Weed Biological Control
Progress Report 2023
CABI in Switzerland
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KNOWLEDGE FOR LIFE
Contents

Notes from the section leader 2

1 Dalmatian and Yellow Toadflax (*Linaria* spp.) 4
2 Houndstongue (*Cynoglossum officinale*) 5
3 Hawkweeds (*Pilosella* spp.) 6
4 Russian Knapweed (*Rhaponticum repens*) 7
5 Garlic Mustard (*Alliaria petiolata*) 8
6 Common Reed (*Phragmites australis*) 9
7 Whitetop or Hoary Cress (*Lepidium draba*) 10
8 Dyer’s Woad (*Isatis tinctoria*) 11
9 Perennial Pepperweed (*Lepidium latifolium*) 12
10 Swallow-Worts (*Vincetoxicum* spp.) 13
11 Common Tansy (*Tanacetum vulgare*) 14
12 Russian Olive (*Elaeagnus angustifolia*) 15
13 Oxeye Daisy (*Leucanthemum vulgare*) 16
14 Field Bindweed (*Convolvulus arvensis*) 17
15 Flowering Rush (*Butomus umbellatus*) 18
16 Tree of Heaven (*Ailanthus altissima*) 19
17 Parrot’s Feather (*Myriophyllum aquaticum*) 20
18 Black Locust (*Robinia pseudoacacia*) 21
19 Understanding the drivers of invasion of cheatgrass (*Bromus tectorum*) 22
20 Japanese Knotweed (*Fallopia japonica*) work in the UK 23
21 Himalayan Balsam (*Impatiens glandulifera*) work in the UK 24

Distribution list 25

Cover photos: Alice Pessina collecting test plants in Dijon, France; Loughlin McQueen collecting common reed at a field site in Switzerland; Laura Kostyniuk setting up an open-field test in the CABI garden.
Notes from the section leader

I am pleased to be sending out the progress report for 2023. The updates from each of the research projects below are testament to the Team, their enthusiasm and dedication for which I am grateful.

On the project front, in 2023, we were able to get two new projects off the ground; the biological control of black locust (Robinia pseudoacacia) for South Africa; and, although not strictly biological control development, a study of the drivers of invasion of cheatgrass (Bromus tectorum), in the hopes that this will guide decisions on the biological control prospects for annual grasses. Some of the other newer projects including tree of heaven (Ailanthus altissima), and parrot’s feather (Myriophyllum aquaticum), have gained traction and are making excellent progress, while the older projects continue to do well and move forward.

It has been an exciting year with several ‘new’ agents making it to their new ‘homes’ abroad. The hoverfly, Cheilosia urbana, on hawkweeds was successfully sent with viable eggs arriving in both Canadian and US quarantine facilities. We were also able to send over the stem and rhizome feeding weevil Bagous nodulosus against flowering rush, which is planned for release in Canada later this summer. The rhizome-feeding tortricid moth, Dichrorampha aeratana and the gall forming eriophyid mite, Aceria angustifoliae were both released in Canada this year against their hosts, oxeeye daisy and Russian olive, respectively. Although our main focus remains North America, it is worth noting that the Team is active in biocontrol projects in other countries such as Pakistan, South Africa and Australia.

The CABI Switzerland Centre has been busy this year hosting several international collaborators. Our first visit was from Mikenna Smith from Wyoming. For over three decades the Wyoming Weed and Pest Council (WWPC) has partnered with CABI and helped fund projects to slow the spread of invasive species in Wyoming. We then had Dr Natalie West (USDA-ARS Sidney, MT) at the Centre to gain experience working with Bagous nodulosus, ahead of possible release in the USA in the near future. David Harris, a PhD student from SUNY ESF, New York State, came over to collect and learn about the defoliating beetle Chrysochus asclepiadeus against Swallow-worts in North America. Finally, we had Dr Peter Toth from the Slovak University of Agriculture in Nitra, Slovakia, visiting to work on field bindweed.

The XVI ISBCW in Puerto Iguazu, Argentina from 7-12 May was certainly a highlight for the Team, especially considering it is the most important global meeting for weed biological control scientists and practitioners and it usually takes place every 4 years. The presentations by CABI scientists covered aspects of our research including project updates as well as policy and regulations in relation to the Nagoya Protocol. In addition to the presentations, several of us were involved in workshops, covering aquatic weed biocontrol, how do we deal with oligophagous candidate agents and promoting weed biocontrol in Europe. All in all, it was an extremely successful meeting, with several opportunities for networking, developing new projects, expanding existing ones and discussing new research ideas. There was also plenty of time to rekindle old friendships and develop new bonds, the ISBCW group really does represent the global weed biological control family.

An important aspect of our work is education and raising awareness of invasive species and biological control as a sustainable option for their management. At CABI we pride ourselves on the summer internship program where we take on students from around the world to learn and engage with weed biological control through practical work on current projects. This summer we have six students from, Switzerland, Italy, Peru and Canada. In addition, Alice Pessina completed her MSc on the host-finding behavior of a candidate biological control agent against parrot’s feather and Lauréline Humair has started her PhD part-time, studying the role that biological control of tropical weeds plays in temperate climates. I would also like to mention that the NAISMA biological control committee, through the NAISMA program, developed a free BioControl 101 short course that will be available online soon, so go check it out.

To finish off, I would like to take this opportunity to thank my colleagues, both staff and students at CABI, our international and local collaborators and funders for their support, without which none of this would be possible.

