Annual Report 2023

Biological control of flowering rush, *Butomus umbellatus*

Patrick Häfliger, Laura Kostyniuk, Loughlin McQueen, Cornelia Clișca, Sarah Thomas, Daisuke Kurose and Philip Weyl

May 2024

KNOWLEDGE FOR LIFE

Cover photo: Summer student Laura Kostyniuk searching for *Bagous nodulosus* adults on an artificial pond.

CABI Ref: IVM10092
Issued: May 2024

Biological control of flowering rush, *Butomus umbellatus*

Annual Report 2023

Patrick Häfliger[1], Laura Kostyniuk[1], Loughlin McQueen[1], Cornelia Cloşca[1], Sarah Thomas[2], Daisuke Kurose[2] and Philip Weyl[1]

[1]CABI
Rue des Grillons 1, CH-2800 Delémont, Switzerland

Tel: ++ 41 32 421 4870
Email: p.haefliger@cabi.org

[2]CABI
Bakeham Lane, Egham, Surrey, TW20 9TY, UK

Tel: ++ 44 1491 829030
Email: s.thomas@cabi.org

Sponsored by in 2023:
Bureau of Land Management, Idaho
Ministry of Forests, British Columbia, Canada
Montana Department of Natural Resources and Conservation through Missoula County
US Army Corps of Engineers
USDA-ARS

Table of contents

Summary

1. Flowering rush (*Butomus umbellatus*) is a perennial aquatic plant of European origin that was introduced to North America as an ornamental over 100 years ago. It has developed into an aggressive invader of freshwater systems especially in the Midwestern and western states of the USA and in western Canada. Since no effective control methods are currently available, a biological control project was initiated in spring 2013, and CABI in Switzerland subcontracted to conduct surveys for natural enemies in the area of origin of flowering rush. Currently, our work focuses on a weevil in the genus *Bagous,* an agromyzid fly and one fungal pathogen. This report summarizes data collected by CABI in 2023.

2. In July 2022, the USDA-APHIS Technical Advisory Group (TAG) recommended release of the weevil *Bagous nodulosus* for the USA, and the Canadian Food Inspection Agency approved field release in Canada. After establishment of a rearing colony in quarantine at the USDA-ARS lab in Sidney, Montana, in 2022, Natalie West hand-carried 100 additional weevils collected in Slovakia in 2023 to Sidney. The first weevils reared in quarantine are planned to be released in Canada in spring 2024.

3. In 2023, host-specificity testing of the agromyzid fly *Phytoliriomyza ornata* progressed well. A total of 26 test species were exposed, and none of the 40 species tested so far supported the development of larvae, confirming the very narrow host range of the fly. A few replicates are needed to complete host-specificity tests in 2024. A petition for release should be ready to be submitted in winter 2024/2025.

In addition, another impact experiment using three different populations of flowering rush (US triploid, US diploid, European triploid) was carried out. We found a strong impact of the fly with up to 68% reduction in total biomass for diploid plants.

4. Research with the white smut continued with the isolate collected in Romania in 2021. Difficulties with obtaining successful infection of Romanian plant material have been resolved, although still not fully understood. Testing of North American populations with the isolate (IMI507227) has since continued and an additional population (Montana, genotype 1) has been shown to be susceptible to the smut. We remain hopeful that the smut will be an important additional biological control agent for flowering rush.

1. Introduction

Flowering rush (*Butomus umbellatus* L.) is a perennial aquatic plant that grows along lake shores and in slow-moving bodies of water, irrigation ditches and wetlands in temperate Europe and Asia. In several European countries, the plant is considered rare and endangered (Stöhr *et al.*, 2006; Raabe *et al.*, 2011). Fluctuating water levels favour the plant. It usually grows as an emergent with upright foliage in water up to 60–80 cm deep (Hroudová, 1989). In North America, where *B. umbellatus* was introduced more than 100 years ago as an ornamental, the common emergent form is found in water up to 3 m deep and, in addition, submerged populations with flexible leaves suspended in the water column are known in water up to 6 m deep (Jacobs *et al.*, 2011). Flowering rush is now considered an aggressive invader of freshwater systems and is becoming an increasing problem in the Midwestern and western states of the USA and in western Canada. Some impacts include reduced recreational opportunities along rivers and lake shores by interfering with boat propellers, swimming and fishing (Jacobs *et al.*, 2011). In addition, the invasion provides habitat for unwanted species such as the great pond snail (host of swimmers' itch) and introduced predatory fish species such as largemouth bass, northern pike and perch (Jacobs *et al.*, 2011).

Two ploidy levels are known for *B. umbellatus*: diploids (2*n* = 26) and triploids (2*n* = 39) and both have been introduced in North America. The diploid populations are more frequent, especially in the Great Lakes region, while triploids appear to have a wider geographical distribution, which is probably due to their use in and escape from horticulture (Kliber and Eckert, 2005; Lui *et al.*, 2005). Molecular analysis including North American and European populations was carried out by Dr John Gaskin, US Department of Agriculture – Agricultural Research Service (USDA-ARS), Sidney, MT. In AFLP analysis, he found six different genotypes in North America, but most populations belong to 2 genotypes (Gaskin *et al.*, 2021). Genotype 1 (triploid) is most common, especially in the Northwest, while genotype 4 (diploid) is only found in the Northeast (Figure 1).

Figure 1. North American collections of *Butomus umbellatus* and their AFLP genotypes (Gaskin *et al.*, 2021). Genotypes (1–6) are noted next to location points.

Diploids produce abundant fertile seeds, whereas triploids produce far fewer and sterile seeds (Krahulcová and Jarolímová, 1993). Despite heavy investment in seed production by diploids, little or no evidence of sexual recruitment was found in North America, suggesting predominantly clonal reproduction via bulbils (Fernando and

Cass, 1997; Kliber and Eckert, 2005; Lui *et al.*, 2005), whereas North American triploids invest heavily in a large, carbohydrate-rich rhizome and appear to only propagate by rhizome fragmentation (Thompson and Eckert, 2004; Brown and Eckert, 2005).

