Weed Biological Control

Progress Report 2024

CABI in Switzerland
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Contents

Cover photos (left to right): Léa Emery collecting European frogbit from a field site in France; Max Bailey setting up an open-field test in Germany; Julia Lamoureux and Cornelia Cloşca with dyers woad in the CABI garden.

Philip Weyl

Notes from the section leader

As every year, I am pleased to be sending out the progress report for 2024. I hope that you will enjoy the updates from each of the research projects presented below. I really appreciate the dedication of the Weeds Team to the research projects they are involved in, which we hope will result in the safe and sustainable management of invasive weeds.

Some exciting news for 2024 is that we have been able to get two new biological control projects off the ground, which include **blueweed, *Echium vulgare*** and **European frogbit, *Hydrocharis morsus-ranae***. On the back of the European frogbit project we have also started surveying water soldier, *Stratiotes aloides*, given its taxonomic relatedness and overlapping distribution. The team has made some really nice progress on these targets already, as you will read in the relevant project sections. The ongoing projects are, as usual, making good headway, however, we did struggle with the weather this year. The spring and summer were particularly cool and wet, which mainly affected the seed feeders because synchronizing the flowering period of the target with the non-targets and insect activity, proved to be extremely challenging.

With the current trajectory of many of the projects we continue to get agents close to the petitioning process. These include agents against **Russian knapweed**, **dyers woad**, **common tansy**, **garlic mustard**, **flowering rush** and **black locust**. As for the agents which have been permitted for release in Canada, there are signs of early establishment for the Russian olive mite, *Aceria angustifoliae* and the root- and rhizome-feeding tortricid moth, *Dichrorampha aeratana,* which were reared and initially released under the guidance of Dr Rosemarie De Clerck-Floate and her team in 2023. There has also been a massive push to release thousands of individuals of the stem-mining moths against phragmites in Canada, which has been **very successful**, see the recent video update. Unfortunately, the approval process in the US continues to take time (upwards of 3 years) to obtain a permit for release. It is my understanding that everything is being done to move agents along in the process and I hope that we will see some fresh biological control agents on the ground soon.

As much as possible, we try to host visitors and collaborators on our projects at the CABI Centre in Switzerland. In May, we hosted Dr Andrew McConnachie from the NSW Department of Primary Industries and Regional Development visiting for a week to work on **oxeye daisy**. In addition, we also hosted Dr Ian Knight and Dr Michael Greer from the US Army Corps of Engineers, to kick off the **European frogbit** project. I invite you to check out the project pages below.

Invasive species management, especially using biological control, is, certainly a team effort and stakeholder engagement, and transfer of knowledge and experience is vital in a successful program. The team here at CABI Switzerland is proud of the efforts we make in education and raising awareness of invasive species and biological control. In addition to the conference presentations and outreach we do, this year we have six summer students passing through our lab, originating from Switzerland, Italy, Canada and the US. We also have short-term internships for school and university students, and spend time teaching school children in the region about invasive species and biological control. We hope that our efforts and those of our partners and collaborators will fuel classical biological control going forward especially in an era of an increasingly discerning public but with greater awareness of human and environmental health.

I would like to take this opportunity to extend my gratitude to our international and local collaborators and funders for their support as well as my colleagues, both staff and students of the Weeds Team at CABI, without which, none of this would be possible.

I hope that you enjoy reading about the progress we have made this field season and feel free to reach out to me or any of the project scientists if you have any questions about the projects.

All the best

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1 Blueweed (*Echium vulgare*)

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Blueweed, *Echium vulgare* (Boraginaceae) is a biennial to short-lived perennial plant originating from Eurasia. This species was introduced to North America sometime during early settlement and spread widely since then. In western Canada, it is currently listed as noxious in Alberta, and in central and southern regions of British Columbia. It also is declared noxious in several states of the US, including Idaho, Washington and Montana. Blueweed invades a wide range of habitats including pastures and rangelands. Being unpalatable to livestock, it is left to displace desirable forage plants in otherwise productive prairies and rangelands. Steps to control this weed by biological means have not yet been taken in North America. However, seven insect biological control agents were introduced to Australia against the closely related species Paterson's curse, *Echium plantagineum*, where at least three of them have contributed to the successful control of this pasture weed. Since these insect species are also associated with blueweed in Europe, they may have potential as biological control agents for this weed in North America, provided they are host-specific enough. In 2023, CABI received funding from the British Columbia Ministry of Forests to conduct a literature survey for potential biological control agents for blueweed. Based on the available information on their impact and narrow host range, the root-feeding weevil *Mogulones geographicus* and the eriophyid mite *Aceria echii* were prioritized for further studies. *Mogulones geographicus* is already successfully used as a biological control agent for Paterson's curse in Australia. In spring 2024, we focused our efforts on identifying collection sites of both species and on understanding their biology in order to establish methods for host-specificity testing.

Root-feeding weevil *Mogulones geographicus* in a flower of *Echium vulgare*

1.1 *Mogulones geographicus* (Col., Curculionidae)

In June, we surveyed several blueweed sites between Delémont and southern Germany for *M. geographicus*. We found weevils at several sites where we observed them on the upper parts of the plants, where they were feeding on closed flowers and in recently opened flowers. We collected approximately 50 weevils, which are currently being kept on potted plants. We expect the weevils to become reproductively active in autumn.

Echium vulgare showing potential symptoms of the eriophyid mite *Aceria echii*

1.2 *Aceria echii* (Acari, Eriophyidae)

The biology of the eriophyid mite *Aceria echii* is unknown, but it is reported to completely change the morphology of blueweed and drastically reduce flowering and seed output. We already identified a collection site in summer 2023 from where we collected mite-infested leaf material in spring 2024 to inoculate 9-month and 3-month old potted blueweed plants. Although the inoculations were successful on the majority of the plants, no visible impact was observed on any of the plants and the 9-month old plants flowered normally. The 3-months old plants are only expected to flower in spring 2025 and we plan to monitor these plants until then.

