



Weed Biological Control

Progress Report 2022

CABI in Switzerland Rue des Grillons 1, CH-2800 Delémont, Switzerland

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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 Japanese Knotweed (Fallopia japonica) work in the UK	21
19 Himalayan balsam (Impatiens glandulifera) work in the UK	22
Distribution list	23

Cover photos: Lauréline Humair (CABI) and Rodrigo Diaz (LSU) collecting *Phytobius vestitus* in Louisiana; Cornelia Closca collecting *Ceutorhynchus turbatus* in southern Switzerland; Christine Petig setting up an open-field test in the CABI garden.



Hariet L. Hinz



Philip Weyl

Notes from the section leader

Travel restrictions related to COVID-19 were more or less lifted this spring, so we had a busy start to the field season with several field trips, also to Asia (Kazakhstan) this time. As seems to become the norm now, weather conditions were again abnormal with a cold snap in late March and April, and heavy and aggressive thunderstorms in May and early June, which negatively impacted some of our work.

On 7 April, the Team participated in the traditional Quadripartite meeting between CABI, BBCA in Italy, and the CSIRO and USDA-ARS-EBCL labs in Montpellier France. Due to still unclear COVID measures at the time of planning, the meeting was held virtually and included presentations of ongoing work from each of the labs as well as discussions on common challenges such as insect shipments, the Nagoya Protocol and classical biological control in agricultural systems.

On 26 and 27 April, we also participated in the yearly meeting of the USDA-APHIS Technical Advisory Group meeting (TAG), also held virtually. As usual the meeting was extremely informative and useful to attend. Among others, Bob Pfannenstiel (USDA, APHIS) gave an update on current petitions in the pipeline, the tribal consultation process and the process of introduction of approved agents to Alaska. Additional presentations covered biocontrol of invasive grasses, improving predictions of non-target attack, and potential restrictions of the use of fungal pathogens (e.g. Puccinia on Canada thistle) that are already present in the U.S.

On the petition front I have the pleasure to report on several approvals of petitions with CABI involvement. Three agents were approved this spring and summer for field release by the Canadian Food Inspection Agency (CFIA), the eriophyid mite *Aceria angustifoliae* for control of **Russian olive**, the root-mining moth *Dichrorampha aeratana* on **oxeye-daisy**, and the rhizome and leaf feeding weevil *Bagous nodulosus* on **flowering rush**. *Dichrorampha aeratana* and *B. nodulosus* were also recommended by TAG for release in the USA. A big "Thank you!" you goes again to our North American partners for their time investment, which does not stop with the submission of the petition, but requires continuous engagement during the regulatory process.

Petitions currently in preparation include the seed-feeding weevils *Ceutorhynchus constrictus* and *C. peyerimhoffi* on **garlic mustard** and **dyer's woad**, respectively.

Another highlight is confirmed field establishment of the *Archanara* moths on **common reed** in Canada. Meanwhile rearing of the moths was continued at CABI and was particularly successful for *A. neurica*, resulting in over 900 pupae, and over 20'000 eggs! 400 pupae were sent to Ottawa, Canada in June 2022 for additional field releases.

Finally, I would like to take the opportunity to inform you that with my new role as Global Director Invasives within CABI, I am currently handing over responsibility of the weed section to my colleague Philip Weyl. Philip has been working in the weeds section with CABI since 2016. Philip grew up in Malawi and Zimbabwe and did his PhD at Rhodes University in South Africa. He therefore has a very versatile background and is also engaged in the development work of CABI (classical biocontrol of parthenium in Pakistan). He has also become very active in project development and is naturally taking over more and more responsibilities. I therefore have no doubts that the hand-over process will be smooth and bring new life and ideas to the weeds Team! For the time being I will however stay involved in more strategic decisions and will also continue reviewing reports and petitions. I loved being responsible for the weed section and I honestly admit that it is not easy for me to let go.

I now wish you an interesting read!

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As Hariet already mentioned, I will be taking over as leader of the weeds section at CABI Switzerland. I am extremely excited about this and look forward to the challenges that my new role and responsibilities will present, and looking forward to driving the team forward as we take on new weeds and agents in the future. I am extremely fortunate that we have a very dedicated Team of scientists and technicians in the weeds section at CABI and I have no doubt that we will continue to excel in the work we do and continue to develop biological control agents to tackle invasive weeds worldwide. I also look forward to continue working with Hariet and am happy that she will remain involved as much as she can, in the weeds Team.

I hope that you enjoy the following updates and please do not hesitate to contact us if you have questions or comments.

Philip Weyl

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





Adult Rhinusa pilosa on Linaria vulgaris

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*. In the second half of March, 42 females were copulated and on 9 April, two field cages were established for two of the genotypes (A3, B6). Additionally, four genotypes (A1, A4, A8, A13) were set up in 23 framed cages planted with yellow toadflax. Unfortunately, the rearing of *R. pilosa* was compromised by an unprecedented long cold period during April and the first half of May with temperatures below 5°C. This situation affected activity of the weevils and resulted in a strong reduction in oviposition and gall formation, not only in our rearing, but also at field sites. Between 10 April and 13 May, we were only able to collect 144 galls during three collection trips. The two field cages and 23 framed cages produced a total number of 80 and 138 galls, respectively. The number of resulting *R. pilosa* adults will only be known after gall dissections, planned for mid-August.

