

# Weed biological control in the European Union: from serendipity to strategy

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Received: 31 March 2017 / Accepted: 17 September 2017 / Published online: 20 October 2017  
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**Abstract** Biological control of weeds is a globally recognised approach to the management of some of the most troublesome invasive plants in the world. Accidental introductions of agents accounted for all weed biological control agent establishments in the European Union until 2010, but these examples include some current or emerging control successes both large and small, from the redistribution of the weevil *Stenopelmus rufinasus* Gyllenhal (Coleoptera: Curculionidae) for the control of small outbreaks of *Azolla filiculoides* Lam. (Azollaceae), to the large scale control provided by the cochineal insect *Dactylopius opuntiae* (Cockerell) (Hemiptera: Dactylopiidae), used against some problematic prickly pears (*Opuntia* spp. (Cactaceae)), and the ragweed beetle *Ophraella communa* LeSage (Coleoptera:

Chrysomelidae), against common ragweed, *Ambrosia artemisiifolia* L. (Asteraceae), which are providing benefits to an increasing number of Member States of the European Union. Recent programmes involving the intentional introduction of biological control agents against target weeds including *Fallopia japonica* (Hout.) Ronse Decr. (Polygonaceae), *Impatiens glandulifera* Royle (Balsaminaceae) and *Acacia longifolia* (Andrews) Willd (Fabaceae) show a shift from luck to judgement in the European Union. The inclusion of new weed targets on the European Invasive Species Regulation should lead to a growth in the profile and use of biological control which would be assisted by the publication of any successes from the few intentional introductions covered in this paper.

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Handling Editors: Mark Schwarzländer, Cliff Moran and S. Raghu.

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**Keywords** Regulation · *Fallopia japonica* · *Ambrosia artemisiifolia* · *Opuntia ficus-indica* · *Azolla*

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## Introduction

Biological control of weeds is a recognised and widely applied tool in several regions of the world (Clewley et al. 2012; Suckling and Sforza 2014), but the European Union (EU) is a noticeable exception (Sheppard et al. 2006). This is surprising considering the extensive use of biological control agents (BCAs) in glasshouses (Minks et al. 1998; Eilenberg et al. 2000), for which Europe is a leading region and the use of at least 176 species of exotic arthropods that have not been confined in glasshouses but released, against pests of agriculture, across Europe (Gerber et al. 2016). The reasons for this are manifold and have been discussed by Sheppard et al. (2006) and Shaw et al. (2011) but a prime issue appears to remain the general ignorance of the potential of classical biological control of weeds amongst policy makers, which is exacerbated by their risk aversion mind-set. The purpose of this account is to document selected examples of weed biological control to illustrate the long history of its inadvertent practice in Europe and then to highlight its recent (since 2010) intentional and successful implementation.

Beneficial but accidental introductions of weed biological control agents in the EU

Accidental introductions of phytophagous arthropods against invasive weeds are not uncommon and it is often difficult to trace the origin of such introductions. They may be revealed during agent redistribution such as the first discovery in North America of *Urophora quadrifasciata* (Weigen) (Diptera: Tephritidae) on spotted knapweed, accidentally redistributed in seed heads believed to only contain the officially approved *Urophora affinis*, or because of post-release evaluation studies on officially released BCAs, such as the case of *Diorhabda* beetles (Coleoptera: Chrysomelidae) on saltcedar, *Tamarix* species (Tamaricaceae), which involved the misidentification of the agents.