Typical collection site of *Mogulones geographicus* in Switzerland

Bagous sp. (possibly *B. puncticollis*) collected on European frogbit in Hungary

2 European frogbit (*Hydrocharis morsus-ranae*)

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European frogbit, *Hydrocharis morsus-ranae,* is a free-floating macrophyte in the Hydrocharitaceae, with heart-shaped leaves and small white flowers. It is native through much of Europe and parts of temperate Asia as well as North Africa. In the native range European frogbit has declined or has been extirpated throughout parts of its range and is of conservation concern in several areas. It was introduced into North America as an ornamental in the early 1930s, with the initial introduction into Ottawa thought to originate from the Zurich Botanical Gardens, Switzerland. Now it is most abundant in eastern North America. In the USA, the invasion is most abundant in northern New York and southeastern Michigan, Vermont and Maine, while in Canada it is prevalent in southeastern Ontario and western Quebec. It has also recently been reported on the west coast of North America, where it is established in Washington, and is an A list noxious weed in California. In 2024, CABI received funding from the United States Army Corps of Engineers to initiate a biological control program. In addition to this, water soldier, *Stratiotes aloides*, also introduced in North America (currently only in Canada), will be considered during surveys in the native range given the phylogenetic relatedness and geographic overlap with European frogbit.

Currently there are no biological control agents already developed for European frogbit, but several herbivores have been recorded in the native range. Many are reported as polyphagous, however, there are some that are worth further investigation including the weevil, *Bagous puncticollis*, a complex of *Hydrellia* flies and the leaf spot pathogen, *Tracya hydrocharidis.*

Hydrellia sp. mining in the leaf of European frogbit.

2.1 Field surveys

In spring and summer 2024, we focused our efforts on identifying survey locations and making initial collections of genetic samples, and herbivore diversity at the sites. Between May and July of 2024, we surveyed a total of 48 sites for European Frogbit in Switzerland, Germany, Netherlands, France, Hungary, Serbia and Greece. For water soldier, we have visited 12 sites, three in Switzerland and nine in Germany. We were able to collect genetic samples as well as assess herbivore diversity from at least 33 of these sites. In addition, we also collected demography data from several of those sites with Dr Ian Knight and Dr Mike Greer from the US Army Corps of Engineers. For all herbivore species, expert identification would be required to confirm the identity. We plan to initiate studies on the biology of the different herbivores to better understand how rearing and potential testing may proceed.

2.2 *Hydrellia* spp. (Dipt., Ephydridae)

By far the most prevalent and widespread herbivore on both weed species are the *Hydrellia* flies. We found larval mines in the leaves of both European frogbit and water soldier at nearly all sites surveyed so far. Larvae can easily move from one leaf to another and usually pupate in leaf petioles. However, the larvae seem to be adaptable for pupation and we have observed puparia in the leaves when the petioles are too thin. We have already obtained our first egg batches from lab emerged adults which we plan to use to establish a rearing colony and to understand the biology of the species. All adults reared out from various sites will be sent for identification to an Ephydrid specialist.

Stratiotes aloides, in a pond in Europe

2.3 *Bagous puncticollis* and *B. binodulus* (Col., Curculionidae)

We have been able to collect *Bagous* weevils from two sites, one from European frogbit in Hungary, suspected to be *B. puncticollis* and one from water solider suspected to be *Bagous binodulus.* In addition, at one site in Germany we found *Bagous* eggs in petioles of European frogbit. We are currently trying to rear the larvae on *H. morsus-ranae.*

Dr Ian Knight (USACE) with Lauréline Humair and Patrick Häfliger collecting demographic data for European frogbit in Germany

3 Parrot's feather (*Myriophyllum aquaticum*)

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

The rearing of *Lysathia* sp. is going well under quarantine conditions. Host-range testing is progressing with the addition of one new non-target species; in total we have tested 14 non-target species, 12 in the Haloragaceae, one in the Hydrocharitaceae and one in the Onagraceae families. As we expanded the test list to include related families in the Saxifragales, additional plants were obtained in the Penthoraceae*,* Crassulaceae and Onagraceae.

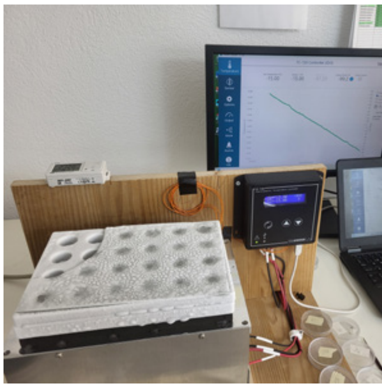
This year, we were able to test under no-choice and single-choice conditions *Haloragis erecta*, originating from New Zealand but present in western North America. Similar to other emergent Haloragaceae, *H. erecta* supported limited oviposition under no-choice conditions, while under single-choice conditions, *Lysathia* sp. chose parrot's feather 100% of the time for oviposition. As in previous tests, as complexity increases, *Lysathia* sp. tends to be specific under lab conditions. Undergraduate students from University of Neuchâtel conducted a multiple-choice test in an arena mimicking a natural complex environment (adding natural features such as rocks, other plants species). Overall, *Lysathia* sp., was extremely selective for *M. aquaticum* with a clear preference for its natural host, which is very encouraging.

Lysathia sp. setup on parrot's feather for oviposition

3.2 *Phytobius vestitus* (Col., Curculionidae)

The rearing of *Phytobius vestitus* is currently going very well, however, over winter, the rearing slows down despite being in quarantine. This has forced us to prioritize the testing of *P. vestitus*, while *Lysathia* sp. can be tested all year long.

This year, we have tested four new non-targets species under no-choice oviposition tests, which gives a total of 13 non-target species, 11 in the Haloragaceae, one in the Hydrocharitaceae and one in the Onagraceae tested so far. As with *Lysathia* sp. There was limited oviposition on all non-targets apart from *H. laevigata* which received no eggs. In single-choice oviposition tests, we were able to test five species this year. Of the eight species tested to date, female *P. vestitus* preferentially chose *M. aquaticum* except for *Myriophyllum hippuroides,* which was equally chosen. In multiple-choice oviposition tests, with *M. heterophyllum* and *M. hippuroides*, the control, *M. aquaticum*, was significantly more often chosen. After refining the larval development testing methods, we were able to obtain the first reliable no-choice larval development results with 48.8% of eggs successfully developing to adults on the control, 53.1% on *M. hippuroides*, 25% on *M. crispatum*, 16.2% on *H. erecta*, and 0% on the submerged species *M. alterniflorum* and *M. pinnatum.*



The chill plate setup in the CABI quarantine for measuring the critical thermal minimum (CT min), chill coma and lower lethal limit.