In contrast to *R. pilosa*, the warm period during March enabled earlier oviposition of *R. rara*, without negative effects on gall development and larval development. On 14 June, a total of 475 *R. rara* galls were collected from *L. dalmatica* and *L. genistifolia* at two sites in eastern Serbia. The galled plants were individually planted and a total of 1,388 adults of *R. rara* were collected during the first week of July.

1.2. Mecinus spp. (Col., Curculionidae)

In 2022, work concentrated on establishing new rearing colonies of *Mecinus heydenii* and *M. peterharrisi*. In spring 2022, 52 adults of *M. heydenii* (30 females) survived overwintering, with which we were able to produce 569 offspring. For *M. laeviceps*, field collections resulted in 42 adults (21 females), which were used for rearing and fitness experiments. The number of resulting *M. laeviceps* adults will only be known after dissections, planned for mid-August. For *M. peterharrisi* 144 adults survived overwintering and were used for rearing and fitness experiments. In mid August, a collection trip for *M. peterharrisi* is planned to the Pelagonian plateau in North Macedonia to reinforce the rearing colony.

In 2022, survival and fitness trials were continued with the two native North American species *Nuttallianthus canadensis* and *Sairocarpus virga*. Both *M. laeviceps* and *M. peterharrisi* were set up on six plants (replicates) of each of the two North American species in addition to controls. Detailed results will be presented in the 2022 annual report.



Ivo Tosevski watering field collected R. pilosa galls induced on Linaria vulgaris

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 3,000 *M. borraginis* larvae collected in 2021, about 580 adults emerged in October 2021, 380 in December 2021 and an additional 260 in April 2022 which were set up on houndstongue rosettes and/or shipped to the USA. At the end of May, beginning of June, about 130 females were retrieved and reset on 72 flowering-seeding houndstongue plants. By mid-July, fruit bearing inflorescences were covered individually with gauze bags and vials attached to the end of each bag to collect mature larvae leaving the fruits. More than 1,700 *M. borraginis* larvae emerged and were separated into cups (30 individuals per cup) and placed in an underground insectary for adult emergence in autumn 2022 and spring 2023.

Over autumn 2021 and spring 2022, a total of 775 adults of *M. borraginis* were sent to the quarantine facility at the University of Idaho (UoI) run by Mark Schwarzländer. Currently work has focussed on developing the most effective way to rear *M. borraginis* under quarantine conditions to maximise space and number of adults.



Cynoglossum officinale seed pod with an egg (white arrow) of Mogulones borraginis



Mogulones borraginis adult on a flower of Cynoglossum officinale



A dense patch of *Pilosella officinarum* in the Black Forest.

3 Hawkweeds (Pilosella spp.)

Ghislaine Cortat and Roel D'Haese

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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 the second biotype carries Wolbachia, which is thought to be responsible for the biotype differentiation. Batches of galls harvested from lab infested plants in 2021 were taken out of cold storage every second week in May 2022. Adults emerging from these were used in host range tests and for rearing.

In 2022, emergence rates of *A. pilosellae* ex *Pilosella* spp. were variable (1-80%, on average 38%) and the sex ratio was well balanced. We exposed 13 test plant species (five North American (NA) natives and five ornamentals or crops) in no-choice tests. The tests are ongoing, but so far galls have only been found on *P. caespitosa*. Final results will be presented in the annual report.

In 2022, no adults of *A. pilosellae* ex *P. officinarum* emerged from our rearing. This might be due the bad weather during gall development in 2021, which made it difficult to harvest the galls at the right developmental stage. We are planning to collect galls in the Swiss Jura during the summer, and in case this fails, we are considering importing galls from the AAFC, Lethbridge colony.

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 genus *Pilosella* and *Hieracium*. *Hieracium argutum* was the only test plant attacked when exposed to *A. pilosellae* ex *P. officinarum* in choice tests exposing a test plant together with the natural host (control). Provided we can grow a sufficient number of plants in 2023, we are planning to conduct an open-field test with *H. argutum*.

3.2 Cheilosia urbana (Dipt., Syrphidae)

In 2022 the first *C. urbana* individual was observed in the CABI garden on 13 April. On 11 May we collected 34 females in the Black Forest in Germany, and adults were prepared for shipment the same day. They arrived and were unpacked at Summerland Research Centre on 16 May. Upon arrival, the adults were dead, but each female had laid about two dozen eggs, unfortunately, no larvae hatched from the eggs. On 18 May, a second field collection took place at the same site, however, the season was probably too advanced and only ten females were collected. These died within two days and the shipment to Bozeman, MT was therefore cancelled.