Several techniques are currently used for flowering rush control, such as mechanical control, planting desirable aquatic plants, managing water levels or chemical control, but all have to be repeated over several years, are costly, unsustainable and may involve high environmental risks (Jacobs *et al.*, 2011). Thus, a biological control project against flowering rush was initiated in spring 2013 on the initiative of Jennifer Andreas (Integrated Weed Control Project, Washington State University, USA), and CABI in Switzerland was subcontracted to conduct surveys for potential biological control agents. Because flowering rush is the only species in the family Butomaceae, the chances of finding very specific biological control agents are very high. We found six insect species in the literature recorded as monophagous on *B. umbellatus* and started working on four of them, two weevils and two flies. Currently, we are concentrating on the weevil *Bagous nodulosus* and the agromyzid fly *Phytoliriomyza ornata*. In 2016, we also started working on the white smut *Doassansia niesslii* in collaboration with plant pathologist Carol Ellison at our UK centre. Since 2020, Sarah Thomas and Daisuke Kurose have been continuing her work. Eleven years after start of the project, the first agent, *B. nodulosus*, is ready to be released in Canada in spring 2024.

2. Work Programme for Period under Report

The work plan for 2023 is outlined below. Results are reported in subsequent sections.

***Bagous nodulosus* (Coleoptera, Curculionidae)**

- Collect additional adults from the field in Slovakia;
- Continue to improve rearing method and increase rearing colony;
- Send more weevils to the USDA-ARS lab in Sidney, MT, to increase the rearing colony in quarantine for subsequent shipments to Canada.

***Phytoliriomyza ornata* (Diptera, Agromyzidae)**

- Completing host-specificity tests;
- Set up another impact experiment, testing the effect of the fly on both diploid and triploid flowering rush populations;
- Maintain a rearing colony at CABI.

***Doassansia niesslii* (Basidiomycota)**

- Investigate and resolve the infectivity issue with isolate IMI507227 on the Romanian flowering rush population;
- If not resolved, then consider a field survey to the site in the Netherlands and/or return to Romania to recollect from the site in the Delta Danube;
- Continue to develop methods for mass production of sporidia in liquid culture to enable a more reliable and efficient method for inoculum production;
- Continue to test the pathogenicity of the Romanian isolate to North American genotypes of flowering rush;
- Depending on the infectivity towards North American genotypes, undertake host-range testing with the Romanian isolate.

3. *Bagous nodulosus* GYLLENHAL (Coleoptera, Curculionidae)

After approval for field release of *Bagous nodulosus* in Canada by the Canadian Food Inspection Agency, and after the recommendation for release for the USA by the USDA-APHIS Technical Advisory Group (TAG), we focused our work on collections and rearing of this weevil. Dr Natalie West (USDA-ARS, Sidney, MT) visited CABI between 26 April and 23 May 2023 to share experiences, in preparation for rearing in quarantine and future field release in the USA.

Plate 1. *Bagous nodulosus* on flowering rush leaf in our rearing pond.

3.1 Field collections

During a field trip to Slovakia with Dr West between 10 and 11 May 2023, a total of 180 weevils were collected. About 100 of these weevils were hand-carried back to the USA for rearing in the quarantine facilities of the USDA-ARS lab in Sidney, MT. About a dozen weevils will be used for DNA analysis. The remaining weevils were used for rearing at CABI.

Plate 2. Dr Natalie West (USDA-ARS, Sidney, MT) searching for *Bagous nodulosus* in a channel in Slovakia.

3.2 Rearing

3.1.1 Rearing in artificial pond

Between the end of April and beginning of June 2023, we collected 144 weevils from our artificial pond system (11 m × 2 m; 50 cm deep) filled with potted flowering rush plants. The numbers are comparable to 2022 (132) and are about the average number of weevils found in the last couple of years (minimum 70, maximum 200). This is a mainly self-sustaining rearing colony. Although we always collect and remove all weevils we can see on daily collections in spring, the weevils seem to be able to lay enough eggs to allow continuous development of weevils. The only maintenance work consists of removing snails, weeding and replacing plants that become pot-bound and stop growing large leaves. We have started to place leaves that may contain weevil eggs, and weevils that have stopped ovipositing into the pond. Whether this helps to increase the output of the artificial pond remains to be seen in summer 2024.

3.1.2 Rearing under confined lab conditions

Although our rearing on potted plants grown in an artificial pond works very well, it is necessary to have rearing techniques in place that work well under confined conditions for rearing in quarantine before release in North America. Unfortunately, this seems to be a difficult task. Earlier attempts to rear *B. nodulosus* by transferring newly hatched larvae onto potted plants resulted in success rates of only 3–6%. And exposing plants to ovipositing adults mostly resulted in only one weevil developing per plant. In 2021, we developed a rearing method that was looking promising. However, the recorded high success rate of up to 20% developing to third instar larvae or pupae was not sustained, and development to adults was only 4.6% in 2022. Nevertheless, it was

applied for rearing in quarantine in Sidney, MT, and we also used it in 2023 while trying to improve it.

METHOD First instar larvae were transferred into cut leaf pieces of flowering rush and kept in tight-sealing Petri dishes (5.4 mm diameter) with a moist filter paper and allowed to develop. After 1–2 weeks, second instar larvae emerging from these leaves were transferred into leaves of potted plants. For this, leaf tips were cut off approximately 15–20 cm above the soil, larvae were transferred with a paintbrush into a prepared hole in the remaining leaf base, and the hole was closed with Parafilm. A total of 125 larvae were transferred this way into 26 plants (internal L2, Table 1).

To test less time-consuming methods, we also transferred a total of 21 second instar larvae just onto the surface of three plants (external L2), and 240 first instar larvae onto the surface of 18 plants (external L1). Since keeping plants submerged in outdoor containers and a pool did not help to increase development success in 2022, plants were kept in buckets filled with water up to soil level in 2023. Plants were covered with fine mesh gauze bags and kept for 1–3 weeks in the lab, before being moved outside and placed alongside a greenhouse. Nineteen plants were dissected 1–3 months after transfer to record the number of larvae developed to third instar, pupa or adult. The remaining plants were regularly checked for emerging adults.

We also transferred about 300 second instar larvae into leaf pieces in 100 larger Petri dishes (diameter 9 cm) to try to obtain full development in Petri dishes. Dishes were checked daily, and exiting larvae were transferred to new leaf pieces.

RESULTS Of 962 first instar larvae transferred into cut leaf pieces of flowering rush, 232 (24%) (36% in 2022, 39% in 2021) successfully developed within 1–2 weeks to second instar larvae. The decrease in success over the years is negatively correlated with the number of larvae transferred. Thus, the more larvae we use, the higher the mortality, likely due to a time constraint limiting care of each individual larva.

Table 1. Rearing success of different methods of larval transfers on plants in 2023.