3.3 Thermal physiology

Both *Lysathia* sp. and *P. vestitus* originate from regions that are characterized by relatively warm climates, Brazil and Louisiana, while the target range northwestern North America is more temperate. Thus, in parallel to the host specificity testing we are obtaining a better understanding of the thermal physiology of the candidate agents and the potential implications for the program. We initiated the thermal testing with the critical thermal minimum (CT min) and the chill coma using a chill plate system. We are at the very beginning for *Lysathia* sp. but preliminary results suggest a CT min close to freezing (0.20 ± 0.13 °C) and a chill coma just below freezing (-0.39 ± 0.17 °C), with the lower lethal limit for short exposure (3 minutes) between -7 and -8 °C. While *P. vestitus* had a CT min below freezing (-0.56 ± 0.31°C) and a chill coma at -1.26 ± 0.22 °C. The chill plate can only reliably go down as far as -15 °C and after 3 minutes exposure there was still almost 100% adult survival, so the lower lethal limit has not yet been determined for *P. vestitus*.

A mix of non-targets plants and environmental features to increase complexity of the multiple-choice test to approach a natural setting.

Adult feeding damage by *Bagous nodulosus* on a plant in the impact experiment.

4 Flowering rush (*Butomus umbellatus*)

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4.1 *Bagous nodulosus* (Col., Curculionidae)

Between 11 April and 14 June 2024, we were able to collect over 220 weevils from our pond population, of which 46 were sent to Canada for setting up a nursery pool. We continue to rear the weevils at CABI to maintain a healthy population by transferring first instar larvae onto potted plants of flowering rush. An exciting development is the first release of weevils in Canada in June from individuals reared in quarantine.

In May, we set up an impact experiment releasing 12 pairs of *B. nodulosus* into two pools each. Two additional pools were set up as controls, and each pool contained six frames (60x60 cm) filled with potting soil with three different plant densities (1, 4, and 9 plants of flowering rush). Already 2.5 months after the weevils were released, a clear impact was visible, with the exposed plants being much smaller than the controls. In addition, there were much fewer flowers in the exposed pools, 0.6 flowering stems per frame vs. 7.5 flowering stems per frame, respectively. We plan to measure above and below ground biomass of the plants later this summer.

Leaf pieces of the Romanian flowering rush infected by *Doassansia niesslii*.

4.2 *Phytoliriomyza ornata* (Dipt., Agromyzidae)

We had planned to complete host-specificity tests in 2024 using mainly the second generation of *P. ornata*. Unfortunately, the offspring of the flies which emerged in April were mostly overwintering puparia, and only a few flies of the second generation emerged. This meant we could only set up 18 replicates with eight test plant species. We hope to be able to set up more replicates with the third generation of flies, and still to be able to start writing a petition for release this year. To date, no development was found on any of the 39 species tested, confirming the narrow host range of *P. ornata.*

In addition, we set up another small impact experiment, including both diploid and triploid plants from US populations. The main purpose was to confirm whether the large impact seen on diploid plants in 2023, would also be seen on triploid plants.

4.3 *Doassansia niesslii* (Fungi, Doassansiaceae)

Experimental work conducted in 2023 to investigate the apparent inconsistent infection of the Romanian *B. umbellatus* population with the smut (IMI507227) was successful. We now have a further understanding of the smut's life cycle and have achieved infection of several North American *B. umbellatus* populations, including South Dakota, Montana, Wisconsin (genotype 1), New York, Ohio and Maine (genotype 4) and Bouchie Lake, Canada (genotype 2). We are currently testing further replicate plants from these populations and in addition, the Minnesota population (genotype 5). Host-range testing of closely related species is also planned as well as further developing our understanding of the smut's life cycle.

Summer students Max Bailey and Julia Lamoureux taking measurements on the impact experiment with *Bagous nodulosus*.

5 Oxeye daisy (*Leucanthemum vulgare*)

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5.1 *Dichrorampha aeratana* (Lep., Tortricidae)

First releases of the rhizome-feeding tortricid moth, *Dichrorampha aeratana,* took place at field sites in British Columbia and Alberta in June 2023 and a new generation of moths has been observed at one site in spring 2024. Releases of the moths in Canada continued in 2024. In the US, the USDA-APHIS Technical Advisory Group (TAG) recommended release of *D. aeratana* in 2022, but a release permit has yet to be issued.

In May, we sent 1600 eggs to Dr Rosemarie De Clerck-Floate (AAFC, Lethbridge) and 700 eggs to Dr Jeffrey Littlefield (Montana State University in Bozeman) to supplement their rearing colonies. In addition, Dr Andrew McConnachie (New South Wales Department of Primary Industries) hand-carried 1400 eggs back to Australia to re-establish a rearing colony under quarantine conditions. At CABI, we are continuing to maintain a rearing colony of *D. aeratana* to facilitate future shipments to North America and Australia.

Female of the rhizome-galling tephritid fly *Oxyna nebulosa.*

5.2 *Dichrorampha consortana* (Lep., Tortricidae)

In 2024, we restarted the work with the stem-mining moth *Dichrorampha consortana*. From 13 to 17 May we visited field sites in Norway where we had found *D. consortana* during a previous collection trip in 2018. Rosettes with larvae were collected and approximately 100 females and 100 males emerged from the collected material. First-instar larvae were transferred onto potted oxeye daisies to study the biology of *D. consortana* and to establish a rearing colony. In addition, no-choice larval development tests with several non-target species and a multiple-choice cage test with four Shasta daisy cultivars were set up.

5.3 *Oxyna nebulosa* (Dipt., Tephritidae)

In May and June, we harvested approximately 3000 galls from the oxeye daisy plants that had been exposed to adults of the rhizome-galling tephritid fly *Oxyna nebulosa* in 2023. In May, 150 of these galls were sent to Dr. Rosemarie De Clerck-Floate to supplement their rearing colony. From the remaining galls approximately 1500 adults emerged between late May and mid-July. We used egg-laying females to set up no-choice oviposition and larval development tests with the few non-target plant species of importance to North America that we had not yet exposed. In addition, we set up two open-field tests with five Shasta daisy cultivars. All plants will be checked for galls in autumn.

