Perspective

Received: 18 November 2022 Revised: 16 January 2023 Accepted article published: 30 January 2023 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/ps.7390

When a plant invader meets its old enemy abroad: what can be learnt from accidental introductions of biological control agents

Heinz Müller-Schärer,[a,b] Yan Sun[b*] and Urs Schaffner[c]

Abstract

Accidental introductions of biological weed control (BWC) agents (i) offer opportunities to assess host use of agents with a potentially broader fundamental host-range than those approved for field release directly in target areas; (ii) urge national authorities to rapidly respond as they may threaten native species or crops, and by this (iii) help advancing post-release studies, a neglected aspect of BWC. Through detailed insights gained from studying the recent accidental introduction of the ragweed leaf beetle *Ophraella communa* into Europe, we derive suggestions for overcoming barriers to adoption of BWC by re-evaluating the predictive power of pre-release studies and, thus, the presently strict criteria for deciding upon their release that might exclude safe and efficient agents. By using the allergenic weed *Ambrosia artemisiifolia* and the accidentally introduced BWC agent *O. communa* as study system, we also hope to raise the awareness of authorities to consider biological control more prominently as a key approach for pest management in the 'One Health' concept, which aims to sustainably balance and optimize the health of people, animals, plants and ecosystems.
© 2023 Society of Chemical Industry.

Supporting information may be found in the online version of this article.

Keywords: importation biological control; pre-release and post-release studies; risk assessment criteria; *Ambrosia*; *Ophraella*

1 ACCIDENTALLY INTRODUCED BIOLOGICAL WEED CONTROL AGENTS: DEFINITION, CHARACTERISTICS, AND OPPORTUNITIES

The number of invasive non-native plants (INNPs) is predicted to further increase in the future,[1] thereby escalating their already severe impacts on nature and human well-being.[2] Management tools have been developed to clear particular sites such as a crop field or a wetland from INNPs or to control early-stage invasions, but are only rarely available to halt or reduce large-scale invasions. Importation biological weed control (IBWC; also termed classical biological weed control), offers a potentially effective tool, especially when combined with other land management interventions.[3] This approach involves the deliberate release of specialist natural enemies, mostly arthropods and pathogens, from the weed's native range. Current practice for selecting the best candidates is based on the agent's host specificity, efficacy, and climatic suitability, but the crucial issue for deciding to import and release an agent remains its narrow host range, best being limited just to the target species in the introduced range.[4] This is easier to achieve for target species that are taxonomically isolated in their introduced range. Finding monophagous species that are also expected to impact their host weed is much more difficult for targets of species-rich families, such as in Asteraceae, which generally are represented in most plant communities, but which also constitute a significant amount of INNPs.[5] Various reviews have dealt with the methodology to assess the fundamental host range (the list of plant species on which a herbivore can complete its full lifecycle or specific stages of its lifecycle) and ecological host range (the subset of plant species from the fundamental host range that are actually used under field conditions) of IBWC agents (see Müller-Schärer and Schaffner[4] and references cited therein). Pre-release studies need to be feasible regarding costs and time needed, and prediction of an agent's host use after its introduction into a new range will always entail some level of uncertainty. In practice, agents that could fully develop on non-target native plants or on crops under no-choice conditions have been refused,[6] as in the case of *Ceratapion brevicorne* (Illiger), a biological control candidate of yellow starthistle (*Centaurea solstitialis* L.). This weevil was most likely not approved for field release in the United States due to data showing that its fundamental host range includes the crop plant safflower (*Carthamus tinctorius* L.); yet the weevil has never been recorded attacking safflower

* Correspondence to: Yan Sun, College of Resources and Environment, Huazhong Agricultural University, 430070 Wuhan, China, E-mail: yan.sun@mail.hzau.edu.cn

a Department of Biology, University of Fribourg, Fribourg, Switzerland

b College of Resources and Environment, Huazhong Agricultural University, Wuhan, China

c CABI, Delémont, Switzerland

under field conditions, suggesting that the risk to this non-target plant may be insignificant.[6,7]

Introductions of non-native species into a new continent have increased in recent years as part of the huge global exchange of goods and human travel. As part of such introductions, accidental introductions of potential biological weed control (BWC) agents have also increased, which we define here as unintentionally introduced natural enemies, which are already used for biological control of an INNP in another part of the invaded range. Unapproved deliberate releases may also be considered as accidental introductions, as no one would claim responsibility for such a release, and their number are expected to increase, if regulations for IBWC are non-existent or too stringent.[8]

1.1 Characteristics of accidentally introduced biological weed control agents

Accidental introductions of weed biocontrol agents are now common not only for Europe (Table 1), but also in other parts of the world, and contribute to the species' invasion dynamics, just as with other organisms. In general, however, less attention is given to them at border controls, as priorities of national authorities is often given for inspecting imports of known harmful organisms.[9] Treating all non-native species as a biosecurity threat would be the ideal approach to detect, intercept, and eliminate accidental introductions, but this is a prohibitively expensive approach.

Several factors are assumed to favour accidental introductions of BWC agents, including the general increase in international trade and human travel, incomplete surveillance of species that are not associated with live plants, the difficulty to detect or intercept tiny organisms such as the various developmental stages of insects and microorganisms, and traits or developmental stages that allow survival in harsh conditions. Furthermore, accidental pick up of biological control agents may be favoured in the vicinity of traffic and trade hotspots, such as around airports and harbours, or along main international car or train routes, where both host and natural enemy populations often can reach high densities in such ruderal habitats.[10,11] Similarly, accidental invasions frequently start in introduced areas where their host is abundant, often at much higher densities than in the native region. Such high-density host populations are frequently also associated with disturbed habitats around traffic hotspots, offering a bridgehead in the receiving area that facilitates successful establishment and spread of a natural enemy.[12]