Method	# plants set up	Total # larvae set up	Total # adults emerged	% success
External L1	18	240	14	5.8
External L2	3	21	1	4.7
Internal L2	26	125	15	12.0

Rearing success of larvae transferred into and onto plants was not good, with a total of only 30 weevil larvae out of 386 successfully developing to adults (Table 1). Internal transfer of second instar larvae was, with 12% adults, the most successful method (Table 1). However, considering that we had already lost 76% of larvae during the development to second instars, external application of first instar larvae was, with 5.8% adults, more successful and involved much less work.

Rearing success in quarantine in Sidney, MT was similar. After 11 adults successfully developed in 2022, between 20 and 40 adults developed in 2023 and will be shipped to Canada for field release in 2024.

We were not able to improve rearing success by rearing larvae completely on cut leaf sections in Petri dishes. No adult developed in 2023, again because we were not able to invest the time needed to take care of individual larvae.

DISCUSSION The method we had developed for rearing of *B. nodulosus* under confined lab conditions is very time consuming and only worked well for small larval quantities. As soon as rearing on a larger scale is required, the development success decreases, because it is impossible to invest the time necessary to maintain sufficient success. For the moment, we consider transferring first instar larvae onto potted plants (15 larvae per 7-litre pot) as the most efficient way to infest plants in quarantine. Exposing plants to ovipositing females could also be a valuable option.

3.1.3 Rearing trial in pool

To test possibilities for rearing in a smaller unit and more controlled conditions than a pond, 46 weevils were released in a pool (2 × 4 × 0.8 m) half filled with potted flowering rush and test plants (Plate 3a). All weevils were marked with nail polish, in order to be able to distinguish weevils that developed in the pool from weevils released in the pool in 2024 (Plate 3b). However, unfortunately the marks tended to fall off some weevils.

Plate 3. Pool with potted flowering rush after release of 46 weevils (a), and marked weevil found four weeks after release in pool (b).

In spring 2024, we will collect all weevils found in this pool. Only if more weevils are found than originally released, will we know for sure that this would also work as a rearing method. This will be especially interesting, since we are planning another impact experiment in 2024 using four pools and different plant densities.

3.3 Preparations for impact experiment in 2024

In September 2023, we set up four pools (2 × 3 × 0.66 m) for an impact experiment with *B. nodulosus* and filled them with rainwater. In spring 2024, we plan to set up six trays in each pool with flowering rush rhizomes of different densities (2 trays × 1 rhizome, 2 × 4 rhizomes, 2 × 9 rhizomes). We plan to release approximately 30 weevils in each of two of the pools. The other two pools will serve as controls without weevils. Half of the trays will be harvested and analysed in fall 2024, the other half in fall 2025.

Plate 4. Four pools prepared for impact experiment with *B. nodulosus*.

4. *Phytoliriomyza ornata* (MEIGEN) (Diptera, Agromyzidae)

The agromyzid fly *Phytoliriomyza ornata* is another insect with potential as a biological control agent of flowering rush. Apart from records of *Butomus umbellatus* as the only host plant, little is known about the life history of *P. ornata* from the literature. Eggs are laid in the leaf epidermis and hatching larvae feed downwards to the leaf base in an inconspicuous mine. Mature third instar larvae feed from the leaf base up again in a wider mine for 20–40 cm, where the puparium is formed. If the adult is to emerge that year, the puparium forms below an emergence window created by the larva, while larvae that develop to overwintering puparia do not create windows, since leaves will have decomposed before adults emerge the following year. Our investigations indicate that the fly can have two to three generations per year. Each generation produces transparent puparia that emerge the same year, and black overwintering puparia. We found in addition brown puparia that also emerge in the same year (Plate 5b). The development of overwintering puparia in each generation seems to be a strategy to allow survival of a population also in dry periods, when no plants are available for oviposition and larval development.

Plate 5. *Phytoliriomyza ornata* female (a) and three types of puparia found: transparent and brown (emerging the same year), and black (overwintering) (b).

4.1 Rearing

Between 22 April and 3 June 2023, 1428 flies (653 females, 745 males) emerged from 1656 overwintering puparia, resulting in the usual good emergence rate of 83% (81% in 2022). Emerged flies were used for host-specificity tests, an impact experiment and to maintain our rearing colony.

METHODS Like last year, we used flowering rush plants grown with emergent leaves for rearing and control plants in experiments, because leaf quality was easier to keep optimal for rearing purposes. Between 26 April and 30 May 2023, 31 flowering rush plants all individually covered with gauze bags were each exposed to two freshly emerged pairs of *P. ornata*. All pots were placed in 10-litre buckets filled with water and kept in the lab for 1–2 weeks before being moved into a shaded open polytunnel. After 1.5–2 months, all plants were dissected for larvae and puparia. Larvae found were transferred onto cut leaf pieces for pupation, and puparia were placed in Petri dishes in a Styrofoam box stored in a wooden shelter at ambient temperatures. From mid-June onwards, Petri dishes were checked daily for emerging flies. Since flies also started to emerge on rearing plants, before dissections, gauze-covered plants were also checked daily for emerging flies. Between 9 June and 12 July, an additional 15 plants were exposed to two pairs of flies of the second generation. To stimulate development of fresh leaves and thereby increase the rearing success of the second generation, plants were cut back 2–3 weeks before exposure. As in the previous two years, we obtained flies from a third generation, and an additional 28 plants were set up with flies between 21 July and 22 August.

Since we observed that some of the unemerged puparia from 2022 were still looking healthy in fall 2023, we kept them for a second overwintering in our wooden shelter.

RESULTS Over 1500 puparia were obtained from rearing plants and from controls in host-specificity tests (Table 2). Half of these are overwintering and available for further tests and rearing in 2024.

Table 2. The number of puparia of *Phytoliriomyza ornata* obtained from rearing and controls in host-specificity tests in 2023.

	Generation	# plants set up	# puparia per plant (mean ± SE)	% adults emerged	% puparia overwintering	% not emerged	Total # puparia
Rearing	1	31	12.4 ± 1.9	46.0	42.3	11.7	385
	2	15	29.7 ± 6.0	38.4	54.1	6.9	445
	3	28	6.0 ± 1.2	10.7	79.9	9.4	169
Tests	1	25	12.5 ± 2.2	36.7	49.2	14.1	313
	2	10	22.1 ± 4.3	57.9	28.1	14.0	221
	3	12	3.5 ± 1.8	2.4	83.3	14.3	42
	Total	121					1575

4.2 Host-specificity tests

Host-specificity tests started in 2019 and were continued in 2020 and 2022. With the large number of overwintering puparia available in 2023, there was high potential for making great progress this year.