Ovipositing female of the root-feeding weevil *Cyphocleonus trisulcatus*.

5.4 *Cyphocleonus trisulcatus* (Col., Curculionidae)

Testing with the root-feeding weevil *Cyphocleonus trisulcatus* for Australia at CABI was completed in 2021. However, we are still maintaining a rearing colony at CABI and in May 2024, we provided approximately 100 weevils to Dr Andrew McConnachie to supplement his rearing colony in the quarantine facility at the New South Wales Department of Primary Industries.

Summer student Chiara Brozzi looking for oxeye daisy rosettes infested with larvae of *Dichrorampha consortana* in Norway.

Mating pair of the leaf beetle *Chrysolina eurina*.

6 Common tansy (*Tanacetum vulgare*)

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6.1 *Microplontus millefolii* (Col., Curculionidae)

Host-specificity testing with the stem-mining weevil *Microplontus millefolii* was completed in 2023 and a petition for field release of this species in Canada and the US is currently under preparation.

6.2 *Chrysolina eurina* (Col., Chrysomelidae)

In spring 2024, we evaluated the open-field test that had been set up in October 2023 with the leaf-feeding beetle *Chrysolina eurina,* exposing the medicinal plant *Tanacetum parthenium,* the native North American plant *Artemisia californica* and the target species, *Tanacetum vulgare,* as a control. In February, we covered all plants individually with gauze bags. Since all *A. californica* plants died over winter, we added an individual seedling of *T. vulgare* to each of the pots with *A. californica* so that the larvae that may emerge from eggs laid on *A. californica* could be recorded later. Between April and June, we checked all plants for larvae and newly developed adults and found on average 12.1 larvae or newly developed adults per plant on *T. vulgare*, 0.5 on *T. parthenium* and 0.3 on the *T. vulgare* seedlings added to the *A. californica* plants.

Since previous open-field tests with the two native species *Tanacetum camphoratum* and *T. huronense* revealed that these species have a higher risk to be accepted for oviposition than the species exposed in autumn 2023, we set up an impact experiment with these two non-target species as well as with *T. vulgare*. The impact experiment is currently under evaluation, but so far, there are no obvious signs of impact by larval feeding on any of the three species. Detailed results will be presented in the 2024 annual report.

Mating pair of the flower-head and stem-mining moth *Gillmeria ochrodactyla*.

6.3 *Gillmeria ochrodactyla* (syn.: *Platyptilia ochrodactyla*) (Lep., Pterophoridae)

In April and May, we conducted no-choice larval transfer tests with the flower-head and stem-mining moth *G. ochrodactyla* using 18 non-target species and *T. vulgare* as a control. These tests were conducted with first-instar larvae originating from *T. vulgare* flower heads that had been collected in Germany in October 2023 and overwintered at CABI. Larval feeding was observed only on the ornamental plant *Leucanthemella serotina*, but not on any of the other non-target species.

On 22 and 23 May, about 1000 shoots attacked by *G. ochrodactyla* were collected in western Germany. From these, about 190 females and 70 males emerged, which were used to set up no-choice oviposition tests with flowering non-target species that had supported partial or complete larval development in previous larval transfer tests. These tests will be evaluated later this summer.

Summer student Madeline Holroyd collecting common tansy stems infested with larvae of *Gillmeria ochrodactyla* in Germany.

7 Hawkweeds (*Pilosella* spp.)

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7.1 *Aulacidea pilosellae* (Hym., Cynipidae)

Two biotypes of this gall wasp are being investigated. We are currently testing a population from Poland of biotype 1, *A. pilosellae* ex *Pilosella* spp. from the northern range (eastern Germany, Poland and the Czech Republic). Biotype 2 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. We are presently focusing on completing the testing with biotype 1. Batches of galls harvested from lab infested plants in 2023 were taken out of cold storage in May 2024. Adults emerging from these were used in host range tests and for rearing.

Hieracium scouleri var. *albertinum* developed galls in a no-choice test in 2005, however, no adults emerged from these. This species was exposed in single-choice tests in 2024. 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).

Females and eggs of *Cheilosia urbana* ready to be shipped.

7.2 *Cheilosia urbana* (Dipt., Syrphidae)

Fourteen *C. urbana* females collected in the CABI garden were shipped to AAFC, Summerland on 6 May. About 400 eggs that were harvested before shipment were also included in the package. The shipment was successful and the eggs were still in good shape with a few live larvae that hatched before they arrived. Although the females died before or shortly after arrival, they laid another 45 eggs during transit. Upon arrival, eggs and larvae emerging from these eggs were transferred onto potted plants for field releases at a later date.

Summer student, Alberto Ossola, field collecting *Cheilosia urbana* in the CABI garden in Switzerland.

Chrysochus asclepiadeus containers for egg harvest.

8 Swallow-worts (*Vincetoxicum* spp.)

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8.1 *Chrysochus asclepiadeus* (Col., Chrysomelidae)

The root-mining beetle *Chrysochus asclepiadeus* can develop and emerge as an adult over 1-3 years. Adults in Franche Comté, France, are active between the end of June and the end of July. After collection, 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 April–May. Plants with larvae are kept for up to three years for adult emergence.

In spring 2024, we found larvae in six of nine *V. hirundinaria,* and 10 of 13 *V. nigrum* (overall three larvae per plant) from tests set up in 2023. While a total of four L3–4 larvae were found in two *Asclepias incarnata* plants*.* This species was not attacked when exposed in an open-field test in 2018. So far, no adults have emerged from plant species outside the *Vincetoxicum* genus exposed in larval transfer tests. A total of 41 adults emerged from *V. hirundinaria* and *V. nigrum* set up in 2021, 2022 and 2023. In July, 100 additional adults were collected by René Sforza (USDA-ARS European Biological Control Laboratory, Montpellier, France) and David Harris (The State University of New York) and these were shipped to CABI. More than 2000 eggs have already been harvested from these adults and neonate larvae will be used in no-choice larval transfer tests.