Wishing you all the best, and happy reading.

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CABI would like to thank the following entities for their support. Without them, our work would not be possible.
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)

In 2022 we continued the rearing of the remaining genotypes of R. pilosa using an adapted protocol due to the unpredictable weather conditions in recent years. The new method involves rearing in smaller mobile cages with 15 L. vulgaris plants planted in trays. This allows the cages to be moved to the greenhouse or covered with plastic sheaths to protect weevils from lower temperatures. A total of 15 copulated females were used for the propagation of three different R. pilosa genotypes (A3, A13, and B6). This method resulted in 402 induced galls. Due to the cold weather during May and June, the weevils are in different development stages inside galls, so the number of adult weevils will only be known after gall dissections in mid-August.

Between 26 April and 15 May, six collection trips were conducted for R. pilosa galls in eastern Serbia. In this period, a total of 71 galls were collected, demonstrating the sparse occurrence of R. pilosa in the field. However, it seems that the cooler weather during April and May also affected the population density of inquilines R. eversmanni, so many of the collected galls were free of attack. This may suggest a recovery of R. pilosa populations in the field in 2024 caused by environmental events in the past three years.

For R. rara, a total of 448 adults survived hibernation (32%) and were used for rearing. Individual potted plants with new shoots from rosettes were set up for oviposition. The method avoided overgrowth and maturation of the shoots, which are not suitable for gall induction. Five copulated females were set up for a duration of 15 days, where every three days the plants were replaced for oviposition. This method was successful, resulting in 116 induced galls, which were dissected on 26 June to retrieve a total of 120 adults.

1.2 Mecinus spp. (Col., Curculionidae)

In 2023, work concentrated on establishing new rearing colonies of Mecinus heydenii, M. laeviceps and M. peterharrisi. In spring, 35 pairs of M. heydenii survived overwintering and were set up in 15 framed cages, each planted with 15 yellow toadflax plants. The dissection of plants is planned for the first half of August. The rearing of M. laeviceps, involved 14 pairs set up between 9 April and 15 May on seedlings of L. dalmatica and L. genistifolia germinated in mid-December 2022. The growth tips of each plant were carefully clipped several times to produce young growing shoots 3–4 cm in length for oviposition. The plants were dissected between 16 and 19 July. A total of 446 adults emerged, an average of 31.9 adults per female. The rearing of M. peterharrisi followed the same method using L. dalmatica ssp. macedonica and L. dalmatica. The results of this rearing will be known after dissection planned for mid-August.

In general, the methods applied for the rearing of Mecinus spp. were successful in 2023. However, the applied methods are time-consuming and confirm that for successful rearing, the phenostage of the plants exposed to the weevils plays a crucial role.
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 1700 *M. borraginis* larvae collected in 2022, about 280 adults emerged in September 2022, and an additional 520 in March 2023 which were set up on houndstongue rosettes and/or shipped to the USA. At the end of May/beginning of June, about 100 females were retrieved and reset on 47 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. About 4200 *M. borraginis* larvae emerged and were separated into cups (30 individuals per cup) and placed in an underground insectary for adult emergence in autumn 2023 and spring 2024.

In spring 2023, a total of 165 adults of *M. borraginis* were sent to the quarantine facility at the University of Idaho (UoI) run by Mark Schwarzländer. Currently, work has focused on developing the most effective way to rear *M. borraginis* under quarantine conditions to maximize space and number of adults.
3 Hawkweeds (*Pilosella* spp.)

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3.1 *Aulacidea pilosellae* (Hym., Cynipidae)

Two biotypes of this gall wasp are being investigated. We are currently testing a population from Poland of the first biotype *A. pilosellae ex Pilosella* spp. from the northern range (eastern Germany, Poland and the Czech Republic). 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 (AAFC) in Lethbridge showed that biotype 2 carries Wolbachia, which is thought to be responsible for the biotype differentiation. The rearing colony of biotype 2 at CABI collapsed in 2022. We are presently focusing on completing the testing with biotype 1. Batches of galls harvested from lab infested plants in 2022 were taken out of cold storage in May 2023. Adults emerging from these were used in host range tests and for rearing.

In 2023, the emergence rates of *A. pilosellae ex Pilosella* spp. were relatively good (33-80%, on average 62%), while no adults emerged from *Hieracium argutum* that developed galls in a no-choice test in 2022. This species was exposed in single-choice tests in 2023. In addition, we exposed three test plant species in no-choice tests. These tests are ongoing and the final results will be presented in the annual report.

Of the 84 species, subspecies and populations tested with *A. pilosellae ex P. officinarum*, and 74 with *A. pilosellae ex Pilosella* spp., galls were only found on the genera *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).

3.2 *Cheilosia urbana* (Dipt., Syrphidae)

In 2023 the period of activity of *C. urbana* was much delayed by a particularly cool and wet April. The first individuals were collected in the CABI garden in the beginning of May and shipped to AAFC, Summerland. Larvae emerging from the eggs upon arrival were transferred onto potted plants for field releases at a later date. The weather remained unstable and the next field collections were postponed until 21 May, by which time the period of activity was coming to an end. Only one individual could be hand carried to the USA by visiting scientist Dr Natalie West who delivered it to Dr Jeff Littlefield, MSU, Bozeman.
4 Russian Knapweed (*Rhaponticum repens*)

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

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

The host range testing has largely been successfully completed with a total of 73 test plant species and/or varieties screened. Of these, only four species (*Plectocephalus americanus, P. rothrockii, Cynara cardunculus* and *C. scolymus*) supported limited adult development. During survival tests with these test plant species, multiple generations were possible, however, it is clear that these plant species are suboptimal hosts with low levels of oviposition coupled with high levels of mortality. In subsequent choice tests the test plant species were attacked to a limited degree and there was 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 cages, under quarantine conditions to have some non-target feeding and oviposition. We have already initiated oviposition latency tests and plan to run host finding behavior tests to better understand the host choice and preference.