Differences in the population make-up between approved releases of BWC agents and accidentally introduced agents (including non-authorized deliberate releases) can be expected (Table 2). Current practice for selecting the best candidates for IBWC is based on the agent's host specificity, efficacy, and climatic suitability, but a population of an accidentally introduced BWC agent might have a slightly broader host range than a population of an in-depth tested, authorized and deliberately released BWC agent. This may pose a potential threat to non-target species (cf. later), and the accidently introduced population may not be the best climatic match for maximum impact on its hosts. Mass rearing for quarantine purposes and non-target testing helps to eliminate parasitoids and virulent pathogens, but it may also result in a loss of genetic variation in BWC agents and to adaptation to laboratory conditions during prolonged rearing.[13] Such domestication will most likely result in a less effective performance in the field, which is not the case in accidental introductions. Furthermore, because of the increased risk of non-target effects, modern guidelines for IBWC request that single populations are separately assessed for potential efficacy and safety before their introduction.[14] Significant among-population differences in biocontrol agents in the native range have been well documented, especially for ecological traits linked to climate and host plant use.[15–17] However, among-population variation can no longer be used to increase genetic diversity and thus increased likelihood of establishment, population build-up and control by collecting biological control agents from distinct populations and subsequently mixing them to promote adaptation post-release. This is because, due to time and financial constraints, mostly only a single population is studied in-depth and subsequently released. Accidental introductions might also be based on single populations, but multiple introductions are also likely, e.g., if they benefit from a certain introduction pathway. Such multiple introductions may stem from genetically distinct populations and admixtures pre-release, then further gain from admixtures post-release (cf. the *Ophraella* example later), resulting in populations with increased standing genetic variation in quantitative

Table 1. Accidental introductions of importation biological weed control (IBWC) agents in Europe (up to 2020)[a]

Target weed	Agents	Country first recorded	Year first recorded	Countries present today	Research team
Azolla filiculoides	*Stenopelmus rufinasus*	United kingdom	*c.* 1920 s	ES, UK	CABI-UK, Generalitat Valencia
Opuntia ficus-indica	*Dactylopius opuntiae*	Spain	<2009	ES	Generalitat Valencia
Ailanthus altissima	*Aculus taihangensis*	Hungary	2016	HU	BBCA, CABI-CH, USDA-ARS-EBCL
Ambrosia artemisiifolia	*Epiblema minutiana*	Israel	2008	ISR	Ministry of Agriculture, Israel
	Tarachidia candefacta	Bulgaria	2009	BU, SR	BBCA/University of Belgrade
	Aceria artemisiifoliae	Serbia	2009	SR, IT, SK	University of Belgrade/BBCA
	Ophraella communa	Italy	2013	CH, HU, IT, SL, HR, RO	University of Fribourg/CABI
Agave americana	*Scyphophorus acupunctatus*	Spain	2007	ES, FR, PO	Generalitat Valencia

Abbreviations: BBCA, Biotechnology and Biological Control Agency, Rome, Italy; BU, Bulgaria; CABI, Centre for Agriculture and Bioscience International, UK and Switzerland; CH, Switzerland; ES, Spain; Generalitat Valencia, VAERSA-Generalitat Valenciana, Spain; HR, Croatia; HU, Hungary; ISR, Israel; IT, Italy; RO, Romania; SK, slovakia; SL, Sierra Leone; SR, Serbia; UK, United Kingdom; USDA-ARS-EBCL, US Department of Agriculture-Agricultural Research Service-European Biological Control Laboratory.

[a] These are the only observed accidental introductions of IBWC agents in Europe known by the authors after checking published records, consulting all accessible reports, and inquiring with experts in this field.

Table 2. Attributes of accidental introductions as compared to authorized releases

Attribute	Deliberate (authorized) releases	Accidental introductions
Host range	Narrow (monophagous)	Might be broader (oligophagous)
Climate match	Selected for	May not
Genetic variation	Rearing culture and mass rearing reduce genetic variation	Does not apply
	Guidelines request testing and releasing of single populations	Multiple introductions likely, increasing genetic variation
Natural enemies	Reared free of natural enemies during quarantine testing before release	Might carry its diseases or parasitoids, hampering population build-up
Post-introduction studies	Often greatly neglected	Rapidly initiated when detected

traits, often even higher than in native populations, and on which selection can then act upon.[18]

If an accidentally introduced BWC agent has a somewhat broader host-range than deliberately released BWC agents, this offers the opportunity to study its realized host range in the field directly in the target area. This, in turn, can provide important insight into what kind of pre-release studies generate the most accurate predictions of the host use or level of non-target attack in the invaded range, and to re-evaluate the criteria used in risk assessment for deciding to import BWC candidates. With too strict selection criteria for importing BWC agents, there is a risk that potentially highly effective agents that might be 'safe' in the introduced range under field conditions are being rejected for importation.

An accidental introduction of a BWC agent also urges national authorities to initiate rapid post-introduction studies as it may threaten wild or crop species closely related to the target invader. Thus, accidental introductions may contribute significantly to a better understanding of the factors affecting the outcome of IBWC, although in principle similar lessons could also be learned from deliberately introduced BWC agents, but such studies are often greatly neglected.[19]

In the following, we will first summarize new insights for biosafety, efficacy, and sustainability of IBWC gained from studies following the recent accidental introduction of the ragweed leaf beetle *Ophraella communa* into Europe. From these findings, we will then derive opportunities for overcoming barriers to adoption of IBWC.

2 NEW INSIGHTS FROM STUDYING THE ACCIDENTAL INTRODUCTION OF *OPHRAELLA COMMUNA*

The North American native *Ambrosia artemisiifolia* L. (*Ambrosia* in the following) has invaded different parts of the world and its spread and impact are likely to increase with changing climate.[20] This plant has raised awareness as a major agricultural weed in spring-sown crops, but mainly also due to its production of a large number of highly allergenic pollen grains, resulting in huge health costs. In Europe, some 13.5 million people suffer from *Ambrosia*-induced allergies, causing costs of €7.4 billion annually.[21] In 2013, we started an interdisciplinary and international research programme with the aim of developing a sustainable management strategy for *Ambrosia* in Europe, which also included a focus on biological control.[22] To our surprise, in 2013, we found large infestations of the North American ragweed leaf beetle *O. communa* L. (*Ophraella* in the following) in southern Switzerland and northern Italy, a species that was not on the list of Europe's prioritized six native North American insect herbivores (plus one rust pathogen) for in-depth studies.[23] This is because the species is reported to be oligophagous and, as a consequence, was also rejected as a BWC agent for *Ambrosia* control in Australia, as host specificity tests revealed that under no-choice conditions *Ophraella* can complete its life cycle on sunflower, *Helianthus annuus* L.[24] (Supporting Information, Table S1). The species was also accidentally introduced into China in 2001, where host specificity studies conducted under field conditions showed that the risk of non-target effects by *Ophraella* on cultivated sunflower is low; adults may occasionally feed on sunflower, but females rarely lay eggs, and larval survival is low[25,26] (Table S1). Since 2007, *Ophraella* has been mass-reared and actively distributed in China and is considered a highly successful biological control agent.[27] For Europe, however, the accidental introduction of *Ophraella* created an urgent need for national authorities to decide whether its establishment should be considered as a fortunate coincidence in the campaign against *Ambrosia* or whether it should be considered as a threat to closely related wild or crop species.[22] Thanks to the then newly established research network composed of experts in weed management, biological control, plant distribution monitoring, plant invasion biology, aerobiology, public health, and economics, a rapid response to this unexpected guest was possible.[28] A series of ecological studies evaluating the safety and effectiveness of this beetle, together with modelling studies to predict future spread and impact were initiated. In addition, novel experimental evolution experiments were started to explore eco-evolutionary outcomes of this weed biocontrol system under present and future environmental conditions.