METHODS Between 25 April and 8 May 2023, 93 test plants and 25 flowering rush plants were each exposed to two freshly emerged pairs of *P. ornata*. An additional 23 test plants and ten flowering rush plants were set up the same way between 30 June and 10 July 2023. Between 28 July and 17 August, flies of the third generation were set up on an additional 17 test plants and 12 flowering rush plants. On each set-up date at least 2–3 flowering rush plants were set up as a control in addition to the test plants. All pots were placed in 10-litre buckets filled with water. Plants were kept in the lab for at least 1–2 weeks. After 6–8 weeks, all plants were dissected for larvae and pupae. Tests were only considered as valid if pupae were found on more than 50% of the controls.

Table 3. Results of no-choice development tests conducted with *Phytoliriomyza ornata* in 2019, 2020 and 2022 and 2023. Numbers in blue are replicates added in 2023 (species marked in red will require additional replicates to complete host-specificity testing).

Plant species	# replicates set up	# replicates valid[b]	Mean # larvae/pupae found per plant (± SE)
Butomus umbellatus (all)	57+47	51+41[1+2+3]	11.4 ± 1.1
Alisma plantago-aquatica	12	8[1]	0
Alisma subcordatum[a]	6	6[1+2]	0
Alisma triviale[a]	3+3	3+2[2]	0
Baldellia ranunculoides	6	3[1]	0
Blixa aubertii			
Carex obnupta[a]	7+1	5[1]+0	0
Ceratophyllum demersum[a]	6	3[3]	0
Damasonium californicum[a]	2+5	2+5[1]	0
Echinodorus berteroi[a]	6	1	0
Echinodorus cordifolius[a]	6+1	5+1[1]	0
Elodea canadensis[a]	6	2[1]	0
Elodea densa	6	6[1+3]	0
Elodea nuttallii[a]	2+6	2+6[1]	0
Glyceria maxima	6	3[1]	0
Heteranthera dubia[a]	6	6[2]	0
Hydrilla verticillata			
Hydrocharis laevigata	7	6[1+2]	0
Hydrocharis morsus-ranae	6	6	0
Iris pseudacorus	5	4	0
Iris virginica[a]	11	8	0
Lythrum salicaria	7	7	0
Myriophyllum spicatum	6	4[1]	0
Najas guadalupensis[a]	5	5[3]	0
Nuphar advena[a]	5	5[2+3]	0
Nymphaea odorata	7	7[1]	0
Oryza sativa	8	8	0
Phalaris arundinacea	2+4	2+3[1]	0
Persicaria amphibia[a]	8+1	5[1+2]+1[2]	0

Plant species	# replicates set up	# replicates valid[b]	Mean # larvae/pupae found per plant (± SE)
Potamogeton amplifolius[a]	6	1[1+2]	0
Potamogeton natans	6	2[1]	0
Potamogeton lucens	1+6	1+0	0
Potamogeton richardsonii[a]	6	3[1+2]	0
Sagittaria cuneata[a]	6	6[2]	0
Sagittaria graminea[a]	6	6[2]	0
Sagittaria latifolia[a]	6	6[2]	0
Sagittaria platyphylla[a]	12	6[2]	0
Sagittaria rigida[a]	12	7[2]	0
Schoenoplectus acutus[a]	8	6[1+2]	0
Schoenoplectus tabernaemontani[a]	7	6[1+2]	0
Stuckenia pectinata[a]	4	1[3]	0
Vallisneria americana[a]	6	6[2]	0
Zizania aquatica[a]	2+6	3[1]	0

[a] Plant species native to North America.
[b] Including three generations: [1-3] first, second and third generation of fly.

RESULTS In 2023, a total of 26 test plant species was exposed with 1–7 replicates each (Table 3). Of the test plants, 18 were new, while replicates for other test species were increased. Results of 48 plants had to be considered as invalid, mostly because the plants were dying for other reasons. In the tests carried out so far, larvae or puparia of *P. ornata* were only found on the control, flowering rush. No signs of larval development have been found on any of the 40 plant species for which we have valid test results so far (Table 3).

On one leaf of *Sagittaria platyphylla* we found a cephalopharyngeal skeleton (mouthpart) of a fly larva. For the moment, we are unable to determine whether this belongs to *P. ornata* or to another fly species developing on *S. platyphyllum*. We plan to repeat test with this species in 2024 and to dissect plants after two weeks in order to detect live larvae. Larvae of *P. ornata* should be easy to distinguish from other fly species.

4.3 Impact experiment with three populations of flowering rush

Preliminary impact experiments conducted in 2020 and 2021 showed slight, but not statistically significant biomass reductions on plants exposed to flies. One reason for failing to show significant impacts could have been the use of plants with too much variability in size. Since we also expect different plant populations to react differently to fly exposure, we designed another impact experiment in 2023, using three different populations of flowering rush and better standardization for plant size at the start of the experiment.

METHODS In March 2023, rhizome pieces (6–10 cm long, each with 2–3 buds) were prepared and fresh weight recorded. Rhizomes of three populations (USA triploid, Montana; USA diploid, New York; Europe triploid, Slovakia) were potted in pairs in 3-litre pots, making sure that the sum of biomass was similar within each population (Table 4). Plants were grown in an unheated greenhouse for 5 weeks in trays (40 × 60 × 20 cm) filled with water (Plate 6a). On 24 April, the number of leaves was counted, and length of the longest leaf was measured for each pot. Pots with fewest leaves were discarded. Between 2 and 4 May, all pots were covered with a fine mesh gauze bag and half of the pots were exposed to two pairs of *P. ornata*. Six pots formed one

replicate with one treated and one control from each of the three populations tested. Twenty replicates were set up, i.e. 120 pots in total, and moved to an open polytunnel (Plate 6b). Because many plants were wilting in July, we decided to analyse half of the replicates earlier than planned. On 19 July, i.e. 2.5 months after set-up, ten randomly selected replicates were dissected, number of puparia found recorded and number and length of leaves measured. Biomass was measured after drying for 48 h at 80°C. The second half of the replicates were analysed on 7 September, 4 months after set-up. Means were compared with an independent samples *t*-test (SPSS 27).

Table 4. Initial plant measurements for impact experiment in 2023 (mean ± SE).