4.2 *Cassida* sp. (Col., Chrysomelidae)

During surveys in 2022 of the Karatal River in south-western Kazakhstan, the tortoise beetle, *Cassida* sp. was collected. Although it remains unidentified, genetic analysis confirmed it is the same species that has been recorded on surveys in 2018 and again in 2019. Unfortunately, we were unable to overwinter a population in the CABI quarantine into 2023, but preliminary host range testing was promising and it would be worth trying to recover this species again on future surveys.
5 Garlic Mustard (*Alliaria petiolata*)

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5.1 *Ceutorhynchus constrictus* (Col., Curculionidae)

Between 1 March and 13 April, 445 adults of the seed-feeding weevil *Ceutorhynchus constrictus* emerged from our rearing colony established in 2022. After testing for oviposition, fertile females were used at CABI for rearing on *A. petiolata* and host range tests with the native North American *Leavenworthia stylosa* and *Stanleya pinnata*. Oviposition of *C. constrictus* was only found on garlic mustard and feeding was mostly superficial on the test plants with limited seed damage. Since feeding with seed damage was found on *B. perstellata* in previous no-choice tests, we exposed this species in a single-choice test, where adult feeding only damaged one *B. perstellata* seed (< 0.1% of the seeds, vs 31% on *A. petiolata*).

In June, about 1100 mature larvae of *C. constrictus* were harvested from garlic mustard (test controls and rearing plants) for adult emergence in spring 2024.

5.2 *Ceutorhynchus scrobicollis* (Col., Curculionidae)

Between May and July, adult *C. scrobicollis* emerged from rearing plants infested with field collected adults in October 2022. So far, 265 old and newly emerged adults were recovered from rearing plants. These weevils were split, with three females and two males kept on potted garlic mustard rosettes for aestivation. Weevils will either be shipped to AAFC in autumn or kept at CABI to maintain the rearing.
6 Common Reed (*Phragmites australis*)

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6.1 *Archanara neurica* and *Lenisa (Archanara) geminipuncta* (Lep., Noctuidae)

In 2023, we continued producing as many pupae and eggs of both moth species as possible to support further field releases in Canada. After the issues using artificial diet in 2022, we decided to rear more larvae on stems this year. About 750 newly hatched *A. neurica* and 2000 *L. geminipuncta* larvae were transferred into stems of *Phragmites australis*. Of these, 165 fourth and fifth instar larvae of *L. geminipuncta* were transferred from stems onto artificial diet. While more than 2100 first instar larvae of *A. neurica* were transferred onto artificial diet.

In general, we observed a higher larval mortality than in other years for both species, both on artificial diet and on stems. We suspect that this could be explained by a decrease in fitness of offspring of moths that had been reared on artificial diet the year before. If further analyses confirm this hypothesis, rearing on artificial diet loses its advantages and may not be recommended in future.

Nevertheless, we were able to produce about 360 pupae of *L. geminipuncta* and 260 pupae of *A. neurica*. In June, over 100 *L. geminipuncta* and 40 *A. neurica* pupae were sent to Ottawa. The remaining pupae are being used for egg production at CABI. So far over 7000 eggs were obtained from *A. neurica*, and about 5000 from *L. geminipuncta* of which a part will be sent to Canada for field release.
7 Whitetop or Hoary Cress (*Lepidium draba*)

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7.1 *Ceutorhynchus cardariae* (Col., Curculionidae)

In spring 2023, we conducted an open-field test with three North American species that had supported gall development under choice tests in previous years. These included *Lepidium paysonii*, *Planodes virginicum* and *Rorippa sinuata*, of which only a single plant of *P. virginicum* showed signs of a single gall and during dissection an L2 larva was recovered. This is in comparison with the control plants which had an average of eight galls per plant. We will send the larvae from *P. virginicum* for genetic identification to be sure it is *C. cardariae*.

In addition to this, we set up a survival test with *Lepidium paysonii*, to see whether the weevil would be able to sustain a population over time. We were able to obtain a total of five females from *L. paysonii*, thus these were split with the same number of males between three plants and control plants were set up in parallel.

We also continued maintaining a rearing colony of the weevil at the Centre. Of the 810 adults that overwintered at CABI from 2022, about 77% survived. The reared and field collected adults were set up on potted plants and about 860 *C. cardariae* emerged in 2023. A consignment of 100 *C. cardariae* females and 50 males were sent to the quarantine facility at the University of Idaho (UoI) run by Mark Schwarzländer in April 2023.

7.2 *Ceutorhynchus turbatus* (Col., Curculionidae)

On 2 June, about 600 adults of the seed-feeding weevil *C. turbatus* were collected in southern Switzerland. Between 2 and 13 June, we established no-choice oviposition tests with nine plants of two non-targets, *Lepidium montanum* and *Aubrietia deltoidea* and five *L. draba* control plants. No eggs were recovered from either of the species. This year we were fortunate enough to be able to set up some development tests with four non-target species and *L. draba* as a control. Apart from the control plants which produced on average 11.8 larvae per plant, no development was recorded on any of the test plant species.
8 Dyer’s Woad (Isatis tinctoria)

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8.1 Ceutorhynchus peyerimhoffi (Col., Curculionidae)

Over 1000 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 5000 larvae were collected and are being kept for adult emergence in 2024.