For our next shipment (likely in spring 2023), we are planning to send eggs carefully packaged in small tubes. This method was tested in a previous shipment and we believe it will work.



Field collection of Cheilosia urbana in the Black Forest, southern Germany

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 initiated the rearing of this weevil in CABI's quarantine facility in Switzerland and are in the process of studying its host range. During surveys in 2022, of the Karatal River in south western Kazakhstan, an additional 21 adults were collected to supplement the rearing in quarantine which has been ongoing since 2019.

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 high levels of mortality. We need to obtain more replicates to get conclusive results. 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 small cages, under quarantine conditions to have some non-target feeding and oviposition, thus, an open-field test is planned for 2023 to fully understand the risk that this weevil might pose to these species. Arrangements for such a test in the native range were made with our local collaborator Dr. Jashenko (Institute of Zoology, Kazakhstan).

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. Currently we believe that it is the same species that has been recorded on surveys in 2018 and again in 2019, however we are awaiting confirmation from the genetic analysis of collected specimens. Preliminary host range testing is promising; however, we are mainly focused on developing a rearing method and understanding the biology of this species in more detail.



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



Cassida sp. collected from Russian knapweed in Kazakhstan in May 2022



C. scrobicollis still covered in phosphorescent powder after overwintering on a rearing plant

5 Garlic Mustard (Alliaria petiolata)

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5.1 Ceutorhynchus constrictus (Col., Curculionidae)

Between 2 March and 15 April 2022, 340 adults of the seed-feeding weevil *Ceutorhynchus constrictus* emerged from our rearing colony established in 2021. After testing for oviposition, fertile females were used at CABI for rearing on *A. petiolata* and host range tests with the native North American (NA) *Boechera perstellata*, *Braya alpina*, *B. humilis* and *Descurainia pinnata* ssp. *nelsonii*. No oviposition was found on *B. humilis* or *B. perstellata*. Eggs were found on four of 15 *D. nelsonii*, but no larvae reached maturity.

In 2017 and 2020, one mature larva emerged from *B. alpina* in no-choice tests, but it did not complete development to the adult stage. So far, oviposition occurred on six of 16 exposed plants under no-choice conditions but without the typical window feeding marks on the pods. No other mature larva emerged from this plant species. We conducted single-choice tests exposing *B. alpina* together with *A. petiolata* in 2020 and 2022. Eggs were only found on the controls and two exploratory feeding holes were found on one of six *B. alpina*.

We therefore conclude that none of the plants tested in 2022 are part of the physiological host range of *C. constrictus*.

In June, about 482 mature larvae of *C. constrictus* were harvested from garlic mustard for adult emergence in spring 2023.

5.2 Ceutorhynchus scrobicollis (Col., Curculionidae)

Between April and July, adult *C. scrobicollis* emerged from rearing plants infested with field collected adults in October 2021. So far, about 50 adults emerged and units of two females and one male are kept on potted rosettes for aestivation. Weevils will either be shipped to AAFC for additional field releases in autumn and/or kept at CABI to maintain a rearing.



Sleeved plants exposed to Ceutorhynchus constrictus in no-choice tests (left), and rearing cages (right)

6 Common Reed (Phragmites australis)

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

In 2022, we focused on producing as many pupae and eggs of both moth species as possible to support further field releases in Canada. After encouraging successes in 2021, we applied a combination of our conventional rearing method using cut stem sections for first instar larvae, and subsequent rearing on artificial diet for 3rd and 4th instar larvae. About 1600 newly hatched larvae of *Archanara neurica* and 2700 larvae of *Lensia geminipuncta* were individually transferred into cut stem sections of *Phragmites australis*. From these, 1300 third instar *A. neurica* and 950 forth to fifth instar larvae of *L. geminipuncta* were transferred onto artificial diet. An additional 800 newly hatched larvae of *A. neurica*, resulting in over 900 pupae, and over 20'000 eggs! However, rearing did not work well for *L. geminipuncta* this year. Many larvae died and only 150 pupae and 2'000 eggs were obtained. This was potentially caused by problems with the quality of the diet at warm temperatures and a virus in the rearing.

In Canada, first establishment of these moths was confirmed during the monitoring of release sites in June 2022. Field releases of eggs, larvae and pupae continued in 2022, with a shipment of about 400 pupae of *A. neurica* sent to Ottawa in June 2022.



Summer student Fleur Fenijn collecting young Phragmites australis stems at a field site close to CABI



Third instar larva of *A. neurica* after having left the rearing stem and before being transferred onto artificial diet.



Summer student Fleur Fenijn with an internode containing a pupal chamber of *L. geminipuncta*



Diaconu Alecu, our collaborator in Romania, collecting *C cardariae*, March 2022

7 Whitetop or Hoary Cress (Lepidium draba)

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7.1 Ceutorhynchus cardariae (Col., Curculionidae)

In spring 2022, we conducted some additional no-choice tests with three native North American (NA) species, *Transberingia virgata, Physaria globosa* and *Physaria saximontana*. The level of attack on the control was good, with an average of 13 galls recorded per plant, while from the test plants, only two replicates of *P. globosa* were attacked, and one adult emerged from one replicate.

