinctoria*)

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8.1 *Ceutorhynchus peyerimhoffi* (Col., Curculionidae)

Over 1,000 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 the latter, over 3,000 larvae were collected and are kept for adult emergence in 2022.

With the host range testing for this weevil coming to an end and the petition for field release being prepared, the remaining tests are limited. Unfortunately, with the cool and wet spring and summer this year, synchronizing the emergence of adults with flowering and seeding dyer's woad, as well as test plant species, was not possible and thus no host specificity tests were conducted.

We were however, able to collect information on parasitism of the weevil 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. From the material collected we currently obtained 147 weevil larvae, and over 150 individual parasitoid wasps from nine morpho-species. The parasitoids will be catalogued and sent for identification as soon as possible.

8.2 *Ceutorhynchus rusticus* (Col., Curculionidae)

A total of 37 plants, from six test plant species and the control were exposed to *C. rusticus* in no-choice oviposition and development tests in autumn 2020. Test plants were regularly checked and any dying plants were dissected. Adult emergence was successful in 2021, with about 76% of the dyer's woad control plants supporting adult emergence with an average of 8.7 adults per plant. Of the six test plant species, we recorded typical *C. rusticus* mining in only one, *Cakile edentula,* however, this species was not able to support adult development.

Two examples of the yet unidentified parasitoids emerged from the material collected in the Abruzzo mountains, Italy

The seed pods collected in the Abruzzo mountains, Italy where *Ceutorhynchus peyerimhoffi* occurs naturally to study the parasitoids associated with the weevil

9 Perennial Pepperweed (*Lepidium latifolium*)

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Larvae of *Ceutorhynchus marginellus* in a gall on *Lepidium latifolium*

9.1 *Ceutorhynchus marginellus* (Col., Curculionidae)

Because there is a certain way forward for the petition of the very similar *Ceutorhynchus cardariae* on hoary cress (see 7.1), we decided to restart host-specificity tests with *C. marginellus* in the quarantine facility at CABI. We exposed a total of seven native North American (NA) species under no-choice conditions. Plants were chosen based on previous test results with *C. marginellus* and/or *C. cardariae.* Adults emerged from six of the seven species.

To determine whether plants would also be accepted for oviposition by *C. marginellus* when they were offered simultaneously with perennial pepperweed, we exposed eight native NA species that had supported adult or gall development in previous no-choice tests under single-choice conditions. These tests were also conducted in the quarantine facility at CABI. Under these conditions, larvae were found on the federally listed *Lepidium papilliferum* (T&E) and on the NA natives *L. davisii*, *L. thiemii* and *L. paysonii*, but generally in much lower numbers than on the simultaneously exposed *Lepidium latifolium*. Detailed results will be presented in the annual report.

We also continued to maintain a rearing colony of *C. marginellus*. From the 700 adults that were kept in incubators set at 2–3°C during winter 2020/2021, 250 females and 200 males survived until March 2021. These weevils were transferred onto potted perennial pepperweed plants to continue our rearing. More than 1,000 weevils emerged from these plants. They are currently being kept in plastic cylinders and regularly fed with leaves of perennial pepperweed or were placed onto potted perennial pepperweed plants.

Plants prepared for single-choice tests with *Ceutorhynchus marginellus*. Pots were afterwards covered with gauze bags and two females were released per pot

10 Swallow-Worts (*Vincetoxicum* spp.)

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

The root-mining beetle *Chrysochus asclepiadeus* can develop and emerge as adult over 1-3 years. We are currently maintaining a rearing colony originating from France. In 2021, emergence was delayed due to adverse weather conditions in spring. Adults (N=11) only emerged from plants infested in 2019 (*Vincetoxicum hirundinaria, V. nigrum and V. rossicum*) and produced few eggs. In July, we therefore field collected about 150 additional adults in Franche-Comté, France. Eggs laid will be harvested regularly and will be used to continue no-choice larval transfer tests.

In 2021, 53% of larvae found on *V. hirundinaria* and *V. nigrum* exposed in 2019, successfully developed into adults. No adult emerged from the single larva found on *Asclepias syriaca.* None of the nine other exposed test plants (7 North American (NA) natives) were attacked.