2.1 Rapid spread and modelling expected impact after the accidental introduction

Since its first records in 2013 in northern Italy and southern Switzerland, *Ophraella* has spread towards the east up to Bucharest, Romania, where it was observed in 2021 (Fig. 1). Despite the fact that studies indicate a high dispersal potential of *Ophraella*,[29] we assume that the fast long-distance spread mainly occurred by air with goods and human travel, as initial observations in Budapest, Hungary, and Bucharest, Romania were made around the airports, where *Ambrosia* is reported to be abundant (Stefan Toepfer, personal communication). We have been collecting beetles since 2013 from the initial populations and along the eastward spread. Genomic analyses are presently underway to identify potential changes in population genetic variation along the spread routes, and to explore which genotypes are actually spreading.

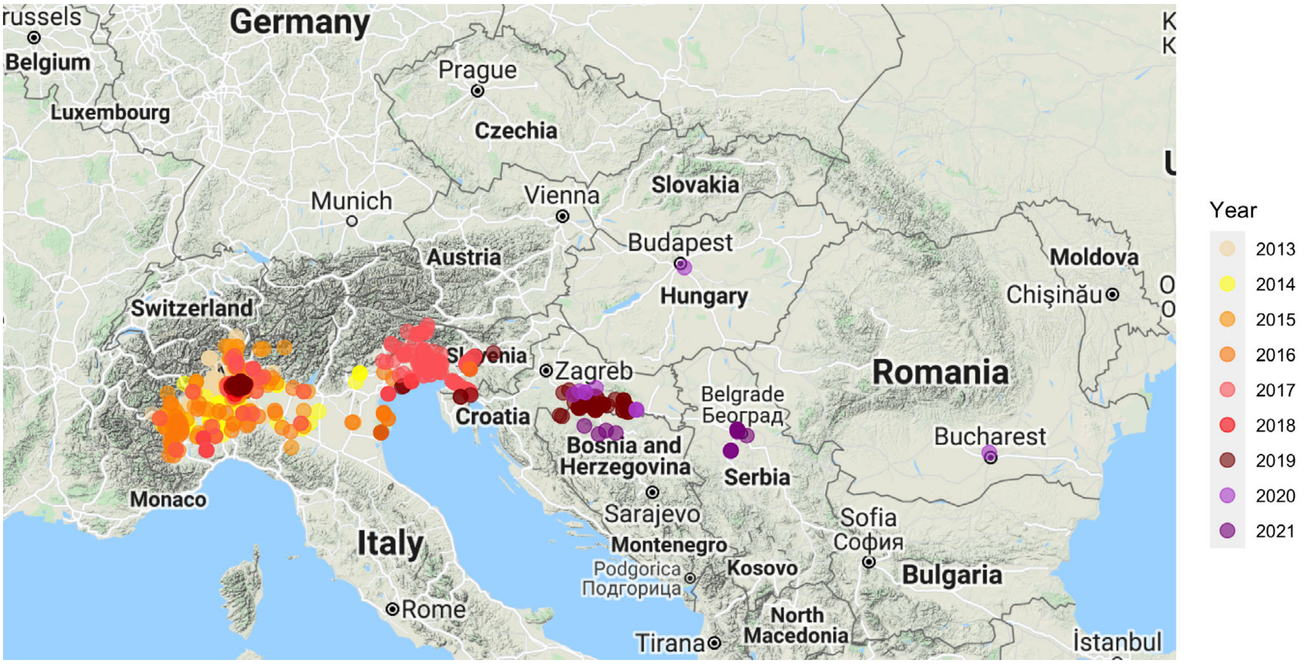

Figure 1. Occurrences of *Ophraella communa* in Europe; colours represent the year that the beetle was recorded at a particular location for the first time. The records do not stem from systematic surveys across years and Europe, but represent a combination of published data, *Ambrosia artemisiifolia* surveys and records from local insect experts.

In addition, based on species distribution models (SDMs) simultaneously carried out for both *Ambrosia* and *Ophraella* under various climate scenarios, Sun *et al.*[20,30] predicted a pronounced northeast spread for *Ambrosia* in both its introduced European and Asian ranges with a reduced total geographic overlap with the beetle under climate change. Further advances have been made by combining SDM with mechanistic (process-based) models by integrating climate-depended vital rates determining the development of *Ophraella*. This led to more reliable predictions of the climatic factors favouring and limiting the beetle's population build-up across its suitable European range and the relative importance of those climatic factors on population growth and thus, for estimating its region-specific impact on the target *Ambrosia* populations.[31] Besides northern Italy, where the beetle has already significantly reduced airborne pollen concentrations, our projections suggest that people in countries of the Balkan Peninsula will benefit most from the establishment of *Ophraella*. Our combined modelling studies also allow to derive management strategies. We predicted that an unassisted population build-up of *Ophraella* north of the Alps will not suffice to cause significant impacts on the weed's population dynamics and pollen production, as high levels of damage are only generated during the third or fourth generation of *Ophraella*.[31] To increase the impact of *Ophraella* on *Ambrosia* in regions with less suitable climatic conditions, e.g., north of the Alps, a mass rearing and mass release programme could be implemented, similarly to what is currently practiced in China.[32] For areas predicted to be not suitable for *Ophraella*, bioherbicide-based management approaches or additional insect or pathogen biocontrol agents previously selected by Gerber *et al.*[23] could be studied in detail and eventually be used to complement the integrated management approach against *Ambrosia* in Europe.