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. We were finally able to test Lepidium montanum for development. Of the seven plants set up there was no larvae development recorded compared with an average of 21 larvae emerging from the control plants.

8.2 Ceutorhynchus rusticus (Col., Curculionidae)

A total of 19 plants, from three test plant species and the control, were exposed to C. rusticus in no-choice oviposition and development tests in autumn 2022. Test plants were regularly checked and any dying plants were dissected. Adult emergence was successful in 2023, with all three plants kept for adult emergence supporting development with an average of 12.7 adults per plant. Of the three test plant species exposed, none supported adult development and no typical C. rusticus mining was recorded. We are planning to setup an additional open field test in southern Germany with plant species that have been able to support adult development in previous tests.

Summer student, Elsa Maria Arriarán Silva, placing test plants into the garden bed.
9 Perennial Pepperweed (Lepidium latifolium)

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9.1 Ceutorhynchus marginellus (Col., Curculionidae)

We continued our investigation on the host range of the gall-forming weevil Ceutorhynchus marginellus in the quarantine facility at CABI. We exposed a total of 22 North American native, non-target species and perennial pepperweed plants under no-choice conditions. Although adults emerged from seven of the non-target species (i.e., from Caulanthus lasiophyllus, Streptanthus glandulosus ssp. pulchellus and from five Lepidium species) these were fewer compared to the simultaneously set up perennial pepperweed plants.

Single choice tests were set up with 11 non-target species that supported adult or gall-development under no-choice conditions in 2023, or in earlier years, to determine whether these would also be accepted for oviposition by C. marginellus when simultaneously being offered perennial pepperweed. Under these conditions, limited larvae were found on four North American native species, i.e., Lepidium alyssoides, L. ramosissimum and Streptanthus lasiophyllus and L. barnebyanum, a federally listed endangered species. The number of larvae on the non-target species was always much lower than on the simultaneously exposed control plants.

In April and May, we also sent a total of approximately 1000 adults of C. marginellus to Mark Schwarzländer at the University of Idaho for studies on the host-finding behavior of this weevil. In addition, we continue to maintain a rearing colony of C. marginellus in our quarantine facility.
10 Swallow-Worts (Vincetoxicum spp.)

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10.1 Chrysochus asclepiadeus (Col., Chrysomelidae)

The root-mining beetle *Chrysochus asclepiadeus* can develop and emerge as an adult over 1-3 years. We usually collect adults in Franche Comté, France, once a year, between the end of June and the end of July. These are stored in containers with cut shoots of swallow-worts and eggs are regularly collected to conduct no-choice larval transfer tests with freshly hatched larvae. The infested plants are kept over winter in an unheated greenhouse (above freezing temperatures). The roots and soil are checked for larvae the following year in May. Plants with larvae are kept for up to three years for adult emergence.

In May 2023, we found larvae in six of 10 *V. hirundinaria*, and 26 of 33 *V. nigrum* (overall 3.5 larvae per plant). No larvae were found in any of the 11 test plant species exposed in 2022. So far, adults (n=15) emerged from three *V. hirundinaria* and three *V. nigrum* infested in 2021, and two *V. nigrum* infested in 2022. In July, we field-collected approximately 120 additional adults.

In tests conducted between 2019 and 2022, no adults emerged from a total of five larvae found on *A. incarnata*, *A. rubra*, *A. syriaca* and *A. tuberosa*. Larval survival after about eight months on these test plants was less than 2% vs. about 20% on the controls. None of the fifteen other exposed test plants (10 North American (NA) natives) were attacked. These are encouraging results, similar to previous tests with the Ukrainian population of the beetle.
11 Common Tansy (*Tanacetum vulgare*)

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11.1 *Microplontus millefolii* (Col., Curculionidae)

Host-specificity testing with the stem-mining weevil *Microplontus millefolii* is almost complete, but so far, we were not able to expose a large number of the North American native *Tanacetum huronense* under multiple-choice conditions. The majority of the host-specificity testing was conducted by Dr Margarita Dolgovskaya and her team (Zoological Institute Russian Academy of Sciences, St Petersburg). Since only a few plants of *T. huronense* produced stems in St Petersburg, we conducted an open-field test with this species as well as the closely related native species, *T. camphoratum*, at CABI in Switzerland using a *M. millefolii* population collected in northern Germany. The experiment was evaluated in early July and an average of 15.2 eggs and larvae were found on *T. vulgare*, 3.6 on *T. camphoratum* and 2.5 on *T. huronense*. The attack rates on both target and non-target plants were higher than what was found in previous tests conducted in St Petersburg.

11.2 *Chrysolina eurina* (Col., Chrysomelidae)

In spring 2023, we evaluated the open-field test that had been set up in October 2022 with the leaf-feeding beetle *Chrysolina eurina*, exposing *T. huronense* and *T. camphoratum* and the target species, *T. vulgare*, as a control. Between April and June, we found on average 3.0 larvae or newly developed adults per plant on *T. vulgare*, 2.8 on *T. camphoratum* and 1.8 on *T. huronense*. The numbers found on *T. vulgare* in 2023 are about three times lower than what was found in the open-field test set up in 2021 and may be explained by the smaller size and lower quality of the *T. vulgare* plants used in the current test. Our results indicate that it cannot be excluded that the beetle would attack native *Tanacetum* species to a certain degree if released as a biocontrol agent in North America.