All plants used in no-choice larval transfer tests in 2020 were searched for larvae in spring 2021. Thirteen test plant species had been exposed (11 NA natives), and *V. hirundinaria*, and *V. nigrum* were used as controls. Larvae were found on about 30% of *V. hirundinaria* and none of *V. nigrum.* Alive larvae were also found on one plant each of the native NA species *Asclepias incarnata* and *A. rubra* and one dead larva was found on *A. tuberosa. Asclepias incarnata* was not attacked under open-field conditions during the test set up in 2018. The low survival of larvae, particularly on controls, could be due to fungal growth on the eggs resulting in poor fitness of first instar larvae. Egg sterilisation trials will be undertaken in 2022.

Storage of adult *Chrysochus asclepiadeus* for egg harvest

10.2 *Euphranta connexa* (Dipt., Tephritidae)

Due to very limited funding, it was decided to discontinue any work on this seed-feeding fly for the time being.

Summer student Léna Jego field collecting *Chrysochus asclepiadeus* in France

11 Common Tansy (*Tanacetum vulgare*)

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Larvae of *Chrysolina eurina* provided with a leaf of *Tanacetum huronense* in a Petri dish

Male of *Platyptilia ochrodactyla* on a flower head of *Tanacetum huronense*

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 early June, a total of 79 females and 54 males were collected in the field. 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 for larvae. So far, larvae were found in all of the dissected *T. vulgare* stems, but not in any of the non-target species.

In addition, an open-field test was set up with the medicinal herb *Tanacetum parthenium* and with *T. vulgare*. Twelve potted plants of both species were exposed on a meadow and a total of 24 females and 24 males were released. The plants were dissected for larvae one month after the weevils were released. Unfortunately, larvae were only found in one *T. vulgare* plant and empty mines likely caused by *M. millefolii* were found in two additional plants. No larvae or empty minds were found in any of the exposed *T. parthenium* plants.

In mid-July approximately 400 stems presumably infested with *M. millefolii* were collected in the surroundings of St Petersburg. The larvae are currently leaving the stems and are being regularly collected and placed in cups filled with soil for pupation. The adults are expected to emerge in August and will be sent to Dr. Rosemarie De Clerck-Floate, Agriculture and Agri-Food Canada in Lethbridge.

11.2 *Chrysolina eurina* (Col., Chrysomelidae)

In spring 2021, we re-established population viability tests with the two North American native species *Tanacetum camphoratum* and *T. huronense* as previous tests had given inconclusive results. We transferred first instar larvae that emerged from eggs collected from beetles reared on *T. vulgare* to leaves of *T. vulgare*, *T. camphoratum* or *T. huronense*. We also transferred first instar larvae that emerged from eggs collected from beetles reared on *T. camphoratum* to leaves of *T. camphoratum*. The population viability tests confirmed that larval survival is reduced on the two native North American species compared to *T. vulgare*, but there was no further reduction in larval survival when *C. eurina* was continuously fed with *T. camphoratum* for a second generation.

11.3 *Platyptilia ochrodactyla* (Lep., Pterophoridae)

On 27 and 28 May, we collected about 1,000 shoots infested with *P. ochrodactyla* in western Germany. The emerging females were released in an open-field test with flowering plants of *T. camphoratum*, *T. huronense*, *T. parthenium* and *T. corymbosum*. The flower heads of test and control plants will be dissected for larvae about two months after the exposure to *P. ochrodactyla*.

Margarita Dolgovskaya and Sonja Stutz preparing field-collected stems of *Tanacetum vulgare* presumably infested with *Microplontus millefolii* for larval emergence

12 Russian Olive (*Elaeagnus angustifolia*)

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Russian olive trees with newly developing buds ready for the inoculation of mites

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 (CFIA) did not approve its field release, mainly based on concerns over the lack of experimental impact data. We have reviewed the comments by the reviewers and will soon submit additional information to address the points raised and hopefully fill in any gaps which may sway the original decision of the CFIA. In the interim, in the U.S. the Biological Assessment is being prepared and we hope to receive news on the process soon.

In light of the current progress, a fresh culture of the mite was collected in Serbia and is currently being reared at CABI in preparation for future shipments to both Canada and the U.S.