In addition to this, we setup a survival test with previously attacked plants in no-choice conditions, to see whether they would be able to sustain a population over time. Species tested were *Lepidium paysonii and Cardamine breweri*. Only two replicates of *L. paysonii* were attacked and only two adults emerged. These were set up on a new *L. paysonii* plant for aestivation. No galls were formed or adults emerged from *C. breweri*.

We also continued maintaining a rearing colony of the weevil at the Centre. Of the 850 adults that overwintered at CABI from 2021, about 62% survived. In March 2022, we conducted a field trip to Romania and collected more *C. cardariae* to enhance the gene pool of our rearing at CABI. The original site from which we had collected adults over the last few years had not been disturbed and competition by grass significantly reduced the *L. draba* population at the site. Nevertheless, we were able to collect 56 females and ten males over three days. The reared and field collected adults were set up on potted plants and about 1,700 *C. cardariae* emerged in 2022.

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 mid April 2022.

7.2 Ceutorhynchus turbatus (Col., Curculionidae)

On 25 May, about 340 adults of the seed-feeding weevil *C. turbatus* were collected in southern Switzerland. Between 26 and 31 May, we established no-choice oviposition tests with 32 plants; six *L. draba* control plants and 1-5 replicates of nine test plant species. Apart from *L. draba*, one egg was found in one of the *Transberingia virgata* replicates, a species native to NA. Unfortunately, no development tests could be set up this year since the control plants foreseen for these tests did not produce seeds (i.e. all were aborted) for development tests.



Anne Mack collecting Ceutorhynchus turbatus in southern Switzerland

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 3000 larvae were collected and are kept for adult emergence in 2023.

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. Unlike last year, this year, we had a very warm spring and summer, and our control plants aged too quickly, so synchronizing the emergence of adults with flowering and seeding dyer's woad, as well as test plant species, was again challenging. At the end of June with the *I. tinctoria* plants that we were able to delay for two months in a plant incubator, we managed to setup three test species, *Physaria globosa, Rorippa columbiae, Transberibgia virgata* in no-choice conditions and one, *R. columbiae* in a development test. One species, *T. virgata,* was attacked with just two eggs on a single individual under no-choice conditions. No development was observed in any of the five replicates of *Rorippa columbiae*.

8.2 Ceutorhynchus rusticus (Col., Curculionidae)

A total of 26 plants, from seven test plant species and the control, were exposed to *C. rusticus* in no-choice oviposition and development tests in autumn 2021. Test plants were regularly checked and any dying plants were dissected. Adult emergence was successful in 2022, with about 75% of the dyer's woad control plants supporting adult emergence with an average of 5.2 adults per plant. Of the seven 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.

In addition to this, we collected larvae in late winter of 2022 in southern Germany, in order to obtain information on the parasitism of the larvae in the field. From the roots we collected, over a hundred larvae were found upon dissection. No ectoparasitoids were found and no endoparasitoids emerged after successful pupation of weevil larvae, suggesting relatively low levels of parasitism, if any, at this site.



Cornelia Closca collecting roots of dyers woad in search of parasitized C. rusticus larvae in southern Germany



Summer student Anne Mack retrieving *C. peyerimhoffi*



Galls of Ceutorhynchus marginellus on Lepidium latifolium



No galls on *Lepidium papilliferum* plant exposed to *Ceutorhynchus marginellus* in single-choice test.

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 *C. marginellus* in the quarantine facility at CABI. We exposed a total of 13 native North American (NA) non-target species and perennial pepperweed under no-choice conditions. Adults emerged only from one of the non-target species (*Lepidium montanum*). These are very encouraging results.

To determine whether plants that supported adult or gall-development under no-choice conditions in 2022, or in earlier years, would also be accepted for oviposition by *C. marginellus* when simultaneously being offered perennial pepperweed, we exposed seven native NA species under single-choice conditions. Under these conditions, larvae were found on two non-target species, i.e. *Lepidium fremontii* and *Lepidium alyssoides*, but generally in much lower numbers than on the control. We also repeated single-choice tests with the federally listed *Lepidium papilliferum* which had been accepted for oviposition in single-choice tests conducted in 2021 when *L. papilliferum* plants were placed next to perennial pepperweed. In 2022, we repeated these tests in larger cages to increase the distance between the two species to approximately 60 cm. Under these conditions, larvae were only found on perennial pepperweed, but not on *L. papilliferum*. These results are very encouraging and may indicate that *L. papilliferum* is unlikely to be accepted for oviposition under field conditions.

In spring 2021, we also continued a population viability test to investigate whether adults that had emerged from *Lepidium paysonii* in spring 2021 and that were since then continuously fed with *L. paysonii* would lay fertile eggs and establish a new generation. This test revealed that multiple generations are possible and it cannot be excluded that *C. marginellus* may establish on *L. paysonii* in the absence of perennial pepperweed, provided it will be accepted for oviposition under natural conditions.