In tests conducted between 2019 and 2022, no adults emerged from a total of eight larvae found on *Asclepias 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 17 other exposed test plants (11 native to North American) were attacked. These are encouraging results, similar to previous tests with the Ukrainian population of the beetle.

Summer student Alberto Ossola checking *Vincetoxicum hirundinaria* for adult *Chrysochus asclepiadeus* emergence.

9 Field bindweed (*Convolvulus arvensis*)

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9.1 *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 and in 2024, we selected a new site in southern Germany. On 18 June, six North American native test plant species and *Convolvulus arvensis* as the control, were planted into the soil at the edge of a maize field. The plants were recollected on 23 July and dissections are ongoing and results will be presented in the annual report.

For the no-choice tests in the lab, about 220 pupae were field collected in 2023, of which 46 flies and 77 parasitoids emerged in 2024. Due to male and female synchronization problems and low longevity, only seven tests could be established. In order to obtain adults to conduct additional testing in the lab in 2025, additional infested *C. arvensis* plants will be collected in southern Germany and dissected for puparia later this summer.

Melanagromyza albocilia on field bindweed.

9.2 *Microsphecia brosiformis* (Lep., Sesiidae)

This root-mining sesiid moth is difficult to collect owing to its low densities in the field. This year we were able to collect four females of *M. brosiformis* on 15 July from east Serbia yielding over 200 eggs which were used for no-choice tests. The garden population established over the years within the experimental garden of the Institute for Plant Protection in Zemun, Serbia was thus used for the open-field tests.

For the no-choice larval development tests, eggs were transferred on 30 July, onto potted plants of seven native North American species: *Convolvulus equitans*, *Calystegia macrostegia*, *C. sepium* ssp. *sepium*, *C. stebbinsii*, *C. occidentalis*, *Ipomoea barbatisepala* and *I. hederifolia*. Any remaining eggs were used to maintain the *M. brosiformis* population on bindweed plants under common garden conditions.

Under open-field conditions a multiple-choice oviposition and larval development test was performed on an 8 m x 8 m surface. Potted plants of three North American native species *Convolvulus equitans* (6 plants), *Calystegia macrostegia* (6 plants), and *Calystegia subacaulis* (4 plants) which showed some larval development in no-choice larval development tests in 2023, while six randomly selected *C. arvensis* from the plot will serve as controls. This test was set up on 27 June and the first male attracted with pheromone lure was observed on 6 July. The population density of *M. brosiformis* inside the experimental field is good this year. In addition, we set up another choice test on a 6 m x 6 m plot with only the native *C. macrostegia* owing to the high survival rate of *M. brosiforme* larvae under no-choice conditions.

Quentin Donzé setting up an open-field test in southern Germany with *Melanagromyza albocilia*.

Multiple-choice oviposition and larval development test with *Microsphecia brosiformis* under open-field conditions with three North American native bindweed species set up in Serbia.

10 Russian knapweed (*Rhaponticum repens*)

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

The host range testing has largely been successfully completed with a total of 74 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. Following on from this, the oviposition latency tests also showed oviposition towards the end of the experimental period, suggesting that these plants may not be sought out in the field for oviposition, providing evidence that these are likely outside of the ecological host range of the weevil. The petition for field release has been initiated and we are planning to have a version ready for submission during the winter of 2024/25.

10.2 Field surveys

In 2023 during surveys in Armenia a root-feeding moth in the genus *Fulvoclysia* was located at one site. A fieldtrip was planned for July 2024 to collect material in an attempt to setup a quarantine colony, however, the trip was cancelled due to safety concerns at the time. We hope to be able to plan a trip to the site soon to collect this species.

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

11 Garlic mustard (*Alliaria petiolata*)

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11.1 *Ceutorhynchus constrictus* (Col., Curculionidae)

In February and March, over 900 adults of the seed-feeding weevil *Ceutorhynchus constrictus* emerged from our rearing colony established in 2023. After testing for oviposition, fertile females were used at CABI for rearing on *A. petiolata* and host-range tests. The native North American *Leavenworthia stylosa* was exposed in no-choice tests, which resulted in one egg, however, this is likely contamination given the roundish shape not typical of a *Ceutorhynchus* weevil egg. The egg was kept in ethanol for DNA analysis. The adult feeding was mostly superficial with limited seed damage. Since feeding with seed damage has been observed 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 (0.2% of the seeds, vs 12% on *A. petiolata*).

In June and July, over 1600 mature larvae of *C. constrictus* were harvested from garlic mustard (test controls and rearing plants) for adult emergence in spring 2025.

Single-choice test exposing *Boechera perstellata* with garlic mustard as a control to *Ceutorhynchus constrictus*.

11.2 *Ceutorhynchus scrobicollis* (Col., Curculionidae)

Between May and July, adult *C. scrobicollis* emerged from rearing plants infested with field-collected adults in Germany in September 2023. A total of 467 adults were recovered from these plants. Most of the weevils will be kept on potted garlic mustard rosettes for aestivation, and 50 females and 40 males are being stored in containers in our wooden garden shed. Weevils will either be shipped to AAFC in autumn or kept at CABI to maintain the rearing.

Summer student Alberto Ossola changing the garlic mustard leaves in *Ceutorhynchus scrobicollis* in aestivation cylinders.

12 Whitetop or Hoary cress (*Lepidium draba*)

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Heavily galled hoary cress plant exposed to *C. cardariae* under open-field conditions.

12.1 *Ceutorhynchus cardariae* (Col., Curculionidae)

In spring 2024, we conducted an open-field test with five North American species that had supported gall development in cho ice tests in previous years. These included *Caulanthus inflatus, Planodes virginicum, Paysonia densipila, Physaria globosa* and *Rorippa sinuata.* Only two replicates of *Caulanthus inflatus* showed signs of a single gall with no adult emergence, while on the control plants there were an average of 3.6 galls per plant. Furthermore, we were able to test *Physaria globosa* in single-choice conditions, with no signs of *C. cardariae* attack.

In addition to this, we set up a survival test with 10 replicates of *Rorippa sinuata*, *Cardamine breweri, P. virginicum* and three replicates of *Lepidium oblongum*, to see whether the weevil would be able to sustain a population over time. We were able to obtain a total of one female from *P. virginicum* and one female from *C. breweri.* These were reset on new plants of the same species and control plants were set up in parallel.