In March 2023, we also continued to set up no-choice larval development tests with 37 non-target species and *T. vulgare* as controls. We transferred five first-instar larvae each, to Petri dishes containing a leaf of a non-target plant or of *T. vulgare*. On most of the non-target species all larvae died before reaching their second instar, but a few larvae exposed to the North American natives *Artemisia californica*, *Matricaria discoidea* and *M. occidentalis* developed to their fourth instar and for three additional species a few larvae developed to their second or third instar. Development to adult was only successful on *T. vulgare*.

11.3 *Gillmeria ochroductyla* (syn.: *Platyptilia ochropectyla*) (Lep., Pterophoridae)

In April, no-choice larval transfer tests were also conducted with the flower-head and stem-mining moth *Gillmeria ochroductyla* using 15 non-target species and *T. vulgare* as a control. Larval feeding was observed on nine of the non-target species, but complete development to the adult stage was only observed on three of them (*Matricaria occidentalis*, *M. discoidea* and *Cotula coronopifolia*).
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 2019. In 2020, both the USDA-APHIS Technical Advisory Group (TAG) and the Canadian Biological Control Review Committee recommended release of the mite. In spring 2022, the Canadian Food Inspection Agency (CFIA) approved field release of the mite in Canada. In October 2022 a pure culture of the mite was sent over to Dr Rosemarie DeClerck-Floate in Lethbridge. This spring the mite was released from their quarantine at two sites in southern Alberta and two sites in British Columbia using a technique of wrapping the buds in parafilm. There appears to be the first signs of galling on the trees under outdoor conditions, which is promising.

*Aceria angustifoliae* leaf galls in Canada. Photo credit: Rosemarie De Clerck-Floate.
13 Oxeye Daisy (*Leucanthemum vulgare*)

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13.1 *Dichrorampha aeratana* (Lep., Tortricidae)

A petition for field release of the root- and rhizome-feeding tortricid moth, *Dichrorampha aeratana*, was submitted to the Canadian and U.S. authorities in 2021 by Rose DeClerck-Floate and Jeff Littlefield, respectively. In June 2022, the USDA-APHIS Technical Advisory Group (TAG) recommended release, and in July 2022, the Canadian Food Inspection Agency approved release in Canada. First releases of the moth took place in Canada at field sites in British Columbia and Alberta in June 2023.

In May, we sent approximately 1000 eggs of *Dichrorampha aeratana* to Rose DeClerck-Floate to supplement the rearing colony at AAFC in Lethbridge. We also provided approximately 500 eggs to Jeff Littlefield, who used them to set up a rearing colony at the quarantine facility at Montana State University in Bozeman, Montana. At CABI, we are continuing to maintain a rearing colony of *D. aeratana* in order to facilitate future shipments to North America and Australia.

13.2 *Oxyna nebulosa* (Dipt., Tephritidae)

In May and June, we harvested more than 3000 galls from the oxeye daisy plants that had been exposed to adults of the rhizome-galling tephritid fly *Oxyna nebulosa* in 2022. Between the end of May and mid-July more than 1500 adults emerged from these galls. We used egg-laying females to set up no-choice oviposition and larval development tests with 21 non-target plant species of importance to North America and two open-field tests with five Shasta daisy cultivars. 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 2024.

13.3 *Cyphocleonus trisulcatus* (Col., Curculionidae)

Testing with the root-feeding weevil *Cyphocleonus trisulcatus* at CABI has been completed in 2021. We are still maintaining a rearing colony at CABI to be able to provide weevils to Australia (Andrew McConnachie, New South Wales Department of Primary Industries) in 2024.
14 Field Bindweed (*Convolvulus arvensis*)

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14.1 *Melanagromyza albocilia* (Dipt., *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 field conditions at a site where the fly occurs naturally. Open-field tests have been conducted in southern Germany since 2017.

Eleven test plant species (eight North American natives and three ornamental or cultivated species) and *Convolvulus arvensis* as the control, were planted into the soil at the edge of a cereal field. Owing to an anticipated early crop harvest, we harvested the experimental plants 1-2 weeks earlier than usual, and plants were in better shape than in 2022. Dissections are ongoing and results will be presented in the annual report.

Of the 457 pupae that were field collected in 2022, so far, 69 flies and 120 parasitoids emerged in 2023. No-choice tests on potted plants are being conducted. In part, due to male and female synchronization problems and low longevity, only a dozen tests could be established. In order to obtain adults to conduct additional testing in the lab in 2023, infested *C. arvensis* plants will be collected in southern Germany and dissected for puparia later this summer.

14.2 *Microsphecia brosiformis* (Lep., *Sesiidae*)

This root-mining sesiid moth is hard to collect in the field, thus in recent years we have been making an effort to establish a rearing colony by transferring eggs onto specially prepared field bindweed plants under common garden conditions. The obtained eggs were used in host-specificity larval development tests with native *Convolvulaceae* plants from North America.