Population	Treatment	Rhizome weight at start[a]	# leaves at start[b]	Max length at start[b]
Europe triploid	Flies	17.8 ± 0.0	11.8 ± 0.6	32.6 ± 1.3
	Control	17.9 ± 0.1	11.8 ± 0.5	31.9 ± 1.3
	Stats	*t* = -0.055 *P* = 0.510	*t* = 0.000 *P* = 1.000	*t* = 0.750 *P* = 0.684
USA triploid	Flies	22.6 ± 0.4	11.3 ± 0.8	31.5 ± 1.2
	Control	22.6 ± 0.3	10.2 ± 0.9	31.5 ± 1.4
	Stats	*t* = -0.005 *P* = 0.991	*t* = 1.053 *P* = 0.392	*t* = 0.658 *P* = 0.763
USA diploid	Flies	21.5 ± 1.2	13.3 ± 0.6	37.9 ± 1.4
	Control	21.6 ± 1.4	13.4 ± 0.7	38.4 ± 1.6
	Stats	*t* = -0.085 *P* = 0.963	*t* = -0.100 *P* = 0.915	*t* = -0.450 *P* = 0.834

[a] Measured in March 2023.
[b] Measured on 24 April 2023.

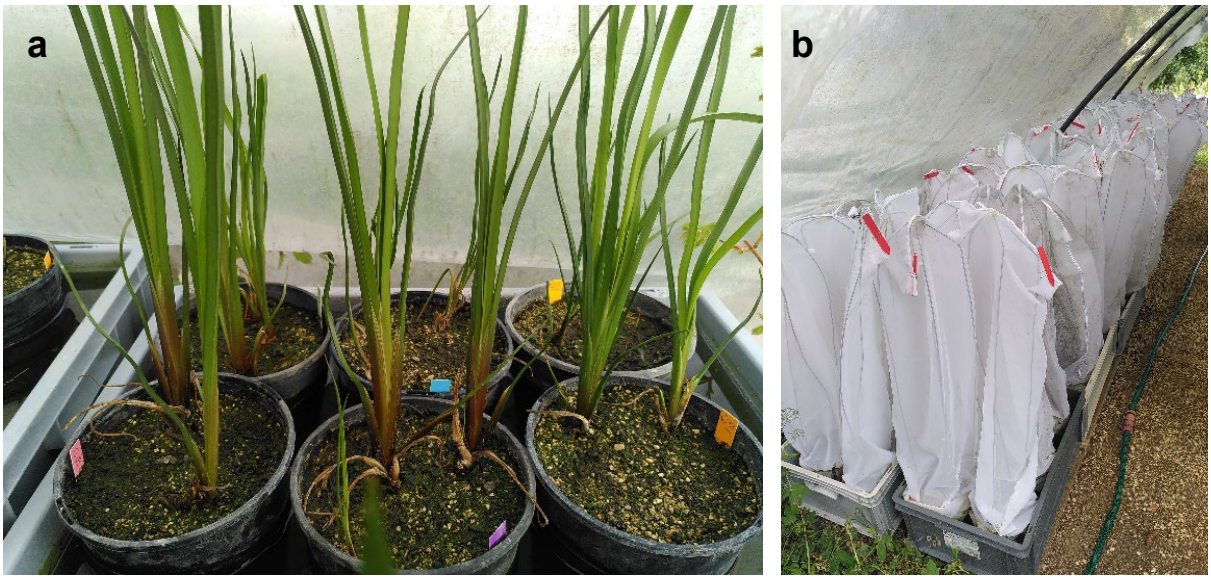

Plate 6. One replicate of plants during set-up of impact experiment in 2023 (a), and set-up in open polytunnel (b).

RESULTS Of the plants analysed after 2.5 months, leaf length was significantly reduced for all three populations by about half (39–60%), while other parameters were

only reduced in diploid USA plants (Table 5). Four months after set-up, most plants, including controls, were completely rotten above-ground. Nevertheless, parameters measured followed a similar trend to the first measurement. Again, diploid USA plants were most-consistently negatively impacted (Table 6). For instance, total biomass was reduced by 37% after 2.5 months, and by 68% after 4 months. A significant reduction in below-ground and total biomass of 33% and 37% respectively was also observed for the European triploid populations (Tables 5 and 6).

Table 5. Results of impact experiment with *P. ornata* in 2023 (first half analysed after 2.5 months).

Population	Treatment	# leaves	Leaf length (cm)	Above-ground biomass (g)	Below-ground biomass (g)	Total biomass (g)	# puparia per plant
Europe triploid	Flies	9.7 ± 1.8	20.5 ± 3.4	0.8 ± 0.2	4.6 ± 0.9	5.4 ± 1.0	6.1 ± 1.9
	Control	10.2 ± 1.4	33.6 ± 2.8	0.9 ± 0.2	5.0 ± 0.2	5.8 ± 0.3	-
	Stats	*t* = 0.500 *P* = 0.829	*t* = 13.139 ***P* = 0.008**	*t* = 0.058 *P* = 0.819	*t* = 0.308 *P* = 0.678	*t* = 0.438 *P* = 0.665	
USA triploid	Flies	7.6 ± 2.3	11.6 ± 4.1	0.3 ± 0.1	5.8 ± 0.3	6.1 ± 0.3	0.9 ± 0.4
	Control	12.0 ± 2.5	28.8 ± 3.5	0.8 ± 0.2	5.9 ± 0.4	6.7 ± 0.5	-
	Stats	*t* = 4.375 *P* = 0.223	*t* = 17.122 ***P* = 0.007**	*t* = 0.486 *P* = 0.074	*t* = 0.085 *P* = 0.853	*t* = 0.570 *P* = 0.308	
USA diploid	Flies	20.3 ± 3.8	28.1 ± 4.1	2.0 ± 0.5	3.4 ± 0.6	5.4 ± 1.1	13.8 ± 2.6
	Control	20.4 ± 2.3	56.7 ± 3.5	3.4 ± 0.3	5.1 ± 0.5	8.6 ± 0.7	-
	Stats	*t* = 0.100 *P* = 0.982	*t* = 28.587 ***P* < 0.001**	*t* = 1.447 ***P* = 0.023**	*t* = 1.721 ***P* = 0.042**	*t* = 3.168 ***P* = 0.024**	

Table 6. Results of impact experiment with *P. ornata* in 2023 (second half, analysed after 4 months).