In an international and interdisciplinary research initiative, SDMs were then combined with the expected number of *Ophraella* generations across its environmental niche to estimate its potential impact on *Ambrosia* pollen integrals at the European level. These projections revealed that *Ophraella* will reduce the number of patients suffering from *Ambrosia* induced allergies by 2.3 million and the health costs by €1.1 billion per year, once the beetle has colonized its entire geographic niche in Europe.[21]

2.2 Exploring host specificity and efficacy

The establishment of *Ophraella* in northern Italy was used to contrast non-target field surveys and open-field host specificity tests with laboratory no-choice and choice oviposition and larval development tests carried out in both the native and introduced ranges. In 2015, a non-target field survey was conducted with both native and non-native plants within the Asteraceae tribe Heliantheae. A total of 15 non-target plant species were surveyed in 25 localities in Italy and three in Switzerland, where both *Ambrosia* and *Ophraella* were abundant.[33] Besides *Ambrosia*, *Ophraella* was found to complete its life cycle also on *Ambrosia trifida* L., *Xanthium strumarium* L. and *Helianthus tuberosus* L., and to cause punctual feeding damage on sunflower and three native plant species in the Asteraceae family, i.e., on *Centaurea nigrescens* Willd., *Buphthalmum salicifolium* L. and the endemic *Xerolekia speciosissima* (L.) Anderb. In a second extensive field survey specifically covering 18 populations of nine native endangered and potential non-target species,[34] found *Ophraella* adults on a single individual of *Bidens cernua* L. In a common garden field experiment in northern Italy in an area with high *Ophraella* densities, leaf damage by adults was highest on two other Asteraceae species, *Pentanema helveticum* (Weber) D.Gut.Larr. and *Dittrichia graveolens* (L.) Greuter (both in the Tribe Inuleae), but only the latter, which has recently become invasive in Western Europe, sustained all life stages of *Ophraella* in the common garden and also in laboratory experiments that involved *D. graveolens*, *P. helveticum*, *P. britannicum and P. salicinum*, and *Centaurea nigrescens*. In a

Table 3. Distribution of egg-batches and damage levels after 9 weeks (mainly by adults) on seven plant accession (*Ambrosia* and two closely related crop species *Guizotia abyssinica* and *Helianthus annus*, with one of the sunflower varieties offered in three phenostages: cotyledon, four-leaf and 6–8 leaf stage) established at four locations in northern Italy (three) and southern Switzerland (one), where both *Ambrosia* and *Ophraella* occur naturally

Plant accession		Percentage Damage (%)			Number of egg batches		
		May–July	July–September	September–November	May–July	July–September	September–November
Ambrosia artemisiifolia		10.12 ± 1.5	27.09 ± 3.35	47.9 ± 4.88	2.69 ± 0.93	4.63 ± 0.57	0
Helianthus annuus	Sunrich orange	0.29 ± 0.19	5.76 ± 1.29	9.85 ± 1.8	0.02 ± 0.01	0.17 ± 0.07	0
	Iregui	0.13 ± 0.1	1.09 ± 0.75	8.18 ± 2	0	0	0
	Girasole small	0.06 ± 0.03	0	14.4 ± 1.79	0.01 ± 0.01	0	0
	Girasole medium	0.06 ± 0.02	5.59 ± 1.17	7.89 ± 1.54	0	0.48 ± 0.12	0
	Girasole large	0.09 ± 0.05	5.13 ± 1.03	6.54 ± 1.21	0	0.16 ± 0.06	0
Guizotia abyssinica		0	0	0.97 ± 0.27	0	0	0

Note: The field experiment was carried out in three cohorts over the 2016 growing season of *Ambrosia*. Data are based on seven plant individuals per plant accession and experiment, arranged in a 3 m-by-3 m Latin-square design within a 7 m-by-7 m mowed plot (see Bustamante[33] for further details).

no-choice field cage experiment in northern Italy, each two first instar larvae were transferred to 18 *Ambrosia* and 18 sunflower var. PR64H42 (average height of 40–60 cm), singly enclosed inside clip-on cages and one placed on an upper and one on a lower leaf. The results after 4 weeks showed that *Ophraella* performed better on *Ambrosia*, with 66.7% developing into pupae on *Ambrosia*, while only 38.9% on young sunflower plants.[33]

Following these results, a series of open field experiments was then initiated at sites with natural occurrences of *Ambrosia* and *Ophraella* in northern Italy and southern Switzerland using different experimental designs under choice and no-choice situations and in early and late cohorts along the *Ambrosia* growing season. Kadima[35] carried out common garden experiments in five sites in Italy and one site in Switzerland, using besides *Ambrosia artemisiifolia* also *Ambrosia trifida*, *Artemisia annua* L., *H. annuus*, *H. tuberosus* and *Zinnia elegans* Jacq. 1793. Under choice conditions a very low amount of *Ophraella* egg batches was found on non-target plants and none of the stages were found on *Artemisia annua* and *Z. elegans*. In general, abundance of adults increased throughout the season, most prominently in the Latin square experiment in Rovio (southern Switzerland), where *Ophraella* adults were able to severely damage sunflower plants late in the season (end of September). It should be noted, though, that commercially grown oil sunflower is already harvested by end of August, when damage levels were minimal (see also Table 3). In a parallel study, *Ambrosia*, five sunflower varieties (one with two phenostages) grown for different use and with different harvest times, i.e., PR64H42 (oil), PR64H42 (oil), PR64H42 (cultivated), Sunrich (ornamental), Iregui (green manure), and *Guizotia abyssinica* Niger (green manure) were grown in three experimental gardens in northern Italy and one in southern Switzerland and repeated as three cohorts from May to November 2015 (Table 3). Number of *Ophraella* egg batches and damage was much higher on *Ambrosia*, but damage was also found on all the plants in different percentages and significantly higher in the third cohort.