For no-choice larval development tests, eggs were transferred on 15 July 2022, onto potted plants of 11 native North American species and four *C. arvensis* (North American populations). The rest of the eggs were used to maintain the *Microsphecia brosiformis* population on bindweed plants under common garden conditions. The rest of the tested plant species did not support early larval development in the “first step testing” where plants were dissected in mid-November 2022. The other plant species were set up in the “second step testing” to assess larval development after hibernation. Dissection of the plants on 25 May 2023 revealed successful larval development in all tested bindweed populations; *Calistegia sepium* of European origin and the North American native, *Calystegia macrostigma*. Later this summer, larval development tests will be set up with an additional eight North American native *Convolvulaceae* species from the genera *Convolvulus*, *Calystegia* and *Ipomoea*.
15 Flowering Rush (*Butomus umbellatus*)

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15.1 *Bagous nodulosus* (Col., Curculionidae)

Between 25 April and 23 May, Dr Natalie West (USDA-ARS Sidney, MT) was at the Centre to gain experience working with *Bagous nodulosus* in view of possible releases in the USA. Between 27 April and 5 June 2023, we were able to collect 142 weevils from our pond population and about 170 additional adults were collected in Slovakia between 9 and 11 May. Of these, 100 weevils were hand carried by Natalie West to the USDA-ARS quarantine facility in Sidney, Montana to supplement the current rearing. In the ARS quarantine, overwintering of weevils worked very well with nearly 100% survival in spring 2023 of the adults that were set up in late 2022. In June 2023, the first batch of 10 weevils reared in the USA were sent to Canada for the first releases at a nursery site in Ontario. An additional 20 weevils which overwintered in quarantine were also sent to Canada for rearing.

A total of 45 weevils were marked and released in a pool with potted flowering rush plants to determine whether they are able to establish in pools. We hope that this will be successful and can expand this into next year with additional pools for an impact experiment.

We also continue to rear the weevils by transferring both first and second instar larvae on to potted plants of flowering rush.

15.2 *Phytoliriomyza ornata* (Dipt., Agromyzidae)

From 1600 overwintering puparia of the fly *Phytoliriomyza ornata*, 1390 flies emerged in spring 2023 (87%). These flies are being used to continue host-specificity tests and for maintaining a rearing colony. In addition, we set up another impact experiment, including both diploid and triploid plants from US and European populations. We had 20 replicates for each treatment with a total of 120 plants. Half of the plants were exposed to two pairs of flies. Due to the high levels of impact already observed after the first generation of the fly, 10 replicates will be analyzed end of July, and depending on results, the other half, in August/September.

So far, over 85 rearing and control plants, and a total of 116 replicates with 23 test plant species were set up. Still no development was found on any of the 37 species tested, confirming the narrow host range of *P. ornata*.

15.3 *Doassansia niesslii* (Fungi, Doassansiaceae)

In September 2021, a field trip to Romania (Somova, Danube delta) was carried out by CABI colleagues, and a new isolate of *D. niesslii* was found on *B. umbellatus*. On return to the laboratory in the UK, the smut was successfully isolated from the infected plant material and was subsequently cultured on agar (IMI507227).

Five North American (NA) populations of flowering rush, including those belonging to genotypes 1 and 4 have been tested with this new isolate to date. Symptoms were observed on the South Dakota population (genotype 1) and on the New York population (genotype 4) but not on the populations from Montana, Maine or Ohio.

As symptoms are not always consistently observed on Romanian control plants, additional experimental work is needed to ensure that further testing of the NA populations is effective and reliable.
16 Tree of Heaven (*Ailanthus altissima*)

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16.1 *Aculus taihangensis* (Syn: *Aculus mosoniensis*)

(*Acari, Eriophyidae*)

The eriophyid mite *Aculus taihangensis* was initially identified as *Aculus mosoniensis*, but has recently been synonymized with *A. taihangensis*. This vagrant mite is native to China but has also been found in Europe and the US. Work with *A. taihangensis* is being done at BBCA in Rome, Italy.

Host-specificity tests are currently being conducted with several non-target species native to North America under laboratory conditions. For this, plants are being grown from seeds and once they are about 15-20 cm tall, each of them is being inoculated with 20-30 mites by attaching a tree of heaven leaf infested with *A. taihangensis*. Three weeks after inoculation, each plant is visually checked under a stereo-microscope. After a total of six weeks, the plants are harvested and the mites extracted and counted.

In June, tests were completed with *Aesculus glabra* and *Leitneria* sp. Six weeks after inoculation, tree of heaven seedlings had an average of about 30,000 mites while at the same time, an average of only about 30 live mites were found on seedlings of *Leitneria* sp. and no live mites were found on any of the *A. glabra* seedlings. Symptoms of the mites (i.e., deformed leaves) were only observed on tree of heaven, not on any of the non-target species. Host-specificity testing with additional non-target species is still ongoing and detailed results of all tests will be presented in the annual report.
17 Parrot’s Feather (*Myriophyllum aquaticum*)

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17.1 *Lysathia* sp. (Col., Chrysomelidae)

The rearing of *Lysathia* sp. is going well under quarantine conditions. Host range testing continued with new non-target species; in total we currently have 12 non-target species in the Haloragaceae family available at CABI Switzerland. This year we were able to test *Myriophyllum hippuroides*, a North American native that is closely related to *Myriophyllum aquaticum*, under no-choice conditions. Similar to other emergent non-target species, *M. hippuroides* supported limited oviposition, and, under multiple-choice conditions, there is a clear preference for parrot’s feather. We collected emergent plant material of *M. heterophyllum* in Dijon, France, and *M. verticillatum* in Biel, Switzerland, to run multiple tests with those two species. A long-term, multiple choice experiment we called the “Japanese garden” ran from September 2022 and was stopped in May 2023. The aim was to mix the target and a few non-target species in the same pot and see how the insect would react. Preliminary results showed that although there was some minor feeding on non-target species, egg-laying only occurred on parrot’s feather. By the time the experiment was terminated, the parrot’s feather population was virtually non-existent and the population of *Lysathia* sp. had died out, suggesting that the non-target plants could not support a population of *Lysathia* sp. New “Japanese gardens” have been set up with only North American species as non-targets. This experimental design is promising, simulating a more complex host selection process that the insect could experience in the field.