The latter case of *Diorhabda elongata* (Brullé) is particularly interesting as this taxon was introduced

into the USA in 1999 to control saltcedar (De Loach et al. 2003), but turned out to be a group of five sibling species with different geographical origins ranging from the Mediterranean region to Asia (Tracy and Robbins 2009). Fortunately, all five chrysomelid species defoliate saltcedar populations in the south-eastern USA and contributed to the successful control of these widespread invasive trees. It is also possible that an agent may arrive from a neighbouring country where it has been released. The fly *U. quadrifasciata* was officially released in Canada against varied knapweed (Asteraceae) targets, and was found across the border in Montana, Oregon and Washington, USA in seed heads of spotted knapweed, *Centaurea stoebe* L. (Story 1984). It is now considered to be widespread in more than 50% of its plant host's invasive range in Canada and the USA. Furthermore, it is possible that an agent may arrive through accidental transportation from its native range: the weevil, *Larinus carlinae* Olivier (formerly *L. planus*) (Coleoptera: Curculionidae), that feeds on seed heads of *Cirsium arvense* (L.) Scop. (Asteraceae) was accidentally introduced into the USA from Europe (Wheeler and Whitehead 1985).

Of the 19 species of weed BCAs believed to have been released accidentally in North America, 17 are credited with having a significant impact on their respective "target" weeds. Only two, *L. carlinae* and *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae) have had adverse effects (Suckling and Sforza 2014). The weevil *L. carlinae* found on *C. arvense* in the 1960s may be useful for controlling seed production to prevent large areas of infestation from expanding further (Drlik et al. 2000). However, it has been shown to attack a congeneric native thistle, *Cirsium undulatum* var. *tracyi* (Rydb.) S.L. Welsh, in Colorado (Louda and O'Brien 2002). The most publicised and notorious case occurred when *C. cactorum* arrived in Florida in 1989 from the Caribbean islands where it had been released to control invasive *Opuntia* species (Cactaceae) (Zimmermann et al. 2001). Upon colonizing Florida, and more recently Mexico, it has started feeding on native *Opuntia* species and is considered by many as a serious failure of biological control safety. It is important to note that such unintentional introductions have been relatively rare (Suckling and Sforza 2014). Some unintended arrivals have been shown to have impressive positive impacts and the EU has been in receipt of such species, all of which have been

intentionally applied elsewhere in the world against their target weeds, as is documented below.

*Opuntia ficus-indica* (L.) Mill. (prickly pear)  
(Cactaceae)

The prickly pear cactus, *Opuntia ficus-indica*, is a well-known perennial succulent widely planted as a fruit and fodder crop, but which has also become a problematic invader around the world. It has become invasive in Spain, particularly in areas of high disturbance, near urban areas and abandoned fields (Vilà et al. 2003; Padrón et al. 2011) but also in natural areas where it competes with native vegetation and severely modifies habitats and landscapes. One of its natural enemies, *Dactylopius opuntiae* (Cockerell) (Hemiptera: Dactylopiidae), is a sap-sucking insect, commonly known as a cochineal. Nine species are known from the Americas (De Lotto 1974; Guerra 1991) and five have been reported from Mexico (Chávez-Moreno et al. 2009). All of them feed on cacti (Mann 1969; De Lotto 1974) and exhibit marked host specificity. For instance, at least two biotypes of *D. opuntiae* exist, each with a restricted host range: the *stricta* biotype feeds on low growing *Opuntia stricta* (Haw.) Haw. whereas the *ficus* biotype is associated with tree-like *O. ficus-indica* (Githure et al. 1999; Volchansky et al. 1999).

*Dactylopius* species are now distributed across several regions of the world due to intentional introductions aimed at starting up a pigment industry (Lounsbury 1915) or controlling infestations of different *Opuntia* species (Zimmermann and Moran 1991; Hosking et al. 1994; Foxcroft and Hoffmann 2000; Klein 2002). However, *D. opuntiae* has also spread, presumably accidentally, to Israel (Spodek et al. 2014) and Spain by unknown routes. In the latter country, it was first recorded in Hellín (Murcia) in 2007 (Llorens Climent 2009), from where it expanded rapidly along the coastal Mediterranean areas of Spain, tracking the almost continuous distribution of its introduced host plant *O. ficus-indica* (Sanz Elorza et al. 2004; Serrano-Montes et al. 2016). Dispersal rates of the insect and any injury to the cactus host have been recorded following the intentional infestation of prickly pear populations with *D. opuntiae*-laden cactus cladodes (V. Deltoro, unpublished data). The results show that dispersal on infested plants took place 15 days after inoculation and spread to