In April, we also sent approximately 300 adults of *C. marginellus* to Mark Schwarzländer at the University of Idaho for studies on the host-finding behaviour of this weevil. In addition, we continue to maintain a rearing colony of *C. marginellus* in our quarantine facility.



Set up of single-choice test with Lepidium papilliferum in a large cage.

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 up to three years for adult emergence.

In May 2022, we found second to fifth instar larvae in three of ten *V. hirundinaria*, and seven of nine *V. nigrum* (overall 0.8 larvae per plant). Two larvae were also found in one of ten *Asclepias incarnata*. In 2022, adults (N=3) only emerged from one *V. hirundinaria* infested in 2020. In July, we field collected about 50 additional adults.

In 2022, 60% of larvae found on *V. hirundinaria* in spring 2021 (plants infested in 2020), successfully developed into adults. No adult emerged from three larvae found on *A. incarnata*, and a single larva found on *A. rubra* and *A. tuberosa*. None of the ten other exposed test plants (eight North American (NA) natives) were attacked.

These are encouraging results, similar to previous tests with the Ukrainian population of the beetle.



Vincetoxicum hirundinaria at the Chrysochus asclepiadeus field site in France



Larvae of *Chrysochus asclepiadeus* between second and fifth instar, found on *Vincetoxicum nigrum* eight months after infestation.



Mature larva of Microplontus millefolii in stem of Tanacetum vulgare.



Adult of Chrysolina eurina on Tanacetum vulgare

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 completed and we started summarizing the data in view of submitting a petition for field release. In parallel, additional no-choice oviposition and development tests were conducted with eight test plant species by Dr Margarita Dolgovskaya and her team (Zoological Institute Russian Academy of Sciences, St Petersburg). Individual shoots of test and control plants were exposed to females for several days and the plants are currently being dissected for larvae and data will be presented in the annual report. Unfortunately, it was not possible to conduct the planned open-field test with the North American native plant *Tanacetum huronense* as none of the 44 available plants of this species produced any stems.

Since it is unclear whether enough *T. huronense* will produce stems in St Petersburg in 2023, we are currently looking into options to conduct an open-field test at CABI in Switzerland instead (or in addition). Therefore, we conducted a field trip to France and Germany in mid-June, to visit locations where *M. millefolii* was recorded 10-30 years ago. Two sites were identified where it might be possible to collect enough *M. millefolii* in 2023. Larvae of *M. millefolii* of all sites were sent to Ivo Toševski to verify whether they are genetically identical to those from St Petersburg.

11.2 Chrysolina eurina (Col., Chrysomelidae)

In spring 2022, we evaluated the open-field test that had been set up in September 2021 with the leaf-feeding beetle *Chrysolina eurina* exposing the two native North American species *Tanacetum camphoratum* and *T. huronense* and the target species *T. vulgare* as a control. Between March and May, we found larvae or newly developed adults on 11 of the 12 exposed plants for *T. vulgare* and *T. camphoratum* and on nine of the 12 exposed plants for *T. huronense*. On average, between three and five times fewer larvae or newly developed adults were found on the test plants compared to the control.

These results indicate that *C. eurina* prefers *T. vulgare*, but 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. One factor that would need to be considered is habitat and geographical overlap between *T. vulgare* and the native species. Since none of them are federally listed species, we decided in accordance with our North American partners to continue working with *C. eurina*.



Christine Petig collecting stems of Tanacetum vulgare presumably infested with larvae of Microplontus millefolii

12 Russian Olive (Elaeagnus angustifolia)

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12.1 Aceria angustifoliae (Acari, Eriophyoidae)

Studies with the mite, *Aceria angustifoliae*, suggest that this species is extremely specific and appears to have a significant impact on the reproductive output of Russian olive under natural field conditions. The petition for field release was submitted to both the U.S. and Canada in November 2019. In May 2020, both the USDA-APHIS Technical Advisory Group (TAG) and the Canadian Biological Control Review Committee recommended release of the mite. In spring 2022, the Canadian Food Inspection Agency (CFIA) approved field release of the mite in Canada. In light of this approval, a fresh culture of the mite was collected in Serbia in June 2022 and is currently being reared at CABI in preparation for future shipments to both Canada and the U.S. in late summer/ early autumn this year.

12.2 Temnocerus elaeagni (Col., Curculionidae)

During surveys in May 2022 of the Karatal River in south western Kazakhstan, seven individuals of the weevil we believed to be *Temnocerus elaeagni* were collected. We are awaiting confirmation from the taxonomists on species identity. The adults of this weevil are thought to feed on the foliage of Russian olive while the larvae mine the shoot tips, which would result in fewer fruit bearing stems being produced.