12.2 Additional surveys

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

Typical Russian olive habitat along rivers and water bodies in Kazakhstan

13 Oxeye daisy (*Leucanthemum vulgare*)

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Set-up used to check *Oxyna nebulosa* females for egg-laying before using them in tests

13.1 *Dichrorampha aeratana* (Lep., Tortricidae)

In May, we set up no-choice larval development tests with two non-target species for Australia. These plants will be dissected for larvae in autumn. In addition, we set up a multiple-choice cage test and an open-field test with *Cotula vulgaris* and two Australian Shasta daisy cultivars, which had all supported larval development in previous tests. The plants were exposed to egg-laying females in May and June and will be dissected for larvae in autumn. We also contributed to the petition for field release, which is currently finalized by our North American partners.

13.2 *Dichrorampha consortana* (Lep., Tortricidae)

In spring, plants that had been infested with larvae of *D. consortana* in 2020 were covered with gauze bags and regularly checked for adult emergence. However, only three females, but no males emerged. Therefore, no experiments could be conducted with this species.

13.3 *Oxyna nebulosa* (Dipt., Tephritidae)

In June and July, we harvested more than 1,000 galls from the oxeye daisy plants that had been exposed to adults of the root-galling tephritid fly *Oxyna nebulosa* in 2020. Between mid-June and late July adults emerged from the galls and were set up in no-choice oviposition and larval development tests with several test plant species of importance to North America. 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 2022.

13.4 *Cyphocleonus trisulcatus* (Col., Curculionidae)

In May and June, we set up no-choice oviposition and larval development tests with this root-mining weevil exposing 11 non-target plant species that are either native or of horticultural value in Australia. No larvae were found in any new test species. In addition, in mid-June, we set up an open-field test with seven non-target species and cultivars that supported larval development under no-choice conditions. These plants are currently being dissected for larvae.

Our garden technicians, Florence Willemin and Quentin Donzé, preparing the open-field test with *Cyphocleonus trisulcatus*

14 Field Bindweed (*Convolvulus arvensis*)

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Convolvulus arvensis plants from different years and origins (Europe and North America) grown in the CABI garden

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. In 2021, owing to a long period of rain and violent storms, the test was postponed until the end of June. Seven test plant species (six 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 cereal field. Plants were checked once a week and fitness was recorded. The plants have been harvested on 23 July and are currently being dissected to record feeding and larval development. Wild *C. arvensis* plants growing close to the exposed test plants were also harvested to determine the natural attack rate at the field site. Detailed results will be presented in the annual project report.

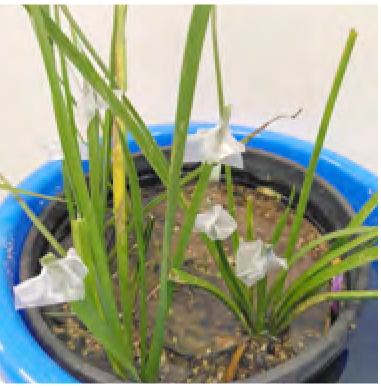
14.2 *Microsphecia brosiformis* (Lep., Sesiidae)

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.

Moth eggs were transferred onto potted plants of five native NA test species and *C. arvensis* in August 2020. The plants were kept in an unheated greenhouse over winter. All plants were dissected in May 2021. None of the test species were found to be attacked, while all field bindweed roots were mined and four larvae were found. This is a very positive result. However, two things need to be improved. Many of the test plants died over winter due to cold weather conditions, and the development on control plants was not satisfactory, although heavy mining and larval canals were found. For these reasons, we will start dissecting plants earlier, i.e. at the beginning of November. This will confirm development of first to third instar larvae. Only plants supporting this early development will subsequently be tested for full development by offering plants with a large enough rhizome to support development of later instar larvae.

Since the species is hard to collect in the field, we tried to establish a rearing colony in 2019 and 2020, by transferring eggs onto specially prepared field bindweed plants under common garden conditions. In 2021, adults started to emerge mid-July and are expected to continue emerging in August. Females will be mated and resulting eggs will be used to continue egg transfer tests.

Microsphecia brosiformis larva mining in *Convolvulus arvensis* root



Cut leaves of flowering rush onto which second instar larvae of *Bagous nodulosus* were transferred to improve rearing success. Cut leaves were taped with parafilm after transfer to make sure that larvae do not "escape".