A previous literature review prioritized the host-specific leaf beetle *Ophraella slobodkini* as a candidate agent for *Ambrosia* control in Europe[23] but rejected its oligophagous congener *O. communa* as mentioned earlier. Lommen *et al.*[36] conducted a comparative no-choice performance assay by transferring first instar larvae onto detached leaves of *Ambrosia* and sunflower, kept under two climatic conditions to explore its fundamental host range and its potential for increasing abundance on these host plants. The results confirmed that *O. slobodkini* does not survive on sunflower, while *O. communa* can survive to adulthood, but develops more slowly on sunflower than on *Ambrosia* (Table S1). Species distribution models, however, predict no suitable area for the establishment of *O. slobodkini* in Europe; this species was therefore excluded from further studies.[20,36]

These studies reflect the oligophagous feeding status of *Ophraella* and confirm that it can complete its life cycle on some closely related species, predominantly alien invasive species, and causes punctual feeding damage on a few native species, observed both in the quarantine as well as in field surveys and experimental gardens under choice and no-choice situations. In quarantine, a complete life cycle was also confirmed on sunflower. However, both *Ophraella* survival and development was greatly reduced on the non-target plants tested, including sunflower, as compared to *Ambrosia*. The fewer *Ambrosia* and the more *Ophraella* present in the open-field experiments, as encountered later in the season, the higher was the damage to non-target plants (Table 3), indicating that their relative abundances greatly determine the risk of non-target attack. In our field common-garden experiments, highest levels of adult feeding damage on sunflower were observed late in the season (end of September), but such damage levels will not occur on commercially grown sunflower due to its earlier harvest. In conclusion, the various *Ophraella* studies found neither indication of substantial damage with yield reduction in sunflower nor evidence of substantial non-target effects that could potentially threaten populations of the tested European plant species. This corroborates well the findings in China which suggest that *Ophraella* is not able to establish persistent populations on cultivated sunflower.[25,26] Furthermore, a large literature survey of phytophagous insects on cultivated and wild sunflower in North America, as well as a field survey in California, from where occurrence of *O. communa* has been reported revealed no records for this beetle[37] (Table S1). Based on these findings, an expert group mandated by the French Ministry of Health, Agriculture and the Environment suggested that the benefits of a natural or human-assisted establishment of *Ophraella* in France could be significant and that an accidentally introduced population in France should not be eradicated.[38] More extended host specificity studies and continued field monitoring are needed before *Ophraella* can be actively spread across *Ambrosia* infested areas in Europe, and these are presently underway.

In summary, these studies clearly show that decisions about importation and release of BWC candidates solely based on no-choice laboratory tests can be misleading and exclude potentially safe and efficient agents. This is not new,[39] but the *Ophraella* data offer one of the best insights to underline this statement, exactly because the beetle has a relatively broad physiological host-range and was nevertheless introduced (accidentally) into a new range. The design of *Ophraella* host specificity studies conducted pre-release were predominantly no-choice laboratory test (Table S1) and full development on non-host plants was then interpretated as a no-go without doing additional studies. Only the study conducted by Dernovici *et al*.[40] assessed vital rates of the different life stages of *Ophraella* on target and non-target species; the authors concluded that *Ophraella* is unlikely to establish permanent populations on sunflower and that potential feeding damage on sunflower is thus likely due to spillover effects (Table S1). The results of this study were largely corroborated by post-release studies. Post-release studies in the introduced ranges in Asia and Europe also measured growth rates on the various test plants and allowed exploration of the ecological host range and concluded that *Ophraella* is highly efficacious and likely safe, although further biosafety studies are needed before the beetle can be deliberately spread.

Populations of a plant invader in the native and the introduced range and those of its potential IBWC agents in the native range may be genetically differentiated among geographically distinct regions. This, in turn, is expected to affect the outcome of their interaction when brought together, and by this the efficacy of the control. In various experiments conducted under controlled conditions, we also explored population differentiation in *Ophraella* regarding efficacy and biosafety. By challenging 11 plant genotypes of *Ambrosia* with larvae of eight genotypes of *Ophraella* (both from various regions in three continents) in a complete factorial design, Sun *et al*.[41] found *Ophraella* genotype to be the main driver of this interaction, i.e., some *Ophraella* genotypes were more effective against most of the plant genotypes. Integrating such bioassays in pre-release studies will give a first indication of the expected efficacy when introducing the best antagonist genotype, and on where to find it. Genotypic mismatch between the biocontrol agent and its target plant can be further exacerbated by future climate change, affecting both biocontrol efficacy and safety. For this, Litto *et al*.[42] performed a common environment experiment with 11 populations of *Ophraella* from the native and introduced ranges and measured larval performance and survival on *Ambrosia* and sunflower under three different temperature regimes mimicking climate change scenarios. In general, with increasing temperatures, they observed faster development and a marginal increase in adult weight but decreasing survival in all populations. The beetles consumed much more *Ambrosia* than sunflower leaves, irrespective of the temperature conditions. This finding further indicates that some of the *Ophraella* populations examined might be able to colonize areas that are heavily infested by *Ambrosia*, or are expected be so under climate warming, but are predicted to be presently unsuitable for the beetle.[42]

2.3 Exploring evolutionary interactions: towards predicting outcomes of species interactions in novel environments

Field evaluations to assess the potential for rapid evolution of traits affecting biocontrol efficacy and host-specificity under novel environmental conditions pre-release are not yet part of weed biocontrol programmes.[19] As environmental impacts caused by climate change, pollution, and habitat destruction are predicted to increase, understanding and predicting eco-evolutionary outcomes of species interactions under novel environmental conditions, such as encountered in the case of weed biocontrol programmes, becomes more challenging, but also more pressing. Potential evolutionary changes in the BWC agent post-release remain a fundamental area of uncertainty associated with biocontrol introductions. Post-introduction monitoring studies of either accidentally or deliberately introduced BWC agents offer an exciting opportunity to better understand eco-evolutionary dynamics of such species interactions in novel environments and help to design more targeted pre-release studies.

Two field studies were initiated in northern Italy in 2016 to assess (i) the beetle's potential to select for beetle resistant/tolerant *Ambrosia* populations under present and future climate conditions (+2.5 °C), and (ii) evolutionary adaptation of *Ophraella* to closely related sunflower. For this, large-caged plots were established (i) with genetically similar *Ambrosia* plants from a wide range of European populations, kept under two temperature regimes and *Ophraella* was released in half of them (beetle as the driver of selection), and (ii) containing either sunflower or *Ambrosia*, and *Ophraella* that had been widely collected across the present distribution in Italy was released in all cages (host plants as drivers of selection). For study (i), genomic and metabolomic changes across generations were tracked in the field populations and plant offspring phenotypes assessed in a common environment. Sun *et al*.[43] found that increased offspring *Ambrosia* biomass in response to warming arose through changes in the genetic composition of populations, while increased resistance to herbivory arose through a shift in plant metabolomic profiles without genetic changes, most likely by transgenerational induction of defences. Importantly, both vigorous and better defended plants were favoured under herbivory and climate warming conditions, indicating that climate warming may decrease biocontrol efficiency and promote *Ambrosia* invasion, with potentially serious economic and health consequences.