We also continued maintaining a rearing colony of the weevil at CABI. Of the 720 adults that overwintered from 2023, about 64% survived. The reared adults were set up on potted plants and about 1000 *C. cardariae* emerged in 2024.

A group of test plants in the open-field test.

12.2 *Ceutorhynchus turbatus* (Col., Curculionidae)

Unfortunately, due to the cold and wet spring conditions, synchronizing the flowering and seed set of the control and test plants with the period when adults were active was not possible. Therefore, despite several attempts at slowing down or speeding up test plants we were unable to perform any work with this species.

Cornelia Cloşca and summer student Julia Lamoureux setting up the open-field test for *C. cardariae,* April 2024.

13 Perennial pepperweed (*Lepidium latifolium*)

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13.1 *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 23 non-target species and perennial pepperweed plants under no-choice conditions. Galls were found on seven of those species, but adults emerged from only three of those (i.e. from the native species *Erysimum asperum* and *Lepidium thurberi* and from *Armoracia rusticana* (horse radish)).

Galls of *Ceutorhynchus marginellus* on *Lepidium latifolium.*

We also set up single-choice tests with 14 non-target species that supported adult or gall-development under no-choice conditions in 2024, 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, larvae were only found on *Lepidium papilliferum*, a federally listed threatened species, but not on any of the other exposed non-target species. The number of larvae found on *L. papilliferum* was 32 times lower than on the simultaneously exposed perennial pepperweed plant.

In March and again in June, we sent a total of approximately 1000 adults of *C. marginellus* to Dr Mark Schwarzländer at the University of Idaho for studies on the host-finding behavior of this weevil.

Gall of *Ceutorhynchus marginellus* on *Erysimum asperum.*

Cornelia Cloşca preparing perennial pepperweed plants for exposure to *Ceutorhynchus marginellus.*

Alice Pessina setting up the open-field test in 2023 for *C. rusticus* in southern Germany.

14 Dyer's woad (*Isatis tinctoria*)

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14.1 *Ceutorhynchus peyerimhoffi* (Col., Curculionidae)

Over 1200 adults of the seed-feeding weevil *Ceutorhynchus peyerimhoffi* emerged from our rearing colony this spring. These adults were transferred onto flowering dyer's woad plants for rearing. Over 4500 larvae were collected and are being kept for adult emergence in 2025.

14.2 *Ceutorhynchus rusticus* (Col., Curculionidae)

A total of 18 plants, from two test plant species and the control, were exposed to *C. rusticus* in no-choice oviposition and development tests in autumn 2023. These plants were regularly checked and any dying plants were dissected. Adult emergence was successful in 2024, with all control plants kept for adult emergence supporting development with an average of 26.6 adults per plant, double in comparison with 2023. Of the two test plant species exposed, none supported adult development but some *C. rusticus* mining from L1 larvae was recorded. In autumn 2024, we are planning to set up an additional open-field test in southern Germany with plant species that supported adult development in previous tests.

Summer student Madeline Holroyd collecting plants to check for adult emergence.

15 Black locust (*Robinia pseudoacacia*)

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Robinia pseudoacacia (black locust) is a deciduous tree native to the south-eastern US and has been extensively transported across the globe. When left unmanaged it often becomes invasive with several detrimental impacts. The tree was introduced into South Africa in the late 1800s and the first naturalized population was recorded in the 1930s. Since then, the tree has established widely out of cultivation and is spreading rapidly in South Africa, especially in the grassland biome where it has a number of negative environmental and economic impacts. Some of the insects associated with *R. pseudoacacia* have been unintentionally introduced into Europe. Currently under study is the gall midge, *Obolodiplosis robiniae* (Cecidomyiidae) which was first recorded in Europe in 2003. The other two agents of interest are the leaf miners, *Parectopa robiniella* and *Macrosaccus robiniella* (Gracillariidae) which were recorded in the 1970s and 1980s respectively.

Summer student Léa Emery collecting *Obolodiplosis robiniae* galls and *Macrosaccus robiniella* mines in Germany.

After the success of the open-field and cage tests in 2023 we repeated the experiments in 2024 with some minor adaptations. This year we placed the herbaceous species only in the cage test and not in the open-field test (to avoid snail and slug damage) and had mainly non-target tree species in the open-field test. For the open-field test, 10 replicates of five non-targets (*Acacia nilotica, Erythrina caffra, Lotus jacobeus, Sesbania grandiflora* and *S. sesban*) and the control were set up at the same site as 2023 near CABI. None of the plants have shown any signs of insect attack yet, however, with the cold and wet spring and summer, several of the plants suffered from fungi and rot. Many individuals are recovering, and the first signs of the insects are visible on the naturally occurring *R. pseudoacacia* trees in the field.

The set-up of the multiple-choice test in cages at CABI.

In mid-June, a multiple-choice test using three cages was set up with seven non-targets, five herbaceous species (*Ornithopus sativus, Securigera varia, Hippocrepis comosa, Lotus corniculatus, Anthyllis vulneraria*), two tree species (*Robinia hispida* and *Sesbania bispinosa*) and control plants originating from Switzerland and South Africa. The tree species were selected to increase the number of replicates from last year. About 1500 galls of the midge *O. robiniae* and about 30 mines of *M. robiniella* were collected in Germany and France. Adults of *O. robiniae* (n = 26) and *M. robiniella* (n = 23) were released into the cages between 20 June and 4 July, unfortunately, to date we have been unable to rear out *P. robiniella.* By early July, the first galls (between 17 and 56) of *O. robiniae* were recorded, however, still no sign of *M. robiniella*. The only other species to show signs of *O. robiniae* attack was *R. hispida* in one cage, with 23 galls, all other non-targets are completely free from attack. The final data will be collected in September and reported in the 2024 annual report.

Open-field test in Switzerland near CABI, surrounded by large *Robinia pseudoacacia* trees which are natural infested with *Obolodiplosis robiniae, Parectopa robiniella* and *Macrosaccus robiniella.*

Giorgia Barbarti (BBCA) inoculating the non-target species *Leitneria pilosa* with *Aculus taihangensis* under open-field conditions.