Population	Treatment	# leaves	Leaf length (cm)	Above-ground biomass (g)	Below-ground biomass (g)	Total biomass (g)	# puparia per plant
Europe triploid	Flies	2.4 ± 1.3	6.3 ± 3.2	0.16 ± 0.08	3.5 ± 0.2	3.6 ± 0.2	0
	Control	2.5 ± 1.1	13.1 ± 7.0	0.48 ± 0.24	5.2 ± 0.6	5.7 ± 0.8	-
	Stats	*t* = -0.100 *P* = 0.954	*t* = -6.795 *P* = 0.390	*t* = -0.313 *P* = 0.241	*t* = -1.774 ***P* = 0.020**	*t* = -2.087 ***P* = 0.032**	
USA triploid	Flies	1.2 ± 0.5	7.8 ± 3.4	0.04 ± 0.02	4.9 ± 0.2	4.9 ± 0.2	0
	Control	4.2 ± 1.9	10.8 ± 6.0	0.43 ± 0.23	6.2 ± 1.2	6.6 ± 1.4	-
	Stats	*t* = -3.022 *P* = 0.154	*t* = -2.980 *P* = 0.661	*t* = -0.390 *P* = 0.127	*t* = -1.281 *P* = 0.284	*t* = -1.672 *P* = 0.236	
USA diploid	Flies	5.1 ± 1.9	26.7 ± 7.1	0.6 ± 0.3	3.3 ± 0.6	3.9 ± 0.9	0
	Control	24.5 ± 4.8	46.1 ± 5.1	3.8 ± 1.4	8.5 ± 2.1	12.3 ± 3.3	-
	Stats	*t* = -19.40 ***P* = 0.003**	*t* = -19.376 ***P* = 0.041**	*t* = -3.183 ***P* = 0.050**	*t* = -5.186 ***P* = 0.035**	*t* = -8.369 ***P* = 0.006**	

4.4 Discussion

Tests carried out so far confirm that *P. ornata* is highly specific to flowering rush. Since oviposition tests were not reliable, all species were tested for potential development. Of the 40 species in 25 genera tested of which 26 species were native to North America, none supported the development of the agromyzid fly. In addition, the larvae have never been observed to leave the plants in search of other plants, thus larval transfer tests were not conducted. Only a few additional species in the genera *Sagittaria*, *Blyxa*, *Hydrilla*, *Najas*, *Stuckenia* and *Nuphar* are planned to be tested for development in 2024 to complete the host-range testing of *P. ornata*.

Although a tendency for reduced above-ground biomass was identified, the two impact experiments carried out in 2020 and 2021 did not show a significant impact on total biomass of flowering rush. There are two possible contributing factors. In 2020, only a single pair of the fly was released per plant which may explain the lack of impact, and in both years only above-ground plant parameters were used to standardize the initial stage of plants (number of leaves and length of longest leaf) and not rhizome size. Together with the low number of replicates this resulted in large variation between replicates and thus no significant differences. Allowing the development of a second fly generation in 2021 led to a 50% reduction of above-ground biomass on triploid plants, and also a tendency for a reduction of below-ground biomass (Häfliger *et al.*, 2023). The experiment from 2023 showed the clearest results with average leaf length being consistently significantly reduced after 2.5 months for all three populations (Table 5), despite the triploid populations not growing well. The impact was significant for the European triploid and USA diploid populations, with a reduction in both the below-ground and total biomass after 4 months of exposure.

Since diploid plants develop much more above-ground biomass relative to below-ground biomass than triploids, we would expect the impact of above-ground herbivores to be different, depending on the ploidy level of the plants. The impact on triploids is uncertain at this stage: it could be higher because fewer leaves are available, or it could be higher because more resources for compensation are available in the rhizomes. Unfortunately, we have so far not been able to test this, either because of the low number of replicates or because of deteriorating plant quality. We believe that our impact experiments so far do not fully reflect the impact capacity of the fly, since plants in the field would be exposed for a longer time period to ovipositing flies, which should reduce their capacity to compensate. The fact that the biomass reduction is observed not only above-ground, but also below-ground (although not always statistically significant), indicates a reallocation of resources from the rhizome to invest in development and growth of new leaves (as was observed with *Bagous nodulosus*, Häfliger *et al.*, 2020).

In addition, the combined impact of *P. ornata* and *B. nodulosus* will likely be additive or even synergistic. Although both species can be found mining in all parts of the leaves, *P. ornata* is found more often on higher parts of the leaves than *B. nodulosus*, which also mines for part of its life cycle in the upper parts of the rhizome.

Based on the expected impact and host-range test results, *P. ornata* is likely to be impactful on flowering rush and highly unlikely that it will be able to develop and sustain a population on any plant species other than flowering rush. We consider *P. ornata* to be an extremely safe and effective biological control agent for flowering rush. Based on our results, a petition for release of *P. ornata* will be initiated with a planned submission in 2025.

5. *Doassansia niesslii* DE TONI (Basidiomycota)

The white smut *Doassansia niesslii* is a leaf pathogen of flowering rush and was identified as an additional potential biological control agent for this invasive plant in 2015 (Häfliger *et al.*, 2016). White smuts are hemi-biotrophic fungal pathogens. This means that their life cycle can only be completed on the host plant, but there is a saprotrophic phase of the life cycle that can grow in culture (on agar). It is this stage which has the potential to be cultured for development as a mycoherbicide (inundative biological control agent).

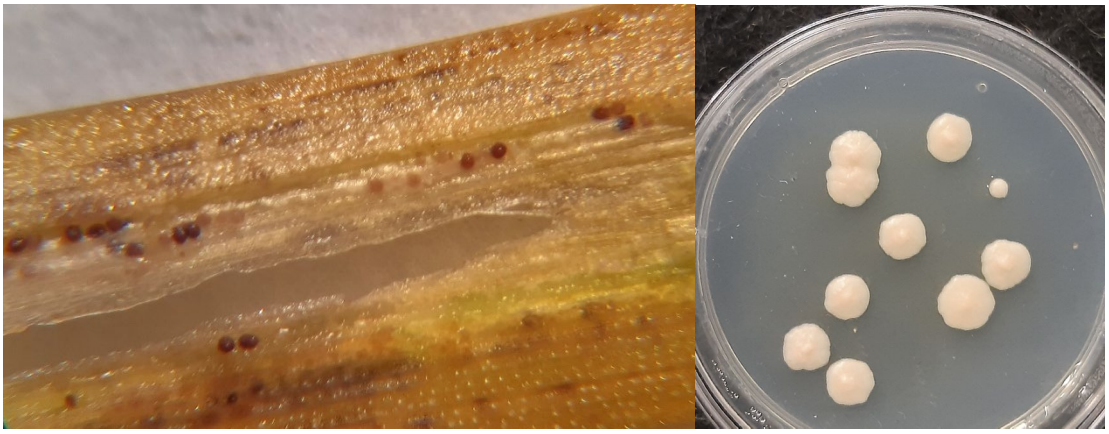

Plate 7. Spore balls (teleomorph stage) of white smut (left); in culture on agar (right).