17.1 *Phytobius vestitus* (Col., Curculionidae)

The rearing of *Phytobius vestitus* started well but we experienced a crash in December 2022 and since then we are struggling to recover high numbers of the weevil. More adults were collected this summer from Louisiana and Texas, USA in order to run more experiments at CABI. Nevertheless, after pre-tests showing that *P. vestitus* was responding better than *Lysathia* sp. to olfactometry tests, we could run tests on the host choice behavior of the weevil. In a Y-tube system, we recorded time spent by the insect in each arm of the olfactometer for 20 minutes. Under no-choice conditions the insect is attracted to parrot’s feather and indifferent to the non-targets, with the exception of *Myriophyllum crispatum*. Under choice conditions, *P. vestitus* significantly preferred parrot’s feather in all tests. These results are promising and compliment the traditional host range testing very well.

Host-range testing was started, but is currently on “stand-by” due to the low number of the insects available. To date, we were able to test eight non-target species under no-choice conditions. Just one non-target, *Proserpinaca palustris*, was tested under single-choice conditions and results are very promising with only one plant out of 10 experiencing limited oviposition. We have been unable to test larval development on any of the non-target species to date. It will be interesting to see if there is the same tendency as for *Lysathia* sp. that is to say whether *P. vestitus* has a specific behavior when the test complexity is increased.
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 for South Africa, led by Dr Grant Martin from the Centre for Biological Control (CBC), Rhodes University.

In Europe, black locust was introduced 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 between 2020-2021, identified three potential biological control agents: two lepidopteran leaf miners, *Phyllonorycter robiniella* (syn. *Macrosaccus robiniella*) and *Parectopa robiniella*, and the leaf rolling midge, *Obolodiplosis robiniae*. The insects are native to North America but are nowadays found in Europe, where the damage may cause premature leaf drop. This offers a unique opportunity to study the host range of these species in Europe for potential introduction into South Africa.

In order to test the host specificity of these potential biological control agents, open field and cage tests exposing non-target species in the same family as *R. pseudoacacia* are currently being run in Switzerland. The open-field test with 10 non-target species was set up in May and will run until late September 2023. To date, galls have been found on three of the six control plants. The larvae have been collected for molecular identification. For the cage tests, seven non-targets and two controls were set up in three outdoor cages in the CABI garden. In this case, we planned to field-collect all three potential biological control candidates and release them into the cages. So far, about 4000 galls of the midge and 500 mines of *P. robiniella* and a dozen mines of *P. robiniella* have been collected, more collections are planned this summer. These galls and mines are checked daily and adults are released more or less equally between the cages. The cage test will be run twice this year, from June to August and from August to October. Any damage on the controls and non-target species will be recorded and insects will be reared out to confirm their identity.
19 Understanding the drivers of invasion of cheatgrass (*Bromus tectorum*)

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In collaboration with Dr Jacob Lucero from Texas A&M University, and Dr Akasha Faust, University of Montana, a biogeographic study has been initiated to better understand the ecological processes determining/regulating the plant invasions outside their native range. In particular, we plan to experimentally evaluate how biotic and abiotic factors affect cheatgrass (*Bromus tectorum*) in its native vs. non-native ranges to determine how these factors contribute to its invasion and potentially to its sustainable control. Plant-fungal interactions, insect herbivory, and cattle grazing will be included as biotic factors, while the abiotic factors considered will be fire and mechanical soil damage. Cheatgrass is an annual grass native to Eurasia but is exotic and extremely invasive across western North American rangelands. The knowledge acquired through this biogeographical study will poise land managers to control cheatgrass invasions by either mimicking population controls that are more effective in the native range, or by disrupting positive feedbacks present mainly in the non-native range.

In May 2023 we were able to initiate the set up of three experimental plots in the native range; two in northern Greece, in collaboration with University of Western Macedonia-Greece, and the third plot in southern Hungary, making use of the CABI satellite station there. The sites were marked out and vegetation surveys were conducted to get a baseline measure of the plant diversity at the site in general, as well as within each subplot, where the different factors will be applied. Moving forward, fire and disturbance, as well as the exclusion of grazing, and treatments against insects and pathogens will be done in October 2023. In addition, we were able to collect the required number of seeds to standardize the seedbank between North America and the native range, and to start the experiments next fall. So far, the setup has been successful and we are looking forward to the next phase.
20 Japanese Knotweed (*Fallopia japonica*) work in the UK

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20.1 *Aphalara itadori* (Hemiptera, Psyllidae)

Japanese knotweed, *Fallopia japonica*, giant knotweed, *F. sachalinensis* and their hybrid, Bohemian knotweed *F. x bohemica* have become serious invasive weeds in North America. A natural enemy from the native range, the psyllid *Aphalara itadori* has been prioritized as a classical biological control agent following extensive research, both in the field and under greenhouse conditions.