neighbouring plants at mean distances of 80–100 cm occurred after four weeks. After 16 months, healthy colonies were found up to 2 km away from the introduction site. The resulting damage apparent after six months was mild chlorosis, but this translated to the loss of up to 50% of cladodes as well as the loss of turgor just four months later. Furthermore, the cochineal attack induced a marked decline in the plant's sexual reproduction, since the outer cladodes of newly infested prickly pears were the first to collapse, leaving mainly lignified stems or old cladodes without fruit production (V. Deltoro, unpublished data). Similar results from South Africa were described by Paterson et al. (2011) for *O. stricta* infested with *D. opuntiae*.

Observations suggest a gradient of cochineal-damage moving from high in the southernmost provinces of the Valencia region where plants are killed, to less damage in more northerly areas, where the plants are still able to produce new cladodes. It is unclear whether this is due to the differing period of residence of the insect or the humidity gradient. It is important to report that no non-target feeding has been observed despite intentional attempts to infect other *Opuntia* species in the field (V. Deltoro, unpublished data), as expected given the well-established host specificity of cactus-feeding *Dactylopius* species (Mann 1969; De Lotto 1974; Githure et al. 1999; Volchansky et al. 1999).

The fact that *D. opuntiae* can be found across a wide area covering the whole Spanish Mediterranean arc suggests the insect is well adapted to the regional climate. Based on experiences in other dry and warm regions of the world where cochineal insect species have been released successfully (Lounsbury 1915; Zimmermann 1981; Zimmermann and Moran 1991; Hosking 1984), it is likely that long distance dispersal of the cactus will be limited, due to the collapse of the outer, fruit-producing cladodes, and this should be followed by a gradual decline in the density of established prickly pear populations and eventually local extinctions. Thus, this unintentional introduction offers the only realistic opportunity to limit the expansion of an invasive plant capable of displacing and preventing the regeneration of native vegetation in dry areas of Spain (Gimeno and Vilà 2002; Sanz Elorza et al. 2004). Nevertheless, the collapse of *O. ficus-indica* populations has also raised some concern in southern Spanish regions, since the plant is

appreciated by some as a component of Mediterranean landscapes, despite its negligible economic importance as a crop (Serrano-Montes et al. 2016). In clear contrast, the Autonomous Government of Catalonia has taken advantage of the opportunity that the cochineal insects provide for *O. ficus-indica* control, and has deliberately introduced them to the Medas archipelago Natural Park, to control this troublesome colonizer.

*Ambrosia artemisiifolia* L. (common ragweed)  
(Asteraceae)

Common ragweed is an annual or short-lived perennial plant that is native to North America. This species has invaded several regions of the world, including Europe, western and eastern Asia, South Africa, Australia and New Zealand (Essl et al. 2015). In Europe, it was introduced with seed imports from North America in the 19th century. Today, *A. artemisiifolia* is particularly abundant in the Pannonian plain, northern Italy and south-eastern France (Essl et al. 2015). Common ragweed is notorious for its impact on human health, due to its highly allergenic pollen, but it is also increasingly becoming a major weed in agriculture. In Europe, the current costs associated with common ragweed impacts on farming and human health are estimated to be of the order of approximately 4.5 billions € per year (Bullock et al. 2012).

Since the 1960s, biological control had been considered as a management option against common ragweed in different parts of the world, including non-EU Member States in Europe (Gerber et al. 2011). The noctuid moth *Tarachidia candefacta* (Hübner) (Lepidoptera: Noctuidae), which was released in Russia in 1969, was the first such intentional attempt to control common ragweed by biological means (Kovalev 1971), but so far with little impact. In the 1970s and 1980s, the leaf beetle *Zygogramma suturalis* (Fabricius) (Coleoptera: Chrysomelidae) was released in Russia, Georgia, Ukraine and the former Yugoslavia (now Croatia) (Julien and Griffiths 1998). First results obtained with this BCA were promising (Reznik 1991), but more recent investigations suggest that *Z. suturalis* is not able to offer effective control of common ragweed (Reznik et al. 2007).