Doassansia niesslii has two states: the sexual or teleomorphic state and the asexual or anamorphic state. The sexual state is a resting spore, forming completely within the leaf tissue (mesophyll) and once the leaf tissue has senesced enters a period of dormancy (over winter) before germination can occur. The resting spores are only liberated by rupture of old and decaying litter. The asexual state forms as pycnidia just under the epidermis and spores are released outside the plant through leaf stomata. These spores germinate immediately and infect new leaf material, causing severe damage throughout the growing season.

5.1 Testing of North American populations of flowering rush with IMI507227

Since March 2022, there have been some difficulties obtaining successful infection of Romanian plant material. Much of 2023 was spent repeating plant inoculations to try and overcome this setback and in October, infection of Romanian plants was achieved once again. It is now thought that different mating types exist, although this is still not fully understood. Testing of North American populations has since continued and the Montana population (genotype 1) has been shown to be susceptible to the smut.

Plate 8. Successful infection of Romanian (left) and Montana (right) plants, achieved after a period of apparent lack of infectivity.

5.2 Smut culture maintenance and storage

The Romanian isolate (IMI507227) is maintained as reported previously (section 5.1 in Häfliger *et al.*, 2021) by infection of Romanian plants and subsequent re-isolation. It is now hypothesized that different mating types are in existence and therefore, multiple sporidial colonies need to be mixed together when preparing inoculum, to allow mating to occur, which is essential for plant infection.

5.3 Discussion and conclusions

Doassansia niesslii is a damaging potential biological control agent for *Butomus umbellatus* in North America. Three isolates (Germany, IMI507029, France IMI507173 and Romania IMI507227) are now deposited in liquid nitrogen at CABI's centre at Egham in the UK. Further development of mass production methods for the smut will continue which will expedite susceptibility testing of North America populations and subsequent host-range testing. It will also be important to undertake further experiments to fully understand the life cycle of this white smut.

6. Work Programme Proposed for 2024

The following work programme is being proposed for 2024.

***Bagous nodulosus* (Coleoptera, Curculionidae)**

- Collect additional adults from the field in Slovakia;
- Carry out an impact experiment using different plant densities;
- Continue to improve rearing method and increase rearing colony;
- Send more weevils to the USDA-ARS lab in Sidney, MT to increase the rearing colony in quarantine in preparation of future field releases.

***Phytoliriomyza ornata* (Diptera, Agromyzidae)**

- Completing host-specificity tests;
- Start writing the petition for release;
- Maintain a rearing colony at CABI.

***Doassansia niesslii* (Basidiomycota)**

- Continue to test North American populations of flowering rush with the Romanian isolate IMI507227.
- Continue to develop methods for mass production of the smut in liquid culture to achieve a more reliable and efficient method for inoculum production and testing;
- Determine infectivity towards North American genotypes, and initiate preliminary host-range testing with the Romanian isolate.

7. Acknowledgements

We thank Florence Willemin and Quentin Donzé (CABI, Switzerland) for propagating and maintaining flowering rush and test plants.

We are very grateful to Jennifer Andreas for acting as chair of the consortium making our work possible. Funding for this project in 2023 came from the Montana Department of Natural Resources and Conservation, the Bureau of Land Management (BLM), Idaho, USDA-ARS and the US Army Corps of Engineers in the USA, and the Ministry of Forests, British Columbia in Canada.

8. References

Brown, J.S. and Eckert, C.G. (2005) Evolutionary increase in sexual and clonal reproductive capacity during biological invasion in an aquatic plant *Butomus umbellatus* (Butomaceae). *American Journal of Botany* 92, 495–502.

Fernando, D.D. and Cass, D.D. (1997) Developmental assessment of sexual reproduction in *Butomus umbellatus* (Butomaceae): female reproductive component. *Annals of Botany* 80, 457–467.

Gaskin, J.F., Andreas, J., Grewell, B.J., Haefliger, P. and Harms, N.E. (2021) Diversity and origins of *Butomus umbellatus* (flowering rush) invasion in North America. *Aquatic Botany* 173, 103400.

Häfliger, P., MacLean, R., Smith, E., Montoro Caceres, M., Streiff, L., Toševski, I. and Hinz, H.L. (2016) Biological control of flowering rush, *Butomus umbellatus*. Annual Report 2015. CABI, Delémont, Switzerland, 16 pp.

Häfliger, P., Guala, M., Trifonov, T., Stettler, P., Bokla, T., Cloşca, C., Toševski, I., Ellison, C. and Hinz, H.L. (2020) Biological control of flowering rush, *Butomus umbellatus*. Annual Report 2019. CABI, Delémont, Switzerland, 30 pp.

Häfliger, P., Nardelli, M., Fattore, S., Rodriguez, M., Cloşca, C., Thomas, S., Kurose, D. and Hinz, H.L. (2021) Biological control of flowering rush, *Butomus umbellatus*. Annual Report 2020. CABI, Delémont, Switzerland, 24 pp.

Häfliger, P., Sarah Birkmire, Fleur Fenijn, Cloşca, C., Thomas, S., Kurose, D. and Hinz, H.L. (2023) Biological control of flowering rush, *Butomus umbellatus*. Annual Report 2022. CABI, Delémont, Switzerland, 16 pp.

Hroudová, Z. (1989) Growth of *Butomus umbellatus* at a stable water level. *Folia Geobotanica et Phytotaxonomica* 24, 371–385.

Jacobs, J., Mangold, J., Parkinson, H., Dupuis, V. and Rice, P. (2011) *Ecology and Management of Flowering Rush (*Butomus umbellatus *L.).* Invasive Species Technical Note No. MT-33. USDA-NRCS Montana.

Kliber, A. and Eckert, C.G. (2005) Interaction between founder effect and selection during biological invasion in an aquatic plant. *Evolution* 59, 1900–1913.