Symptoms of *Aculus taihangensis* on *Ailanthus altissima* 30 days after its inoculation.

16 Tree of heaven (*Ailanthus altissima*)

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16.1 *Aculus taihangensis* (Syn: *Aculus mosoniensis*) (Acari, Eriophyidae)

Work with the eriophyid mite *Aculus taihangensis* is being done at BBCA in Rome, Italy.

In 2024, host-specificity tests were continued under laboratory conditions with five non-target species native to North America as well as several North American populations of tree of heaven. For this, plants were grown from seeds and once they were about 15-20 cm tall, each of them was inoculated with 20-30 mites by attaching a tree of heaven leaf infested with *A. taihangensis*. After a total of six weeks, the plants were harvested, and the mites extracted and counted. Tree of heaven seedlings had on average more than 50,000 live mites per plant and mites were found on all of the tested populations. In contrast, only about 40 live mites were found on seedlings of the North American native species *Castela emoryi*, and zero or less than two mites on the remaining four non-target species. Symptoms of the mites (i.e., deformed leaves) were only observed on tree of heaven but not on any of the non-target species. Host-specificity testing with additional non-target species and additional North American populations of tree of heaven is still ongoing and detailed results of all tests will be presented in the 2024 annual report.

In June, an open-field test was set up with four non-target species native to North America (three of them in the tree of heaven family) and tree of heaven plants as controls. To date, most of the inoculated tree of heaven plants show symptoms typically caused by *A. taihangensis*, while all other test plants remain healthy, without symptoms. Leaves above and close to the inoculation point will be collected in August from each of the inoculated plants, and the number of live mites will be recorded.

To confirm the identity of the live mites found on non-target species they will be sent to the mite specialist Dr Biljana Vidović (University of Belgrade, Serbia) at the end of the field season.

Silvia Barlattani (BBCA) setting up host-specificity tests with the eriophyid mite *Aculus taihangensis* under controlled conditions (left: *Leitneria* sp., right: *Quercus garryana*).

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

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Rhinusa pilosa induced galls on North American *Linaria vulgaris* in spring 2024.

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

In 2024, we continued rearing *R. pilosa* genotypes using an improved rearing protocol carried out in a greenhouse to avoid the unpredictable weather conditions in recent years. A total of 82 copulated females were used for the rearing, of which 35 females were of known haplotype (i.e. haplotype Rp-A3=12 females, Rp-A13=3 females and Rp-B6=20 females). In addition, 30 females of unknown haplotype originating from the laboratory rearing between 2019-2023 and 17 females originating from the field collected in 2023 were used. The rearing resulted in the propagation of over 3500 adults, of which 413 were Rp-B6, 319 were Rp-A3 and 126 were Rp-A13 haplotypes. We will molecularly examine the other adults obtained from each single female to determine their haplotype affiliation.

These results indicate that severe weather conditions during spring heavily affected *R. pilosa* development in previous years and changes in the rearing protocol substantially increased the number of adults.

To supplement the adults obtained in the rearing, several collection trips were conducted for *R. pilosa* galls in eastern Serbia. Between 1 and 12 May. We collected a total of 134 galls, from which 176 adults emerged, indicating a slight recovery of *R. pilosa* populations in 2024. We will use these adults for the genetic diversity study and propagation in the 2025 season.

This year, we supplemented the rearing of *R. rara* with adults from field-collected galls. On 2 June 2024, a total of 150 galls were collected in eastern Serbia from two sites between the towns of Pirot and Bela Palanka. Those galls yielded 297 adults, which were set up for aestivation and hibernation.

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

This spring and summer, we concentrated on establishing rearing colonies and developing mass rearing protocols for *M. laeviceps* and *M. peterharrisi.* The rearing of *M. laeviceps* involved 62 pairs set up between 9 April and 15 May on seedlings of *L. dalmatica* of North American origin (population from Ft. Macleod area, AB, Canada). For oviposition, we carefully clipped the growth tips of each plant several times to produce young, growing shoots 3–4 cm in length. We exposed the plants for oviposition between 10 and 28 April, using two pairs per plant and the plants were dissected on 10 and 16 July. A total of 1560 adults emerged, with an average of 26 adults per female. We reared *M. peterharrisi* using the same method as *M. laeviceps*, using 40 pairs. The rearing of *M. peterharrisi* yielded 362 adults, an average of nine adults per female, which strongly suggests that the rearing of *M. peterharrisi* is a more demanding process, likely related to plant phenology. For the rearing of *M. heydenii,* we used 15 pairs set up on 9 April in 15 plastic containers with potted *L. vulgaris.* The number of adults obtained from *M. heydenii* is still not known because the plants will only be dissected in mid-August.

In general, 2024 saw improvements to the methods used for rearing *Mecinus* spp. However, the applied methods are time-consuming and confirm that for successful mass rearing, the phenostage of the plants exposed to each weevil species plays a crucial role.

Ivo Toševski monitoring the mass rearing of *Linaria* weevils in the greenhouse.

Archanara neurica emerged in plastic cup.

Archanaraneurica resting on a stem in an oviposition cage.

18 Common reed (*Phragmites australis*)

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

In 2024, we continued producing as many pupae and eggs of both moth species, as possible to support further field releases in Canada. Larval hatching of *Archanara neurica* started for the first time at end of March already. About 1750 newly hatched *A. neurica* larvae were transferred into stems of *Phragmites australis*. Of these, 780 third instar larvae of *A. neurica* were transferred from stems onto artificial diet and 575 pupated successfully. An additional 280 pupated on fully reared on stems. For *L. geminipuncta*, 2760 larvae were set up on stems and 370 pupated successfully. We still need to analyze our data to assess the advantages or disadvantages of using artificial diet. At the moment, transferring third instar larvae from stems to the diet seems to be the most efficient rearing technique for *A. neurica*, while rearing fully on stems seems to be best for *L. geminipuncta*.

In June 2024, two shipments with 300 pupae of *A. neurica* and 190 pupae of *L. geminipuncta* were sent to Ottawa. We are aiming at sending up to 10'000 eggs of each species in October 2024.

Summer student Julia Lamoureux collecting *Phragmites australis* stems for moth rearing.