To advance the development of sustainable management strategies for *A. artemisiifolia* in Europe, the

EU-COST Action SMARTER ('Sustainable management of *Ambrosia artemisiifolia* in Europe') was launched in 2012. Emphasis was put on biological control by promoting and coordinating studies on the host-specificity and impact of selected insect and fungal BCAs (Gerber et al. 2011). In 2013, biological control efforts against common ragweed experienced an unexpected boost when the North American leaf beetle *Ophraella communa* LeSage (Coleoptera: Chrysomelidae) was detected in Northern Italy and Southern Switzerland (Bosio et al. 2014; Müller-Schärer et al. 2014). The beetle was first reported close to the international airport of Milano, suggesting that this species was accidentally introduced, a situation reminiscent of the arrival and detection of *Trichapion lativentre* (Béguin-Billecocq) (Coleoptera: Apionidae), at Durban International Airport, South Africa (Hoffmann and Moran 1991). *Ophraella communa* was not prioritized in the SMARTER project, because host specificity tests under laboratory conditions had shown that *O. communa* can complete its life-cycle on sunflower, *Helianthus annuus* L. (Asteraceae) (Palmer and Goeden 1991).

Interestingly, *O. communa* had already been accidentally introduced to another area outside of its native range, i.e. to Japan (Moriya and Shiyake 2001). Since its first detection in Japan in the 1990s it rapidly expanded its distribution over the main Japanese islands of Honshu, Shikoku, and Kyushu (Moriya and Shiyake 2001). From Japan, it spread to Korea (Sohn et al. 2002) and to China. In China, it was first found in the east (Jiangsu province) in 2001 (Meng and Li 2005), from where it continued spreading to provinces in southern China (Zhou et al. 2010). Field studies in China showed, however, that the risk of *O. communa* causing significant damage to sunflower plants in the field is low (Cao et al. 2011; Zhou et al. 2011). Today, *O. communa* and the deliberately introduced moth *Epiblema strenuana* Walker (Lepidoptera: Tortricidae) are mass-reared and actively distributed in China for the biological control of common ragweed (Zhou et al. Zhou et al. 2014). The history of accidental introductions and rapid dispersal by *O. communa* highlights the need for concerted actions by authorities of all European countries in which *O. communa* can establish permanently.

As soon as *O. communa* was detected in the EU, laboratory and open field host specificity and impact

studies were taken up as part of SMARTER to assess the risks and benefits related to the accidental establishment of this beetle. In the first year of its detection, *O. communa* reached high enough densities to completely defoliate and prevent flowering and seed set of most ragweed plants in the Milan area. Bonini et al. (2016) showed that airborne common ragweed pollen levels observed in the Milan area in 2013 and 2014 were approximately 80% lower than in years prior to the establishment of *O. communa*. The decrease in ambrosia pollen observed in the Milan area could not be explained by meteorology in these years, suggesting that the decrease is related to the presence of large numbers of *O. communa* (Bonini et al. 2016). Studies are also underway to assess the non-target risks posed by *O. communa* to sunflower and native plant species. Because of the potentially significant positive impact of *O. communa* on health costs, the French Ministries of Health, Agriculture and the Environment mandated an expert appraisal to assess the efficacy of *O. communa* as a BCA against common ragweed in France (ANSES 2017). In the final document, it was suggested that the benefits of an establishment of *O. communa* to France could be significant, but that further host specificity studies with native plant species are warranted (ANSES 2017).