A new line of the psyllid, collected in Murakami, Niigata Prefecture, Japan in 2019 (Murakami line), was found causing severely curled leaves and this region has a much better climatic match to the invaded range. The first releases of the Murakami line took place onto Bohemian knotweed populations in the Netherlands in 2020; Japanese and Bohemian knotweed populations in 2021 in the UK; and Japanese knotweed populations in 2021 in Canada. For *F. x bohemica* populations in the UK, severe curling damage was observed and adults have successfully overwintered.

To explore the Murakami psyllid behaviors that initiate leaf-roll galls, we conducted field and semi-field experiments. During the field experiments, artificial galls were found to improve psyllid nymphal survival on sleeved branches of Bohemian knotweed, however, this effect was overshadowed by the production of new (galled) leaf material in some branches, which supported significantly more psyllids. In the semi-field experiment, contrary to expectations, both Bohemian and Japanese knotweed plants produced leaf-roll galls and predation did not cause significant psyllid mortality. Our results highlight how the development of leaf-roll galls on Bohemian knotweed can benefit the knotweed biocontrol program, both by suppressing plant growth and by improving psyllid survival through the amelioration of abiotic stressors.

Molecular analysis of the psyllids by next generation sequencing has also commenced with the aim of investigating any potential microorganisms that could be contributing to the leaf-curling damage. Samples of the psyllids ex Kyushu and ex Murakami at various stages (egg, nymph and adult) as well as of heathy and curled leaves of *F. x bohemica* were collected. Preliminary analysis of the psyllid samples showed that the bacterial community in both Murakami and Kyushu psyllid lines is mostly composed of *Sodalis*, however, *Wolbachia*, which has been reported to manipulate insect host reproduction, was only found in the Murakami line. Further data analysis is currently underway.
21 Himalayan Balsam (*Impatiens glandulifera*) work in the UK

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21.1 *Puccinia komarovii* var. *glanduliferae* (Fungi, 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 program 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. Results of a molecular study indicated that the two haplotypes of Himalayan balsam present in Canada are identical to the two most common ones in the UK, supporting the suggestion that the plant was introduced into Canada from the UK. So far, 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 the UK. In some instances, neither of the rust strains has been suitable, necessitating 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 those observed on the positive control plants from the UK. Therefore, additional rust strains are also required for control to be successful in Canada.

During 2020-2022 further strains of the rust were sourced in India and Pakistan. Unfortunately, a strain obtained in 2020 from Karchon, India also failed to infect the Canadian Himalayan balsam populations. In 2021 and 2022, a local collaborator returned to northern Pakistan to collect additional rust specimens. One of the three strains collected in Pakistan in 2022, from Das Kharram, Astore, has been successfully established on UK Himalayan balsam plants in quarantine and is stored in liquid nitrogen. Susceptibility testing of three Canadian populations of Himalayan balsam is currently underway. Further surveys for additional strains will continue in India and Pakistan in 2023.
Distribution list

Adams, Gary
Allen, Edith B.
Anderson, Oscar
Andreas, Jennifer
Asadi, Ghorbanali
Ashton, Leo
Auge, Harald
Barge, Edward
Bautista, Shawna
Baxter, Colden
Bean, Dan
Becker, Roger
Bloem, Ken
Blossey, Bernd
Bon, Marie-Claude
Borkent, Chris
Bourchier, Rob
Bowes, Angela
Bracewell, Ryan
Brenzil, Clark
Brooke, Janine
Brown-Lima, Carrie
Brusven, Paul
Bryce, Christiaëns
Buntrick, Gregory
Butler, Tim
Cappuccino, Naomi
Casagrande, Richard
Cass, Jaemy
Chandler, Monika
Christiaëns, Bryce
Ciopperlik, Matt
Coetzee, Julie
Cofrancesco, Al
Collier, Tim
Colonnelli, Enzo
Cripps, Michael
Cristofaro, Massimo
Dadkhodaie, Ali
Danly, Lynn
Davidson, Alisha
Dean, Jennifer
DeClerck-Floate, Rosemarie
DeLillo, Enrico
DesCamp, Wendy
Desurmont, Gaylord
Detweiler, Cynthia
Diaconu, Alecu
Diaz, Rodrigo
Dige, Greta
Dolgovskaya, Margarita
Dunbar, Rich
Eagar, Aaron
Edmiston, Erika
Eisen, Rochelle
Ensing, David
Faltthauer, Ana
Faist, Akasha
Fee, Mary
Fick, Derrill
Foster, Aaron
Fowler, Simon
Fu, Weidong
Galford, Shayne
Gaskin, John
Ghorbani, Reza
Giantsis, Ioannis
Gibbs, Bary
Goolsby, John
Gould, Joel
Gourlay, Hugh
Gowland, Nathan
Green, Nicole
Greer, Michael
Grevstad, Fritzi
Groenteman, Ronny
Gültekin, Levent
Gurcan, Kahraman
Hanes, Glenn
Hardin, Janet
Harms, Nathan
Harris, David
Haubrich, Greg
Haverhals, Marijka
Hayes, Lynley
Heimpel, George
Helbig, Bruce
Hill, Martin
Hoelmer, Kim
Holtz, Tara
Hudson, Wayne
Hufbauer, Ruth
Hull, Aaron
Jashenko, Roman
Jones, Marian
Jørgensen, Carl L.
Justen, Emilie
Katovich, Jeanie
Katz, Gabrielle
Keever, Kenny
Korotyaev, Boris
Knight, Ian
Landis, Douglas A.
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Lesica, Peter
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