15 Flowering Rush (*Butomus umbellatus*)

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15.1 *Bagous nodulosus* (Col.: Curculionidae)

As in 2020, a sufficient number of weevils could be collected from our artificial pond in the CABI garden to complete all host-specificity tests and carry out further rearing trials. We finally were able to purchase two missing test plant species, *Blyxa aubertii* and *Limnobium spongia*. Unfortunately, *L. spongia* sent by our partners in the U.S. again did not survive the shipment. Host-specificity tests included oviposition and adult feeding tests, no-choice and single-choice establishment tests with larvae, and larval development tests over a longer for three species, where larval feeding and survival had been found during the 5-days establishment tests. No larvae were found to survive 10 days on any of the species tested (*Alisma plantago-aquatica*, *Limnobium spongia*, *L. laevigatum*, and *Hydrocharis morsus-ranae*). All data are being summarized for the petition for field release that is planned to be submitted by our North American partners before the end of the year.

In 2021, we continued trying to improve rearing techniques for *B. nodulosus*. Transferring first instar larvae into cut leaf sections kept in tight closing Petri dishes, and then transferring second instar larvae into leaves of potted plants seems to significantly increase rearing success. Trials are not completed and analyzed yet, but it appears that this combination will be an important step forward to be able to rear this species in quarantine in North America.

15.2 *Phytoliriomyza ornata* (Dipt.: Agromyzidae)

In 2021, we focussed on increasing the rearing colony and improving the rearing success of *P. ornata*. From 23 plants exposed to adults of the first generation, we obtained about 380 pupae (16 pupae per plant, compared to nine in 2020). About 70 pairs of the second generation were setup on 35 additional plants. Overall rearing success will be analysed in August/September 2021.

In addition, we set up another impact experiment. Two pairs of the fly were released onto ten potted, gauze-covered plants; ten control plants were set up in parallel. We will leave plants covered until September, assuming that flies of the first generation will emerge, mate and re-infest plants, thus allowing us to quantify the impact caused by both generations.

15.3 *Doassansia niesslii* (Fungi, Doassansiaceae)

The key issue in the research on the smut fungus *D. niesslii*, is the need to find alternative smut isolates with high infectivity towards the two most common genotypes of flowering rush invading North America, i.e. genotypes 1 and 4.

In 2020, John Gaskin (USDA, ARS) was able to confirm that some of the flowering rush specimens collected in 2019 in Leeuwte, the Netherlands, match with genotype 1 in North America based on AFLP analysis. The assumption is that any *D. niesslii* strain associated with *B. umbellatus* at this location would therefore be pathogenic to genotype 1 plants from North America. Due to COVID-19 related travel restrictions, it has not been possible to visit this site in 2020. Surveys will be conducted as soon as it is possible in 2021 or 2022.

In addition, herbarium samples of *B. umbellatus* from a large geographical range in Europe and Asia were sourced from the Royal Botanic Garden Edinburgh and sent to John Gaskin for further analyses (for details see annual report 2020). Unfortunately, the DNA was too degraded to obtain good AFLP data. Results nevertheless suggest that samples are unlikely to match with any of the six invasive genotypes.

Artificial pond for maintaining test plants, flowering rush and a rearing of *Bagous nodulosus* in Switzerland

16 Yellow Floating Heart (*Nymphoides peltata*)

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In winter, we intensified our literature searches using online databases and monographs to investigate whether there are any herbivores and/or fungal pathogens recorded to be specific to *Nymphoides peltata* in Europe. Our collaborators Dr Mark Volkovitsh and his colleagues (Russian Academy of Sciences, Zoological Institute, St Petersburg) also reviewed the Russian literature for any herbivores associated with *N. peltata*. We found one weevil (*Bagous charbinensi*) and two fungal pathogens (*Septoria villarsiae* and *Uromyces nymphoidis*) that are likely to be specific to *N. peltata* and that may therefore have potential as biological control agents.

The weevil *B. charbinensi* is recorded from China. Nathan Harms from the Army Corps of Engineers, leading the project in the U.S. and collaborators have collected several Bagous species on flowering rush in China and South Korea and it remains to be seen whether *B. charbinensi* is one of them. *Septoria villarsiae* seems to be widespread in Europe and is already present in the U.S. *Uromyces nymphoidis*, a rust fungus, has been recorded from Romania and southern Russia. *Uromyces* species have previously been used as biological control agents and we therefore decided to conduct a specific survey to try and find the rust.

From July to September we plan to conduct several field trips in Europe (i.e. Switzerland, the Netherlands, Romania) to look for the pathogen *U. nymphoidis* and to collect leaves for molecular and chemical analyses. Furthermore, we will collect additional data on the *N. peltata* populations in Europe (e.g. plant density) for comparison with Asian and North American populations.