#### *Azolla filiculoides* Lam (water fern) (Azollaceae)

The *Azolla* water fern is native to the Americas but has become naturalised on most continents worldwide (Lumpkin and Plucknet 1980). In parts of its introduced range, *A. filiculoides* is utilised commonly as a green manure for rice and other crops in certain areas of Asia due to its ability to fix atmospheric nitrogen, and as fodder for livestock. In much of its introduced range, however, *A. filiculoides* is a highly invasive weed that can double its biomass in less than a week (Arora and Singh 2003) to form dense floating mats across freshwater bodies. The impacts of *A. filiculoides* are numerous and include: reduction in dissolved oxygen in the water body and decreased light penetration through the mat, negatively affecting submerged flora and fauna; direct impediment to leisure activities such as angling and boating; threat to livestock and people when mistaken for solid land; impediment to water flow; clogging of pipes, pumps and floodgates (Gratwicke and Marshall 2001; Hill and Cilliers 1999; Janes et al. 1996).

The impacts of *A. filiculoides* in South Africa, following its introduction as an ornamental in 1947, became so significant that a classical biological control programme was initiated against the plant in the mid-1990s. Native range surveys in North America followed by host range testing resulted in the selection and release of the frond-feeding weevil *Stenopelmus rufinasus* Gyllenhal (Coleoptera: Curculionidae) in 1997 (McConnachie et al. 2003). *Stenopelmus rufinasus* is an *Azolla* specialist, with *A. filiculoides* and *A. caroliniana* Willd serving as host plants in the weevil's native range (Madeira et al. 2013; Pemberton and Bodle 2009). The biological control programme proved incredibly successful, with the weevil reducing the *A. filiculoides* population to a level at which it was no longer considered a problem within three years, with an estimated benefit:cost ratio of 15:1 by 2010 anticipated in a post-release evaluation (McConnachie et al. 2003).

Europe has benefitted from the unintentional introduction of *S. rufinasus*, probably as a stow-away on *A. filiculoides* which was widely sold, until quite recently. The weevil was first reported in France in 1901 (Bedel 1901) and was detected in the Netherlands and in the United Kingdom (UK) in the early part of the century (Florencio et al. 2015; Janson 1921). *Stenopelmus rufinasus* is now widespread and can also be found in Belgium, Germany, Ireland, Spain, Ukraine, Italy and Portugal in association with *A. filiculoides* (Carrapiço et al. 2011; Florencio et al. 2015). Naturalised weevil populations can have a dramatic impact on *A. filiculoides* infestations, but *S. rufinasus* has been found to be a less effective BCA in Europe than in South Africa (Gassmann et al. 2006). It is possible that differences in the climatic conditions, particularly between northern Europe and South Africa, could play a significant role in limiting the impacts of the weevil on *A. filiculoides*, with fewer generations per year, induced diapause and potential mortality over winter and limited dispersal on cooler days year-round. Richerson and Grigarick (1967) estimated that *S. rufinasus* would complete 4–6 generations per year in part of its native range, California, whereas Hill (1998) estimated up to ten generations per year would be possible in South Africa. Parts of southern Europe may be better suited to *S. rufinasus* and could expect good levels of *A. filiculoides* control during the summer months



where the weevil is established. This has been observed in the Valencia region (East Spain) where a precipitous fall in the extent of *A. filiculoides* infestations occurred upon arrival of the weevil in 2011. In the UK, where widespread control of this weed is less consistent, *S. rufinasus* is being mass-reared by the Centre for Agriculture and Biosciences International (CABI) for redistribution. The Department for Environment, Food & Rural Affairs (Defra) classifies the weevil, which has been present in the UK for close to a century, as “ordinarily resident”, so there are no restrictions to its redistribution in England and Wales. During the summer months, the weevil is shipped across the region to be applied to *A. filiculoides* outbreaks, proving to be a highly effective agent, commonly resulting in local eradication of the weed (C. Pratt, unpublished data). Following on from this work, and with similar results, *S. rufinasus* mass rearing and releases have been trialled in the Netherlands and Belgium, along with field assessments of naturalised populations under the EU-funded RINSE (Reducing the Impact of Non-native Species in Europe) programme.