In study (ii), we followed the demography of *Ophraella* by combining genomic (pool-sequence) analyses assessing the molecular diversity and differentiation over *c*. 12 experimental generations in *Ophraella* confined to either *Ambrosia* (four field cages) or sunflower (eight field cages), with behavioural and performance bioassays to evaluate potential population differentiation in host choice and larval performance of the beetles over time from the two plant species. In the sunflower cages, beetle densities rapidly declined, and no beetles could be found after 3 years. Interestingly, after approximately eight generations (2 years of experiments), the genetic composition of the *Ophraella* populations kept on sunflower did not change significantly. Adult preference (damage, oviposition choice) and larval performance (leaf consumption, development time, adult weight and survival probability) showed a higher performance on *Ambrosia* and a strong preference for it independently of the plant species on which the beetles were kept in the field cage. Thus, all results combined indicate little risk of a short-term adaptation of *Ophraella* to sunflower.

To our knowledge, this is the first attempt to rigorously and simultaneously assess the evolvability of the target weed and its biological control agent. We specifically advocate such experimental evolution studies be conducted pre-release to advance biocontrol towards a more predictive, efficient, and sustainable management strategy under changing climatic conditions.

Applying genetic and genomic knowledge to improve biocontrol agents has recently been referred to as 'next generation biocontrol'.[44] We acknowledge that experimental evolution studies might not be feasible for all study systems in due course, being especially suitable for multivoltine species with simple rearing protocols. The case of *Ophraella* is, however, not unique, as several other cases of accidentally introduced BWC agents are known in Europe (Table 1), some of which have multiple generations per year, which offers further opportunities to carry out similar studies.

3 LESSONS LEARNT FROM ACCIDENTAL INTRODUCTIONS FOR OVERCOMING BARRIERS TO ADOPTION OF BIOLOGICAL WEED CONTROL

Through insights gained from the earlier-described case study, we derive suggestions for overcoming barriers to adoption of IBWC by reviewing and extending pre-release studies and thus re-evaluating the presently strict criteria for deciding upon their release, as they might exclude safe and efficient agents. With the 'successful' accidental introduction of *Ophraella*, which led to a significant decrease in allergenic ragweed pollen in northern Italy,[45] we also hope to raise the awareness of authorities to consider IBWC more prominently as an integral and leading management tool against invasive species, as it is more than just reducing weed densities but, besides plant health, also promotes other components of the 'One Health' concept,[46] including human (reduced allergies), plant (e.g. reduced competition with crops) and environment health (reduced environmental pollution through pesticides).

3.1 Extending pre-release studies to better predict safety and impact in the introduced range

IBWC agents accidentally introduced may have a broader fundamental host range in the newly established area than deliberately introduced ones and, thus, allow comparison of host-use and performance of such agents in the introduced range with records from literature surveys or, if available, with predictions from pre-release studies that rejected their introduction. Authorized releases allow testing of hypotheses formulated from pre-release studies, but here, the tested fundamental host ranges tend to be very narrow, and unfortunately, detailed post-introduction studies of deliberate releases are still relatively rare.[3,19] The motivation to carry out post-introduction studies is clearly much higher for accidentally than for deliberately introduced agents, as the latter might pose an immediate risk for closely related native species and crops.

As pointed out earlier, the detailed studies on the accidently introduced ragweed leaf beetle in Europe provide no evidence for substantial non-target effects that could potentially threaten closely related endangered populations of native plant species, nor substantial damage with yield reduction in sunflower. From insights gained through these studies, we derive five suggestions to improve and extend pre-release studies to predict the performance of IBWC agents in the introduced range more realistically.

(1) Predicting biocontrol impact: Combining a statistical (SDM) and mechanistic approach (climate-dependent demographic model) to predict biocontrol impact and economic savings more accurately and spatially explicitly, and to derive region-specific management options.
(2) Performance on non-host species: Full development on non-target species under no-choice laboratory conditions should indicate only which test plant species should be used in further studies on biosafety. The focus should be put on population growth rate rather than on survival on individual non-target plants, as a negative population growth rate of a non-target plant would indicate no sustainable population on this non-target plant (but spill-over effects may occur[39]).
(3) Preference in the field: Conduct host preference assays under various relative abundances of target and non-target plants, using (i) various experimental designs: from choice test with abundant target, but low numbers of non-target species as found in areas heavily infested by the plant invader, up to no-choice condition such as in early crop situations, where the target species has not yet germinated, and (ii) early and late cohorts over the growing season: with few numbers of BWC agents but lots of target individuals early in the season, to situations late in the season, where most targets have been eliminated and BWC agent populations have reached maximum population build-up.
(4) Effectiveness: Identifying highly effective BWC agent populations across a wide range of target genotypes by exploring plant genotype by antagonist genotype interactions. Integrating such bioassays will give a first indication of the probability for an at least initial high efficacy when introducing the best antagonist genotype, and on where to find it.
(5) Evolvability: Assessing (i) the potential of the BWC agent to select for antagonist resistant/tolerant target plant populations, and (ii) the potential evolutionary adaptation of the BWC agent to closely related crop species or rare native plant species, both under present and future climate conditions. Such experimental evolution studies conducted pre-release and under present and future climatic conditions will advance biocontrol towards a more predictive, efficient, and sustainable management strategy.

We acknowledge that at least some of these studies impose extra time and costs to IBWC projects but are surely worth it to not exclude potentially effective agents, as our case study attests.

3.2 A call for considering biological control as an integrated and leading tool in the 'One Health' concept

The detailed studies on the accidently introduced *Ophraella* into Europe document its high impact on *Ambrosia* pollen and seed production resulting in huge economic benefits for human health,[21] and for agriculture.[47,48] Thus, the studies emphasize that IBWC, and biological control in general, not only offer solutions to plant health problems, but also contribute to improved environmental (e.g. through reduced pesticide use) and human health (e.g. reduced allergies). This is well in line with the 'One Health' concept, which is based on the recognition that the health of people is closely connected to the health of animals, plants, and our shared environment.[46] By this, it also contributes to reaching the European Green Deal vision to make Europe the world's first climate-neutral economic area by 2050 (https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=COM%3A2019%3A640%3AFIN). Since the second half of the 20th century, the diversity and quantity of pesticides and other synthetic chemicals released into the environment has been increasing at rates greatly surpassing those of other drivers of global environmental change, including the accumulation of greenhouse gases.[49] Over the next decades, biological control and other nature-based solutions will be key tools in a global attempt to reduce the impacts of environmental pollution on biodiversity, food safety, water and other aspects related to human well-being. Considering the breadth of benefits IBWC – and BWC in general – can generate, we argue that regulations should simultaneously consider the potential risks and benefits of IBWC as compared to other management options, including 'business as usual'.