First signs of Aceria angustifoliae gall formation on the leaves of Russian olive under quarantine conditions at CABI



Philip Weyl beating Russian olive in search of the weevil, *Temnocerus elaeagni* in Kazakhstan



Adult Dichrorampha aeratana



Galls of Oxyna nebulosa on Leucanthemum vulgare

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 November 2021 by Rose DeClerck-Floate and Jeff Littlefield, respectively. In June 2022, the USDA-APHIS Technical Advisory Group (TAG) recommended release of *D. aeratana*, and in July, the Canadian Food Inspection Agency approved release in Canada. Based on comments from TAG, we are currently collecting additional information from the European horticultural industry to confirm that *D. aeratana* is not considered a pest of Shasta daisy in Europe.

In May, we sent eggs of *D. aeratana* to Andrew McConnachie at the New South Wales Department of Primary Industries, Australia which allowed him to re-establish a rearing colony at their quarantine facility. We are also keeping a rearing colony of *D. aeratana* at CABI in order to facilitate future shipments to North America and/or Australia.

13.2 Oxyna nebulosa (Dipt., Tephritidae)

In May and June, we harvested more than 2'500 galls from the oxeye daisy plants that had been exposed to adults of the rhizome-galling tephritid fly *Oxyna nebulosa* in 2021. Between the end of May and mid-July more than 1'000 adults emerged from these galls. We used egg-laying females to set up no-choice oviposition and larval development tests with 20 non-target plant species of importance to North America, multiple-choice tests in field-cages with four non-target species and an open-field test with three 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 2023.

13.3 Cyphocleonus trisulcatus (Col., Curculionidae)

Testing with the root-feeding weevil *Cyphocleonus trisulcatus* at CABI has been completed in 2021. In May 2022, we sent approximately 200 adults to Andrew McConnachie to increase his rearing colony and for additional host-specificity testing. We are also still keeping a small rearing colony at CABI.



Set-up of multiple-choice cage test with Oxyna nebulosa

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. Twelve test plant species (11 North American (NA) natives and one ornamental) and *Convolvulus arvensis* as the control, were planted into the soil at the edge of a cereal field. In 2022, many plants died owing to an alternance of very warm dry weather and violent storms, which caused soil displacement thus baring the roots or burying the shortest plants in soil. Owing to the premature drying of plants and an anticipated early crop harvest, we harvested the experimental plants 1-2 weeks earlier than usual. At the final count, 29 of 50 plants exposed had dried out or were missing. Attack was only found on one *C. arvensis* as adult feeding on the leaves. Due to this very low attack on controls, the test can therefore not be regarded as valid.

Fourteen wild *C. arvensis* plants growing at the edge of the field were harvested upon set up of the experiment and also dissected in the lab. Larvae were found mining the shoots of two plants and a total of seven plants had adult feeding. This confirms that the fly was active at the beginning of the experiment and that the low attack on the exposed plants was due to the adverse weather conditions.

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, and since 2019, we have therefore been trying to establish a rearing colony by transferring eggs onto field bindweed plants in field cages or under common garden conditions. The problem has been that the sesiid larvae need a lot of food and thick enough roots, and many of the rearing plants were killed prematurely by larval mining not allowing full development.

In 2022, the first adults emerged on 5 July. Five females obtained from the rearing plants were successfully copulated and laid a total of 604 eggs during the next 10–12 days. In addition to this, between 13-26 July, four field trips were conducted, and a total of 18 females were collected, which laid another 945 eggs.

The eggs were used in host specificity larval development tests. They were transferred on 15 July, onto potted plants of 12 native NA species and five *C. arvensis* populations from NA. All plants in the test were subsequently kept in a well-ventilated greenhouse. The rest of the eggs were used to maintain the colony on bindweed plants under common garden conditions.



Open-field test with Melanagromyza albocilia in Southern Germany



Copulating pair of Microsphecia brosiformis



Bagous nodulosus adult feeding on leaf of flowering rush.



Summer students Sarah Birkmire and Fleur Fenijn covering flowering rush plants infested with larvae of Bagous nodulosus

15 Flowering Rush (Butomus umbellatus)

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15.1 Bagous nodulosus (Col., Curculionidae)

After having completed all host-specificity testing in 2021, a petition for field release of the weevil *B. nodulosus* was submitted in April 2022. In July, the USDA-APHIS Technical Advisory Group (TAG) recommended release, and in July 2022, the Canadian Food Inspection Agency approved field release in Canada. In April 2022, the first 30 weevils had already been sent to the USDA-ARS Lab in Sidney, Montana, to establish a rearing colony in quarantine, which was successful.

In 2022, we continued testing the rearing techniques developed for *B. nodulosus* in 2021. About 1200 newly hatched larvae were transferred into leaf pieces placed in Petri dishes (diameter 5.4mm). After about a week, 320 emerging second instar larvae were transferred into leaves of 62 potted plants. Dissections of a subset of plants after about six weeks indicated that on average only one larva developed to the adult stage per plant. Thus, this method was less successful than in 2021 and will need to be elaborated further.

In May 2022, an additional 40 weevils were collected in northern Germany to boost our rearing colony at the Centre.