### The strategic use of weed biological control agents in the EU

*Altica carduorum* Guérin-Ménéville (Coleoptera: Chrysomelidae)

In 1969, small-scale caged and uncaged field studies in the UK on the leaf beetle, *A. carduorum*, a natural enemy of *Cirsium arvense* (Asteraceae) from France, effectively became the first release of a classical weed BCA in an EU Member State (Baker et al. 1972). In this case the researchers set out to determine whether *Altica carduorum* appeared capable of establishment in Great Britain. The results were similar to those from Canada (Peschken et al. 1970), in that there was no successful survival over winter. It is not clear what authorisation, if any, was secured or what host range testing was carried out on the beetle prior to this work in the UK. This type of activity would be highly restricted today but the fact that both the source country and the release country are both members of the EU means that those restrictions would be national rather than regional.

*Pteridium aquilinum* (L.) Kuhn  
(Dennstaedtiaceae)

Strategic weed biological control in the EU can be considered to have begun in earnest in the 1980s with a project targeting bracken fern, *P. aquilinum*, for the UK. This project was successful in that highly specific natural enemies from South Africa were identified. However, these agents were never released because the UK authorities requested a prohibitively expensive quarantine field cage to be built to further confirm the agents' specificity. Nowadays this project would probably not be commissioned because some of the fundamental requirements for successful classical weed biological control are not met: the target weed is cosmopolitan, even though its agents are not; and bracken has been credited with providing a habitat for rare and protected lepidopteran species (Pakeman and Marrs 1993).

*Heracleum mantegazzianum* Sommier & Levier  
(Apiaceae)

An implementation plan, developed between 2002 and 2005 under an EU-funded project entitled ‘Giant Alien’, for the biological control of *Heracleum mantegazzianum* (among other common names known as giant hogweed), was an integral part of subsequent research efforts for the sustainable control of this alien weed in Europe. The aim was to evaluate current European guidelines for the importation of exotic organisms. However, no suitably specific agents were found during the project which could have been taken forward through a pest risk assessment for potential future release (Cock and Seier 2007).

*Fallopia japonica* var *japonica* (Hout.) R. Decr.  
(Japanese knotweed) (Polygonaceae)

Japanese knotweed is one of the worst weeds in Europe and certainly the worst invasive plant in Great Britain from an economic standpoint, as it costs that country £165 million each year (Williams et al. 2011), mainly borne by land developers and homeowners. The threat to property posed by Japanese knotweed is considered so great that many banks restrict lending for house purchases if it is found on or near the property and sellers are legally obliged to report its presence to prospective buyers. As a rhizomatous

perennial plant, which is largely clonal and with no real conflicts of interests identified, this was a highly attractive target for biological control. It was, therefore, the subject of the first officially sanctioned release, in 2010, of a WBA in the EU: namely the Japanese knotweed psyllid *Aphalara itadori* Shinji (Hemiptera: Psyllidae). This was the culmination of a research programme which began in the year 2000 with an initial scoping study funded by the USDA Forest Service and the then Welsh Development Agency and continued in earnest from 2003, supported by a consortium of funders.

The psyllid *A. itadori* was found to be the best potential agent of the 186 insect species and more than 40 fungal species found attacking the plant in Japan (Shaw et al. 2009) and was selected to be petitioned for release in England and Wales in 2009. Once it was agreed that licensing should be done under Plant Health Regulations, a Pest Risk Analysis (PRA) was produced which received comments from a wide range of interested parties before being reviewed by the Advisory Committee on Releases to the Environment (ACRE). Further questions were raised by the latter committee regarding possible secondary, tertiary and community level impacts of the release of the psyllid which were addressed by further quarantine studies. The data package was then subjected to a scientific peer review by three anonymous experts, prior to becoming part of a public consultation. Once no further substantive issues emerged, Ministerial approval was sought and subsequently granted for restricted release at a limited number of sites. For the first time in weed biological control history, the release had an eradication plan attached, should anything go wrong. Though this process appears extreme (and is completely impractical) when compared with other more experienced biological control-utilising nations, it should be borne in mind that this was a pioneering activity for Europe and a very cautious approach was justified.