Krahulcová, A. and Jarolímová, V. (1993) Ecology of two cytotypes of *Butomus umbellatus*. I. Karyology and breeding behaviour. *Folia Geobotanica et Phytotaxonomica* 28, 385–411.

Lui, K., Thompson, F.L. and Eckert, C.G. (2005) Causes and consequences of extreme variation in reproductive strategy and vegetative growth among invasive populations of a clonal aquatic plant, *Butomus umbellatus* L. (Butomaceae). *Biological Invasions* 7, 427–444.

Raabe, U., Büscher, D., Fasel, P., Foerster, E., Götte, R., Haeupler, H., Jagel, A., Kaplan, K., Keil, P., Kulbrock, P., Loos, G.H., Neikes, N., Schumacher, W., Sumser, H. and Vanberg, C. (2011) Rote Liste und Artenverzeichnis der Farn- und Blütenpflanzen, Pteridophyta et Spermatophyta, in Nordrhein-Westfalen, 4. Fassung. *LANUV-Fachbericht* 36, 51–183.

Stöhr, O., Wittmann, H., Schröck, C., Essl, F., Brandstätter, G., Hohla, M., Niederbichler, C. and Kaiser, R. (2006) Beiträge zur Flora von Österreich. *Neilreichia* 4, 139–190.

Thompson, F.L. and Eckert, C.G. (2004) Trade-offs between sexual and clonal reproduction in an aquatic plant: experimental manipulations vs phenotypic correlations. *Journal of Evolutionary Biology* 17, 581–592.

Distribution list

Gary Adams
Laurel Baldwin
Edward Barge
Lutz Behne
Chris Borkent
Rob Bourchier
Janine Brooke
George Bruno
Roy Brunskill
Paul Brusven
Steven Burke
Mickie Chamness
Jamie Cass
Brian Clapp
Al Cofrancesco
Tim Collier
Kelly Cooley
Eric Coombs
Craig Dawson
Rose De Clerck-Floate
Wendy DesCamp
Tim Dietzler
Celestine Duncan
Virgil Dupuis
Steve Fleming
Bryce Fowler
Lindy Garner
John Gaskin
Roderick Gilbert
Rafal Gosik
Nicole Green
Michele Hanson
Martin Hanzl
Nathan Harms
Greg Haubrich
Marijka Haverhals
David Heimer
Greg Hensel
Zdenka Hroudova
Carl Jorgensen
Nicole Kimmel
Henrich Klugkist
Roland Krusi
Ricky Lara
Sam Leininger
Liz Lodman
Anna Lyon
Tricia MacLaren
Tiffany Mauro
Alec McClay
Peter McEvoy
Craig McLane
Patrick McPherren
Karin Menke
Mesman Amy
Valerie Miller
Ray Newman
Loretta Nichols
Colin Park
Jenifer Parsons
Kim Patten
Jeffrey Pettingill
Mike Pitcairn
Laura Poile
Tim Prather
Art Preachuck
Carol Randall
Vic Reeves/Robin Kuske
Jasmine Reimer
Peter Rice
Mark Schwarzlaender
Lizbeth Seebacher
Lynn Shuldberg
Gayah Sieusahai
Bridget Simon
Larry Skillestad
Lincoln Smith
Sharon Sorby
Mark Sytsma
Kenneth Thacker
Michael von Tschirnhaus
Kevin Veenstra
Sharlene Vowels
Chip Welling
Mitch Whitmill
Teresa Wickeham
Shannon Williamson
Rachel Winston

Africa

Ghana

CABI, CSIR Campus
No.6 Agostino Neto Road
Airport Residential Area
P.O. Box CT 8630,
Cantonments
Accra, Ghana
T: +233 (0)302 797 202
E:westafrica@cabi.org

Kenya

CABI, Canary Bird
673 Limuru Road,
Muthaiga
P.O. Box 633-00621
Nairobi, Kenya
T: +254 (0)20 2271000/20
E:africa@cabi.org

Zambia

CABI, Southern Africa
Centre
5834 Mwange Close
Kalundu, P.O. Box 37589
Lusaka, Zambia
T: +260 967619665
E: southernafrica@cabi.org

Americas

Brazil

CABI, UNESP-Fazenda
Experimental Lageado,
FEPAF (Escritorio da
CABI)
Rua Dr. Jose Barbosa De
Barros 1780
Fazenda Experimental
Lageado
CEP: 18.610-307
Botucatu, San Paulo, Brazil
T: +55 (14) 3880 7670
E: y.colmenarez@cabi.org

Trinidad & Tobago

CABI, 59 Gordon Street,
Curepe, St. Augustine
TUNAPUNA 331323
Trinidad & Tobago
T: +1 868 6457628
E: caribbeanLA@cabi.org

USA

CABI, One Lincoln Street
24th Floor
Boston, MA 02111
T: +1 (617) 682 9015
E: h.jansen@cabi.org

Asia

China

CABI, Beijing
Representative
Office
Internal Post Box 85
Chinese Academy of
Agricultural Sciences
12 Zhongguancun Nandajie
Beijing 100081, China
T: +86 (0)10 82105692
E: china@cabi.org

India

CABI, 2nd Floor, CG Block,
NASC Complex, DP
Shastri Marg
Opp. Todapur Village,
PUSA
New Delhi – 110012, India
T: +91 (0)11 25841906
E: india@cabi.org

Malaysia

CABI, PO Box 210
43400 UPM Serdang
Selangor, Malaysia
T: +60 (0)3 894329321
E: cabisea@cabi.org

Pakistan

CABI, Opposite 1-A,
Data Gunj Baksh Road
Satellite Town, PO Box 8
Rawalpindi-Pakistan
T: +92 51 9292062/ +92 51
8434979
E: cabi.cwa@cabi.org

Europe

Netherlands

CABI, Landgoed Leusderend 32
3832 RC Leusden
The Netherlands
T: +31 (0)33 4321031
E: netherlands@cabi.org

Switzerland

CABI, Rue des Grillons 1
CH-2800 Delemont
Switzerland
T: +41 (0)32 4214870
E: europe-CH@cabi.org

UK

CABI, Nosworthy Way
Wallingford, Oxfordshire
OX10 8DE, UK
T:+44 (0)1491 832111
E: corporate@cabi.org

CABI, Bakeham Lane
Egham, Surrey
TW20 9TY, UK
T: +44 (0)1491 829080
E: microbialservices@cabi.org
E: cabieurope-uk@cabi.org