15.2 Phytoliriomyza ornata (Dipt., Agromyzidae)

In spring 2022, about 400 flies emerged from overwintering pupae. Another 300 flies emerged from the 2nd generation. Flies were used for host-specificity testing and to continue rearing on potted plants. All setups were done with two pairs of flies per plant. So far, over 120 rearing plants and 83 replicates with 16 test plant species were set up. No development was found on any of the 24 species tested, confirming the narrow host range of *P. ornata.* Over 1200 pupae were obtained from the rearing of the first two generations so far.

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.



Patrick Häfliger collecting Bagous nodulosus in northern Germany

16 Tree of Heaven (Ailanthus altissima)

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

This leaf-deforming eriophyid mite has initially been identified as *Aculus mosoniensis*, but has recently been re-described as *Aculus taihangensis*. Work with this species is being conducted at BBCA in Rome, Italy.

At the end of May, an open-field host-specificity test was established with five non-target species (four of them native to North America) and tree of heaven plants as controls. Plants were inoculated by attaching tree of heaven leaves infested with *A. taihangensis* collected from a natural site in Italy. To date, all 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 were collected at the end of July from each of the inoculated plants, and currently the number of live mites are being recorded and will be identified by a taxonomist.

In addition, an impact experiment with seedlings was established in spring. For this, 20 seedlings of tree of heaven were infested with at least 100 mites in May while 20 additional seedlings are being kept free of mites by regularly applying an acaricide. The plants are being checked every second week to record their height, total number of leaves and the number of leaves with symptoms indicating the presence of the mites. So far, none of the control plant show any indication of mite attack, whereas inoculated plants are already strongly damaged by the presence of the mite. Detailed results will be available by the end of summer.

Furthermore, data collected in 2021 and early 2022 as part of the experiments conducted under controlled conditions to quantify life-history parameters of *A. taihangensis* such as development time and fecundity, are currently being evaluated and will be presented in the annual report.



Garden plot of the host range test conducted with the eriophyid mite Aculus taihangensis.



Tree of heaven seedling 40 days after its inoculation with *Aculus taihangensis*.



Healthy tree of heaven seedling not inoculated with *Aculus taihangensis*.



Adult Lysathia sp. and eggs on a shoot of parrot's feather in the CABI quarantine



Multiple-choice setup of *Lysathia* sp. under quarantine conditions at CABI

17 Parrot's Feather (Myriophyllum aquaticum)

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17.1 Lysathia sp. (Col., Chrysomelidae)

A rearing of *Lysathia* sp. was successfully established in quarantine and host-specificity testing was initiated in October 2021. Under quarantine conditions (23°C, 16:8 light: dark), males (n=12) live about 97 days and females (n=12) 76 days. On average, females have a pre-oviposition period of about six days, and lay 642 eggs (roughly ten eggs per day) over a period of about two months.

Host range testing is currently ongoing. So far, seven plant species have been exposed, including species occurring in western North America. Development to adults of *Lysathia* sp. was possible on several plant species under no-choice conditions, however, often these species are either not selected for oviposition, or have significantly fewer eggs compared to the control under multiple-choice conditions. Survival tests over multiple generations were conducted for all non-target species. Three species, *Myriophyllum proserpinacoides, Proserpinaca palustris* (Haloragaceae) and *Ludwigia grandiflora* (Onagraceae) could, at a very low rate, support a population of *Lysathia* sp. until F3 generation.

To fully understand the host finding behavior of this insect, we are collaborating with the University of Neuchâtel, Switzerland, to run tests on the chemical ecology of the plants to determine whether or not there are shared volatiles that may be triggering the host choice behavior of females.

Additional host range tests on new plant species are in progress and more non-target plants occurring and/or native to western North America are being field collected in Canada, by our collaborator David Ensing, Agriculture and Agri-Food Canada, for future shipments to Switzerland.

17.1 Phytobius vestitus (Col., Curculionidae)

In collaboration with Louisiana State University (Prof. Rodrigo Diaz) and the Army Corps of Engineers (Nathan Harms) a field trip was conducted in Louisiana and about 100 individuals of the stem-mining weevil, *Phytobius vestitus* were collected. We have initiated a rearing of this weevil and are in the process of studying the biology.

It is native to eastern North America and occurs in regions as far west as Oklahoma, USA, as far north as Michigan, USA, and New Brunswick, Canada, and as far south as central Florida, USA. *Phytobius vestitus* appears to be a *Myriophyllum* specialist with its native host probably being *Myriophyllum heterophyllum*. Damage inflicted to the plant by both larval and adult feeding results in wilted shoots and rotting stems.

We plan to initiate host range testing this summer/autumn and, in collaboration with the University of Neuchâtel, run tests on the chemical ecology of the plants (olfactometer tests) to understand the host finding behavior of this weevil.