The psyllid did not perform well in UK conditions, during the restricted five year release programme (2010–2015), and despite proving itself capable of overwintering successfully, populations either failed to establish or did not flourish. This could be due to the founder population having been reared under continual Japanese summer conditions in a growth room for almost 90 generations, but could also be due to abnormal and unseasonal weather experienced in the

UK in each of those years and the fact that releases took place on just one occasion each season on small isolated patches of knotweed. In 2015, further psyllids were re-collected from the same locality in Japan as those in the quarantine culture and are currently undergoing field assessment, having been reared in the lab for several generations. The strategy is to start future release cultures from those newly sourced adults that successfully survive the winter in the field in the UK, so as to select for the hardier individuals. Releases will also take place on multiple occasions throughout the season with overlapping generations, and at riparian sites with large knotweed populations, using various stages of psyllids on potted plants. This approach should increase the chances of successful establishment and spread.

*Acacia longifolia* (Andrews) Willd (long-leaved wattle) (Fabaceae)

*Acacia longifolia* is a small tree or shrub, native to south-eastern Australia, which is invasive in Portugal, South Africa and other regions of the globe (Sanz Elorza et al. 2004). In Portugal, *A. longifolia* invades extensive areas of coastal ecosystems and is replacing native plant communities previously dominated by herbs and small shrubs and creating monospecific woody stands (Marchante et al. 2015). In addition, it changes soil chemistry and functioning (Marchante et al. 2008), and the ecological networks of associated communities (López-Núñez et al. 2017). The invasion by this species also reduces forest productivity, mainly in littoral pine plantations, with consequent negative economic impacts. The extensive production of long-lived seeds is a key characteristic that contributes significantly to the dispersal ability and invasiveness of *A. longifolia* (Marchante et al. 2010).

The first intentional release of a BCA against this weed in Europe occurred in November 2015 when *Trichilogaster acaciaelongifoliae* (Froggatt) (Hymenoptera: Pteromalidae), a host-specific Australian bud-galling wasp, was permitted for use in Portugal. The female wasps lay their eggs on flower buds (and later also on vegetative buds) inducing the formation of galls, instead of flowers, which reduces seed production and curbs the growth of *A. longifolia*. The bud-galling wasp is univoltine (one generation per year) and most of the annual life cycle is spent as eggs, larvae and pupae within the developing galls. The

authorization to release *T. acaciaelongifoliae* in Portugal took 12 years and included host-specificity testing involving 40 plant species (Marchante et al. 2011), several analyses and risk assessments by national (both conservation and phytosanitary authorities) and European (Standing Committee on Plant Health SCOPH, from European Commission and European Food Safety Authority EFSA) entities (more details in Shaw et al. 2016). Following their positive opinion (EFSA PLF Panel 2015a) EFSA went on to make a statement with constructive observations and recommendations on the process of assessing risk (EFSA PLH Panel 2015b).

The collaborative research and release process was carried out by the Centre for Functional Ecology (University of Coimbra) and Coimbra College of Agriculture (Polytechnic Institute of Coimbra) and benefited greatly from the extensive experience from researchers from the Department of Biological Sciences, University of Cape Town, South Africa where the BCA *T. acaciaelongifoliae* has been used successfully for more than 30 years (Dennill 1990). Galls of the BCA, despite being native to Australia, were obtained from South Africa, for both host specificity testing and field releases. Only females were released due to the wasp's parthenogenetic reproduction and, despite the challenge of overcoming asynchrony between the phenology of the wasps (from the southern hemisphere) and target plants (in the northern hemisphere), the first records of establishment are encouraging. After the first releases (in November 2015) at eight locations mostly along the Portuguese coast, by July–August 2016, adult females emerged from galls at half the sites indicating the completion of the wasp's life cycle for the first time in the wild in the northern hemisphere (Marchante et al. 2017). The life cycle took approximately 8–9 months to complete, instead of taking the expected one year after oviposition, as happens in the southern hemisphere, so the prospects are good for successive generations of the BCA to fully synchronise their life cycles to northern hemisphere seasons and with the phenology of the host plant. The second-generation galls resulting from oviposition by wasps emergent in July–August 2016 in Portugal were first observed in February 2017 at some of the sites, indicating that they may also complete the cycle in less than one year: by May 2017 a significant increase in the number of mature galls was observed on those sites (H. Marchante, unpublished

data) showing successful establishment. A second release campaign (still with galls imported from South Africa) took place in November–December 2016, and further field releases are planned (with South African or Portuguese galls depending on the rate of establishment in Portugal) until the agent is established and widespread in Portugal.