19 Houndstongue (*Cynoglossum officinale*)

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Summer student Madeline Holroyd covering the *Cynoglossum officinale* seed pods for the larval emergence of *Mogulones borraginis.*

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 therefore hard to collect.

From over 4000 *M. borraginis* larvae collected in 2023, about 500 adults emerged in November 2023, and an additional 1300 in March 2024 which were set up on houndstongue rosettes and/or shipped to the USA. At the end of May/beginning of June, about 150 females were retrieved and reset on 58 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. Over 1700 *M. borraginis* larvae emerged so far and were separated into cups (30 individuals per cup) and placed in an underground insectary for adult emergence in autumn 2024 and spring 2025.

In autumn 2023 and spring 2024, a total of 1200 adults of *M. borraginis* were sent to the quarantine facility at the University of Idaho run by Dr Mark Schwarzländer. Currently, work is focused on developing the most effective way to rear *M. borraginis* under quarantine conditions to maximize space and number of adults.

Mogulones borraginis adult on a flower of *Cynoglossum officinale.*

20 Cheatgrass (*Bromus tectorum*)

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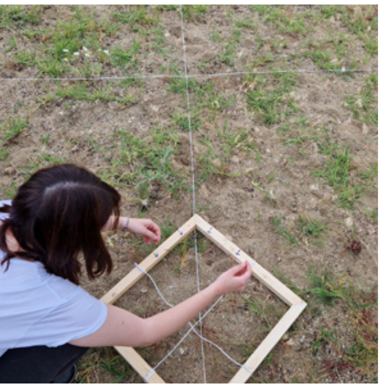
In collaboration with Dr Jacob Lucero from Texas A&M University and Dr Akasha Faust, University of Montana, a biogeographic study has been initiated to better understand the ecological processes determining/regulating plant invasions outside their native range. In particular, we plan to experimentally 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.

This year with the help of our collaborators at the CABI satellite station in Hungary, Dr Stefan Toepfer and students, and the University of Western Macedonia, Greece, Dr Fokion Papathanasiou and Dr Ioannis Giantsis as well as their students we were able to assess the treatments that were performed in October 2023. The treatments included fire and mechanical soil disturbance, as well as the exclusion of grazing, and treatments against insects and pathogens. After sowing of the seeds to artificially create a standardized seed bank, the treatments were left until May 2024. There was a clear treatment effect with fire and disturbance creating the largest visual effect in the short term with several of the sub-plots dominated by cheatgrass. After one season the insecticide and fungicide treatments do not appear to have much effect, however, over the longer term this may change. The vegetation surveys were completed successfully, and the planned insecticide and fungicide treatments are ongoing.

Cheatgrass, *Bromus tectorum* dominating a mechanical soil disturbed sub-plot in Hungary.



Vegetation survey in Prespa, Greece

Cheatgrass clearly dominating the grazing exclusion and fire treated sub-plots at the site in Hungary.

21 Japanese knotweed (*Fallopia japonica*) work in the UK

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21.1 *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. This region has a much better climatic match to the invaded range based on Climex modelling. The first releases of the Murakami line took place into 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. At *F.* x *bohemica* populations in the UK, severe curling damage was observed and adults have successfully overwintered.

The results of the 2022/23 work showed that the development of leaf-curl on *F. japonica* and *F.* x *bohemica* can benefit the knotweed biological control program, both by suppressing plant growth and by improving psyllid survival through the amelioration of abiotic stressors. Based on the results, it is likely that the curling damage on leaves of the knotweed species is induced by the Murakami psyllids; however, the mechanism by which the curling damage is induced by the psyllids has not yet been elucidated. Therefore, molecular analysis of the Murakami psyllids has commenced to search for any as-yet unidentified potential microorganisms that could be contributing to the leaf curling damage. The MinION sequencing platform, which reads DNA by channeling the strand through a nanopore, measuring the electric current that flows through it, was applied. This technology allows for the sequencing of much longer DNA fragments compared to traditional MiSeq, for which results were reported previously. Analysis of the psyllid samples showed that the bacterial community in a female Kyushu psyllid line is mainly composed of the genus *Sodalis* (50%) followed by *Klebsiella* (13%), whereas a female Murakami psyllid line holds 36% of the genus *Sodalis* and 33% of *Wolbachia*. In the Kyushu psyllid line, only 1.4% of *Wolbachia* was detected. Upon sequencing plant samples, it mainly consisted of *Stenotropomonas* on all the samples ex healthy *F. japonica* and *F.* x *bohemica*, *F. japonica* reared with the Kyushu psyllid line and *F.* x *bohemica* reared with the Murakami psyllid line. The data analysis will be completed in the next reporting period.

Heavy curling damage on *Fallopia* x *bohemica* caused by the Murakami line of *Aphalara itadori* in the field.

The Murakami line of *Aphalara itadori* in the field in the UK.

UK field site of *Fallopia* x *bohemia* population released with the psyllid *Aphalara itadori*

Impatiens glandulifera leaf ex Harmondsworth Moor, UK, infected with the strain of the rust *Puccinia komarovii* var. *glanduliferae* ex. Das Khurram, Pakistan.

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

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22.1 *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 across England, Wales and Scotland, where susceptible biotypes of the weed 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 in 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. Assessment of an additional 47 species of specific importance to North America confirmed that the rust is highly host-specific, with no non-target impacts observed.

Two strains of the rust, one originating from India and the other from Pakistan, have been released at 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 2020-2023, following surveys by in-country collaborators, four additional strains of the rust were sourced from India and Pakistan. Unfortunately, the strain obtained in 2020 from Karchon, India, failed to infect the Canadian Himalayan balsam populations and many of the UK populations assessed. A total of three rust stains were collected from northern Pakistan, however, the viability of the spores following import from all sites was found to be very low. Through repeated inoculations a strain from Das Kharram, Astore (IMI 507233), was established on UK Himalayan balsam plants in quarantine. Susceptibility testing of two Canadian populations of Himalayan balsam found that these populations were, at most, weakly susceptible to this strain and plants from a third population could not be tested due to poor viability of seed. A second strain of the rust from Shangrilla Sharda, AJK (IMI 507422) has recently been established under quarantine conditions and will be assessed for its virulence and pathogenicity towards Canadian populations once plants are available.

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