ACKNOWLEDGEMENTS

The authors are grateful to Francesca Marini for input on accidentally introduced mites, and the Young Scientists Fund of the National Natural Science Foundation of China (Grant Number: 32201438 to YS), the Scientific Research Foundation for Returned Scholars, Huazhong Agricultural University (Grant Number: 11042110026 to YS), and the Swiss National Science Foundation (project number 31003A_166448 to HM-S) for funding. US was supported by the Swiss National Science Foundation and the Swiss Agency for Development and Cooperation as part of the Swiss Programme for Research on Global Issues for Development (r4d; Grant Number: 400440_152085) and by CABI with core financial support from its member countries (see https://www.cabi.org/about-cabi/who-we-work-with/key-donors/). This article was presented orally at the workshop 'Overcoming the barriers to adoption of microbial bioherbicides', which took place in Bari, Italy on 25–29 September 2022, and which was sponsored by the OECD Co-operative Research Programme: Biological Resource Management for Sustainable Agricultural Systems, whose financial support made it possible for the authors to participate in the workshop. The opinions expressed and arguments employed in this article is the sole responsibility of the authors and do not necessarily reflect those of the OECD or of the governments of its member countries.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

REFERENCES

1 Seebens H, Bacher S, Blackburn TM, Capinha C, Dawson W, Dullinger S *et al.*, Projecting the continental accumulation of alien species through to 2050. *Global Change Biol* **27**:970–982 (2021).

2 Vilà M and Hulme PE, *Impact of Biological Invasions on Ecosystem Services*. Springer Cham, Heidelberg (2017).

3 Sun Y, Müller-Schärer H and Schaffner U, Fighting neobiota with neobiota: consider it more often and do it more rigorously. *Biol Conserv* **268**:109506 (2022).

4 Müller-Schärer H and Schaffner U, Classical biological control: exploiting enemy escape to manage plant invasions. *Biol Invasions* **10**: 859–874 (2008).

5 Lambdon PW, Pyšek P, Basnou C, Hejda M, Arianoutsou M, Essl F *et al.*, Alien flora of Europe: species diversity, temporal trends, geographical patterns and research needs. *Preslia* **80**:101–149 (2008).

6 Hinz HL, Schwarzländer M, Gassmann A and Bourchier RS, Successes we may not have had: a retrospective analysis of selected weed biological control agents in the United States. *Invasive Plant Sci Manage* **7**:565–579 (2014).

7 Smith L, Physiological host range of *Ceratapion basicorne*, a prospective biological control agent of *Centaurea solstitialis* (Asteraceae). *Biol Control* **41**:120–133 (2007).

8 McFadyen RE, Biological control: managing risks or strangling progress, in *Proceedings of the 14th Australian Weeds Conference*. Weed Society of New South Wales, Sydney, pp. 78–81 (2004).

9 Eschen R, Britton K, Brockerhoff E, Burgess T, Dalley V, Epanchin-Niell R *et al.*, International variation in phytosanitary legislation and regulations governing importation of plants for planting. *Environ Sci Policy* **51**:228–237 (2015).

10 Burke MJW and Grime JP, An experimental study of plant community invasibility. *Ecology* **77**:776–790 (1996).

11 Orbán I, Szitár K, Kalapos T and Körel-Dulay G, The role of disturbance in invasive plant establishment in a changing climate: insights from a drought experiment. *Biol Invasions* **23**:1877–1890 (2021).

12 Weber D, Hajek AE, Hoelmer KA, Schaffner U, Mason PG, Stouthamer R *et al.*, *Unintentional biological control*. CSIRO Publishing, Collingwood, Victoria, Australia (2021).

13 Woodworth LM, Montgomery ME, Briscoe DA and Frankham R, Rapid genetic deterioration in captive populations: causes and conservation implications. *Conserv. Genet* **3**:277–288 (2002).

14 Müller-Schärer H, Bouchemousse S, Litto M, McEvoy PB, Roderick GK and Sun Y, How to better predict long-term benefits and risks in weed biocontrol: an evolutionary perspective. *Curr Opin Insect Sci* **38**:84–91 (2020).

15 Fukano Y, Doi H, Thomas C, Takata M, Koyama S and Satoh T, Contemporary evolution of host plant range expansion in an introduced herbivorous beetle *Ophraella communa*. *J Evol Biol* **29**:757–765 (2016).

16 Hopper K, Roush RT and Powell W, Management of Genetics of biological-control introductions. *Annu Rev Entomol* **38**:27–51 (1993).

17 Mathenge CW, Holford P, Hoffmann J, Zimmermann H, Spooner-Hart R and Beattie G, Hybridization between *Dactylopius tomentosus* (Hemiptera: Dactylopiidae) biotypes and its effects on host specificity. *Bull Entomol Res* **100**:331–338 (2010).

18 Gaudeul M, Giraud T, Kiss L and Shykoff JA, Nuclear and chloroplast microsatellites show multiple introductions in the worldwide invasion history of common ragweed, *Ambrosia artemisiifolia*. *PLoS One* **6**:e17658 (2011).

19 Müller-Schärer H and Schaffner U, Editorial overview: biological control of plant invaders: a continued stimulus and yet untapped potential to link and advance applied and basic research. *Curr Opin Insect Sci* **38**:v–viii (2020).

20 Sun Y, Brönnimann O, Roderick GK, Poltavsky A, Lommen ST and Müller-Schärer H, Climatic suitability ranking of biological control candidates: a biogeographic approach for ragweed management in Europe. *Ecosphere* **8**:e01731 (2017).

21 Schaffner U, Steinbach S, Sun Y, Skjøth C, Weger LA d, Lommen ST *et al.*, Biological weed control to relieve millions from *ambrosia* allergies in Europe. *Nat Commun* **11**:1745 (2020).

22 Müller-Schärer H, Lommen STE, Rossinelli M, Bonini M, Boriani M, Bosio G *et al.*, *Ophraella communa*, the ragweed leaf beetle, has successfully landed in Europe: fortunate coincidence or threat? *Weed Res* **54**:109–119 (2014).

23 Gerber E, Schaffner U, Gassmann A, Hinz HL, Seier M and Müller-Schärer H, Prospects for biological control of *Ambrosia artemisiifolia* in Europe: learning from the past. *Weed Res* **51**:559–573 (2011).