Nymphoides peltata growing in a channel in the Netherlands

Inoculation of *Aculus mosoniensis* on a seedling (top) and symptoms observed 24 days after the inoculation (bottom)

17 Tree of Heaven (*Ailanthus altissima*)

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

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The main biological control agent currently investigated for tree of heaven is the eriophyid mite *Aculus mosoniensis*. The mite was first discovered in Europe by BBCA in 2016 and has so far been recorded in 13 different European countries. Two open-field tests were performed in Italy in 2019 and 2020, exposing 13 nontarget plant species, selected either for their phylogenetic or ecological similarity to the target weed or their economic importance. The results obtained were very promising. *Aculus mosoniensis* was not able to multiply on any of the nontarget species tested, and none of them appears to be a potential host of this mite. Moreover, the mite did not negatively impact any of the nontarget species tested.

Field observations suggest that *A. mosoniensis* can have a significant impact on tree of heaven, inducing vascular necrosis and even death, especially on young shoots. To quantify the impact of the mite, an experiment has been initiated in 2021 in Italy. Plants to be used in the impact experiment were either started as roots, cuttings or seeds. More than 30 roots and 150 cuttings of tree of heaven were collected at the beginning of spring from a natural site known for being mite free, and around 150 seeds. Roots and cuttings were transplanted in pots and arranged in a garden plot. Some of the shoots obtained have been infested with the mite (twice, two weeks apart, with at least 100 mites), while some others are being kept free of mite attack using an acaricide (control group).

Unfortunately, most of the cuttings did not survive, and they were therefore excluded from the experiment. Despite the high number of seeds sowed, not many germinated and only four seedlings could be included in the experiment, two were infested and two kept as controls. The impact observed so far on the two infested seedlings is very promising (e.g. narrow, deformed and twisted leaves, with edges folded or rolled upward lengthwise). A good number of sprouts was obtained from roots and currently, at least 10 of the 28 infested shoots show already signs of mite attack, and the first symptoms have appeared in less than 10 days from the inoculation of the mites. So far, none of the 17 shoots treated with acaricide show any indication of mite attack. Detailed results will be available by the end of summer.

Setup to evaluate the impact of the mite *Aculus mosoniensis* on tree of heaven at BBCA in Rome

18 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.

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. and the stem-mining weevil *Listronotus marginicollis*. We plan to import *Lysathia* sp. from the mass rearing facility, run by the Centre of Biological Control (CBC), Rhodes University in South Africa to CABI Switzerland in the next few weeks and initiate host range testing as well as thermal physiology studies. Currently we have been working on boosting our cultures of parrot's feather in anticipation of the insects to arrive. In addition to this we are sourcing and propagating various test plant species that are considered critical to be tested with these agents in a Canadian context.

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

Parrot's feather, *Myriophyllum aquaticum,* in the CABI quarantine awaiting the arrival of *Lysathia* sp. from South Africa

Myriophyllum heterophyllum collected in Europe for host range testing of *Lysathia* sp.

Robinia pseudoacacia infestation along the Rhine river in southern Germany

19 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 U.S., 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 2020. 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*.

Due to the cool and very wet spring and summer season in 2021, population levels of the known insects associated with black locust have been extremely low and thus far no collections for shipments to South Africa have been possible. During surveys however, leaf rippling and curling was noticed which is unlike what is caused by the known insects. Upon inspection, an eriophyid mite was discovered. We have setup a rearing of this mite in order to obtain information on the biology and impact and to obtain an identification.

Robinia pseudoacacia leaf rippling and curling (damaged leaves left and middle, undamaged leaf right) caused by the presence of an unknown eriophyid mite

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

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Daisuke Kurose and Djami Djeddour inoculating Japanese knotweed plants with *Mycosphaerella polygoni-cuspidati*

20.1 *Aphalara itadori* (Hemiptera, Psyllidae)

A new line of the psyllid, collected in Murakami, Niigata Prefecture, Japan in 2019 (Murakami line), was found causing severely curled leaves. This region has a much better climatic match to the invaded range based on Climex modelling.

A study comparing the performance of the Murakami line on *F. japonica* and *F.* x *bohemica* showed that the rate of development from egg to adult was higher on *F.* x *bohemica* than on *F. japonica*. *F. x bohemica* also suffered more severe curling damage on young leaves on main stems and leaves on side shoots compared with *F. japonica*. The results suggest that the Murakami line prefers, and has a greater impact upon, *F.* x *bohemica* plants.