The collection of the stem mining weevil *Phytobius vestitus* attacking parrot's feather in Louisiana (from left to right: Carlos Wiggins, Rodrigo Diaz and Philip Weyl)

18 Japanese Knotweed (Fallopia japonica) work in the UK

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

18.1 Aphalara itadori (Hem., Psyllidae)

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

A study comparing the performance between the Murakami and a new stock of the original Kyushu lines on both *F. japonica* and *F. x bohemica* showed that the Murakami line is able to produce a higher number of eggs and adults on both plant species compared to the Kyushu line. In addition, the Murakami line caused severe leaf-curling damage on both knotweed species, while the Kyushu line did not show any curled leaves on either of the two species.

An investigation of the relationship between the timing and intensity of nymphal feeding and the occurrence and severity of leaf curling showed that feeding by nymphs needs to occur very early in leaf development in order to induce leaf curling. The results also confirm that a single nymph is enough to induce leaf curling, but that higher nymphal densities can increase the severity, as well as the number of curled leaves. Furthermore, experiments to examine the effects of artificial leaf rolls on the survival of nymphs highlighted the role of leaf rolling in promoting psyllid survival. Those results suggest that leaf curling provides sheltered, humid, microhabitats which aid the survival and development of nymphs.

Release permits for the Murakami line were obtained from authorities in the Netherlands in 2020 and Canada and the UK in 2021. In the UK, the Murakami line was released onto *F. japonica* and *F. x bohemica* populations in June-July 2021. At *F. x bohemica* populations, severe curling damage was observed and adults have successfully overwintered. Further release experiments have commenced in the field with Dr. Ian Jones, a visiting scientist from AAFC, Canada

18.2 Mycosphaerella polygoni-cuspidati (Fungi, Mycosphaerellaceae)

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

Supported by funds from the UK and the Netherlands experimental field trials conducted over three seasons (2019-2021) at CABI UK, confirmed that *M. polygoni-cuspidati* can infect *F. japonica* and to a lesser extent *F. x bohemica* under more natural conditions. Analysis of the collated data showed that higher ambient temperature and lower relative humidity adversely affect disease development and that early season application of the agent likely leads to better disease development due to higher susceptibility of the plants. Further collaboration with private industry is currently being sought in order to further progress research into the development of a mycoherbicide product.



Set up of experimental field trial with *M. polygoni-cuspidati* in the grounds of CABI UK.



Ian Jones releasing the Murakami line of *Aphalara itadori* into sleeved *F. x bohemica* plants in the field



Daisuke Kurose monitoring disease development on knotweed plants inoculated with *M. polygonicuspidati* in the experimental field trial at CABI UK.



Aecia of *Puccinia komarovii* var. *glanduliferae* infecting a Himalayan balsam seedling in the field

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

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19.1 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. 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 the requirement for additional strains to be identified. Assessment of six Canadian populations of the weed found many to be resistant to the strain from Pakistan and for the Indian strain, levels of infection were much lower than that observed on the positive control plants from the UK. Therefore, additional rust strains are also required for control to be successful in Canada. In order to ascertain where in the native range new strains should be sought, a molecular study was conducted. This study has been extended, using a total of 42 leaf samples from 17 Canadian populations in British Colombia. The results indicate that the two haplotypes present in Canada are identical to the two most common ones in the UK, which further supports the suggestion that the plant was introduced into Canada from the UK.

In January 2020, permission to export a new strain of the rust from Karchon, Uttarakhand, India was granted. However, this strain failed to infect many of the UK and all Canadian populations assessed and was therefore deemed unsuitable. In 2021, further surveys were conducted by a local collaborator in the north-eastern region of Pakistan during August-September. Urediniospores and teliospores were observed and specimens were collected and shipped to CABI UK. Unfortunately, germination of urediniospores was very poor and it was not possible to establish this new strain on Himalayan balsam plants in quarantine.

The local collaborator returned to northern Pakistan in July 2022 to collect additional rust specimens. It is hoped that by surveying earlier in the season, a greater quantity of viable urediniospores will be present and a new strain of the rust can be established. Once this is achieved, the susceptibility of Canadian populations of Himalayan balsam will be assessed.



Sonal Varia assessing Himalayan balsam

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Eagar, Aaron Edmiston, Erika Ensing, David Fee, Mary Fick, Derrill Foster, Aaron Fowler, Simon Fu, Weidong Galford, Shayne Gaskin, John Gephart, Sean Ghorbani, Reza Gibbs, Bary Goolsby, John Gould, Joel Gourlay, Hugh Grammon, Arnie Green, Nicole Grevstad, Fritzi Groenteman, Ronny Gültekin, Levent Gurcan, Kahraman Hanes, Glenn Hardin, Janet Harms, Nathan Haubrich, Greg Haverhals, Marijka Hayes, Lynley Hudson, Wayne Hufbauer, Ruth Hull, Aaron Jones, Marian Jørgensen, Carl L. Justen, Emilie Katovich, Jeanie Korotyaev, Boris Lara, Ricky Littlefield, Jeff Locke, Terri Lovero, Angela Luchessa, Scott Maggio-Kassner, Melissa Marini, Francesca Marks, Jerry Marschman, Brian Mason, Peter Mauro, Tiffany

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