24 Palmer W and Goeden R, The host range of *Ophraella communa* Lesage (coleoptera: Chrysomelidae). *Coleopt Bull* **45**:115–120 (1991).

25 Cao Z, Wang H, Meng L and Li B, Risk to nontarget plants from *Ophraella communa* (coleoptera: Chrysomelidae), a potential biological control agent of alien invasive weed *Ambrosia artemisiifolia* (Asteraceae) in China. *Appl Entomol Zool* **46**:375–381 (2011).

26 Zhou Z-S, Guo J-Y, Zheng X-W, Luo M, Chen H-S and Wan F-H, Reevaluation of biosecurity of *Ophraella communa* against sunflower (*Helianthus annuus*). *Biocontrol Sci Technol* **21**:1147–1160 (2011).

27 Zhou Z-S, Chen H-S, Zheng X-W, Guo J-Y, Guo W, Li M *et al.*, Control of the invasive weed *Ambrosia artemisiifolia* with *Ophraella communa* and *Epiblema strenuana*. *Biocontrol Sci Technol* **24**:950–964 (2014).

28 Müller-Schärer H, Sun Y, Chauvel B, Karrer G, Kazinczi G, Kudsk P *et al.*, Cross-fertilizing weed science and plant invasion science to improve efficient management: a European challenge. *Basic Appl Ecol* **33**:1–13 (2018).

29 Tanaka K and Yamanaka T, Factors affecting flight activity of *Ophraella communa* (coleoptera: Chrysomelidae), an exotic insect in Japan. *Environ Entomol* **38**:235–241 (2009).

30 Sun Y, Zhou Z, Wang R and Müller-Schärer H, Biological control opportunities of ragweed are predicted to decrease with climate change in East Asia. *Biodiversity Sci* **25**:1285–1294 (2017).
31 Augustinus B, Sun Y, Beuchat C, Schaffner U and Müller-Schärer H, Predicting impact of a biocontrol agent: integrating distribution modelling with climate-dependent vital rates. *Ecol Appl* **30**:e02003 (2020).
32 Zhou Z-S, Guo J-Y, Chen H-S and Wan F-H, Effects of temperature on survival, development, longevity, and fecundity of *Ophraella communa* (coleoptera: Chrysomelidae), a potential biological control agent against *Ambrosia artemisiifolia* (Asterales: Asteraceae). *Environ Entomol* **39**:1021–1027 (2010).
33 Bustamante JE, *Assessing the Risk of Potential Biological Control Agent of Ambrosia Artemisiifolia to the Closely Related Species*. University of Fribourg, Switzerland (2016).
34 Augustinus BA, Gentili R, Horvath D, Naderi R, Sun Y, Tournet A-MTE *et al*., Assessing the risks of non-target feeding by the accidentally introduced ragweed leaf beetle, *Ophraella communa*, to native European plant species. *Biol Control* **150**:104356 (2020).
35 Kadima G, *Is Ophraella Communa a Risk for Non-target Plant Species?* University of Fribourg, Switzerland (2015).
36 Lommen ST, Jolidon EF, Sun Y, Eduardo JIB and Müller-Schärer H, An early suitability assessment of two exotic *Ophraella* species (coleoptera: Chrysomelidae) for biological control of invasive ragweed in Europe. *Eur J Entomol* **114**:160–169 (2017).
37 Hilgendorf JH and Goeden RD, Phytophagous insects reported from cultivated and weedy varieties of the sunflower, *Helianthus annuus* L., in North America. *Bull ESA* **27**:102–109 (1981).
38 ANSES, *The Efficacy of the Ophraella communa Beetle Used as a Biological Control Agent against Common Ragweed and Assessment of the Possible Associated Risk*. Revised OPINION of the French Agency for Food, Environmental and Occupational Health & Safety, France (2019).
39 Hinz HL, Winston RL and Schwarzländer M, How safe is weed biological control? A global review of direct nontarget attack. *Q Rev Biol* **94**:1–27 (2019).
40 Dernovici SA, Teshler MP and Watson AK, Is sunflower (*Helianthus annuus*) at risk to damage from Ophraella communa, a natural enemy of common ragweed (*Ambrosia artemisiifolia*)? *Biocontrol Sci Technol* **16**:669–686 (2006).
41 Sun Y, Beuchat C and Müller-Schärer H, Is biocontrol efficacy rather driven by the plant or the antagonist genotypes? A conceptual bioassay approach. *NeoBiota* **63**:81–100 (2020).
42 Litto M, Bouchemousse S, Schaffner U and Müller-Schärer H, Population differentiation in response to temperature in *Ophraella communa*: implication for the biological control of *Ambrosia artemisiifolia*. *Biol Control* **164**:104777 (2021).
43 Sun Y, Züst T, Silvestro D, Erb M, Bossdorf O, Mateo P *et al*., Climate warming can reduce biocontrol efficacy and promote plant invasion due to both genetic and transient metabolomic changes. *Ecol Lett* **25**:1387–1400 (2022).
44 Le Hesran S, Ras E, Wajnberg E and Beukeboom LW, Next generation biological control–an introduction. *Entomol Exp Appl* **167**:579–583 (2019).
45 Bonini M, Šikoparija B, Prentović M, Cislaghi G, Colombo P, Testoni C *et al*., A follow-up study examining airborne *ambrosia* pollen in the Milan area in 2014 in relation to the accidental introduction of the ragweed leaf beetle *Ophraella communa*. *Aerobiologia* **32**:371–374 (2015).
46 Essack SY, Environment: the neglected component of the one health triad. *Lancet Planet Health* **2**:e238–e239 (2018).
47 Barnes ER, Knezevic SZ, Sikkema PH, Lindquist JL and Jhala AJ, Control of glyphosate-resistant common ragweed (*Ambrosia artemisiifolia* L.) in glufosinate-resistant soybean [Glycine max (L.) Merr]. *Front Plant Sci* **8**:1455 (2017).
48 Kazinczi G, Béres I, Novák R, Bíró K and Pathy Z, Common ragweed (*Ambrosia artemisiifolia*): a review with special regards to the results in Hungary. I. Taxonomy, origin and distribution, morphology, life cycle and reproduction strategy. *Herbologia* **9**:55–91 (2008).
49 Bernhardt ES, Rosi EJ and Gessner MO, Synthetic chemicals as agents of global change. *Front Ecol Environ* **15**:84–90 (2017).