Host range testing with the Murakami line has been completed for nine test plant species including *Fallopia cilinodis* and *Fagopyrum esculentum* sourced from Canada. No-choice and multiple-choice oviposition tests showed eggs were laid and adults developed on some of the non-target test plant species. However, the results of the host-specificity testing for both the Murakami and the original Kyushu lines of *A. itadori* were very similar. The data was shared with partners from Canada, the U.S. and the Netherlands to support a petition to release the Murakami line into the respective countries.

Release permits for the Murakami line were obtained from authorities in the Netherlands in 2020 and Canada and the UK in 2021. In the Netherlands, the first releases took place on *F.* x *bohemica* populations in 2020 and adults have successfully overwintered at all sites. In the UK, the Murakami line was released into *F. japonica* and *F.* x *bohemica* populations in June-July 2021. The number of eggs and adults and any curling damage at the release sites are currently being assessed. Furthermore, plans are well-established regarding the hosting of Dr. Ian Brown from Canada after August/September 2021.

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

The fungal pathogen *Mycosphaerella polygoni-cuspidati* has been ruled out as a classical agent for Japanese knotweed as it can cause limited disease symptoms and form the first stage of its life cycle on critical UK and US native non-target species. However, the pathogen is currently undergoing evaluation as a potential mycoherbicide. Such mycoherbicide would be based on a single mating-type isolate thereby preventing reproduction, persistence and spread of the pathogen in the field and allowing for targeted application. A European patent held in the name of the Secretary of State for Environment, Food and Rural Affairs, UK, protects the idea in selected countries; a patent application has also been filed in Canada in March 2021. Funds from British Columbia have previously supported the proof-of-concept research.

Supported by funds from the UK and the Netherlands experimental field trials conducted at CABI UK confirmed that *M. polygoni-cuspidati* can infect its host outside the greenhouse. The analysis of data collated over three seasons (2019-2021) will allow us to assess performance of the pathogen under these more natural conditions and inform the future course of research.

Daisuke Kurose and MSc student Tade Irelewuyi releasing the Murakami line of *Aphalara itadori* into a *F. japonica* population

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

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

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

Himalayan balsam is an invasive alien weed in both Europe and North America and can cause serious impacts on biodiversity, river networks and infrastructure. In 2006, CABI-UK initiated a biological control programme for this weed and in 2014, the highly-specific rust fungus *Puccinia komarovii* var. *glanduliferae,* was approved for release. Since then, the rust has been released at sites in England, Wales and Scotland, where susceptible *Impatiens glandulifera* biotypes exist. On fully susceptible plants, the rust is performing well, adapting to local climatic conditions and spreading from the initial area of release. Good leaf infection is frequently observed in the summer and the rust is able to survive the winter and establish in stands of Himalayan balsam the following year.

In parallel to the UK work, the rust is also under evaluation for control of *I. glandulifera* in Canada, where the weed occurs in eight provinces. A total of 47 non-target species of relevance to North America have been tested, with no non-target impacts observed.

Two strains of the rust, one originating from India and the other from Pakistan, have been released at a number of field sites. In order to determine which of the two strains is most suitable for each population, pre-release susceptibility testing is conducted in the glasshouse before release. In some instances, neither of the rust strains has been pathogenic, necessitating the requirement for additional strains to be identified. Assessment of six Canadian populations of the weed found many to be resistant to the strain from Pakistan and for the Indian strain, levels of infection were much lower than that observed on the positive control plants from the UK. Therefore, additional rust strains are also required for control to be successful in Canada. In order to ascertain where in the native range new strains should be sought, a molecular study was conducted. This study has been extended, using a total of 42 leaf samples from 17 Canadian populations in British Colombia. The results indicate that the two haplotypes present in Canada are identical to the two most common ones in the UK, which further supports the suggestion that the plant was introduced into Canada from the UK.

In January 2020, permission to export a new strain of the rust from Karchon, Uttarakhand, India was granted. However, this strain failed to infect many of the UK and all Canadian populations assessed and was therefore deemed unsuitable. In order to tackle these rust-resistant populations, further surveys are to be conducted by an in-country collaborator in the north-eastern region of Pakistan during August-September 2021.

Kate Pollard training collaborators in the Himalayan balsam rust release and monitoring protocol

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