The long process that led to the release of *T. acaciaelongifoliae* has paved the way for new biological control projects. Host specificity testing on two *Melanterius* species (Coleoptera: Curculionidae) targeting other invasive *Acacia* species in Portugal, has been authorized. This was via a much faster process under the National Authorities ICNF, Portuguese Institute for Nature Conservation and Forests, and DGAV, the Portuguese National Authority for Animal Health, Phytosanitation and Food Safety, and will begin in 2017.

*Impatiens glandulifera* Royle (Himalayan balsam)  
(Balsaminaceae)

Following the release of the psyllid for the biological control of Japanese knotweed in the UK, a second invasive weed was targeted for biological control in that country, *Impatiens glandulifera*. This annual plant was introduced to the UK in 1839 as a garden ornamental. Since then, it has spread by seed, both naturally and with human assistance, over much of the UK and other parts of Europe (Beerling and Perrins 1993). Himalayan balsam can tolerate a wide range of environmental conditions, enabling the plant to rapidly form dense monocultures on wasteland, woodland, railways lines and particularly in riparian habitats. As well as directly reducing biodiversity (Hulme and Bremner 2006), especially amongst invertebrate communities (Tanner et al. 2013), Himalayan balsam also lures pollinators away from native plants, decreasing the fitness of native species (Chittka and Schürkens 2001). River banks are laid bare by the weed after it dies back in the winter, which renders them more prone to erosion (Greenwood and Kuhn 2014). Himalayan balsam was considered to be a good target as it is a fleshy annual plant with an apparently limited number of introductions to Europe. At the time, this latter factor was expected to mean that one (or a few) strains of the rust that was utilized as a BCA would be able to infect all populations of the weed in Europe, due to the host's limited genetic



variability. It was also recognised that to control the extensive riparian populations of this annual weed using traditional physical and chemical methods would need the coordination of the multiple land owners on a catchment scale, which is unlikely to be realised.

The search for natural enemies started in 2006 with surveys undertaken throughout the native range of the Himalayan foothills from Pakistan to Nepal. A range of insects and fungal plant pathogens were collected, but most of the insects were found to feed on a broad set of *Impatiens* species. A rust fungus, *Puccinia komarovii* var. *glanduliferae* (Uredinales), was observed to infect Himalayan balsam throughout the areas surveyed, causing significant damage to infected plants both at the seedling stage (stem infection, usually leading to plant death) and to leaves of the remaining maturing plants and, hence, was prioritised for further study (Tanner et al. 2014). An isolate of the rust fungus collected in the Kullu Valley, Himachal Pradesh, India was selected and screened for specificity against 74 plant species and an additional ten varieties of three widely grown ornamental species in the UK (Tanner et al. 2015). Only *I. glandulifera* and *Impatiens balsamina* L. (a non-native ornamental species with very low commercial value) were fully susceptible to the rust.

The licensing procedure that was followed for the Japanese knotweed psyllid (as recorded above) was replicated for the rust including the submission of a PRA to UK regulators, followed by a public consultation. However, the process then differed as microorganisms are not regulated by the UK Wildlife and Countryside Act 1981. In this case the SCOPH, which had only been informed prior to the release of the psyllid rather than consulted, took an interest in the proposal for release and withheld their endorsement of the PRA. The committee signalled its intention to pass the application to the EFSA for further assessment as the PRA only considered the UK as the intended area of introduction and requested the UK Defra Minister to delay issuing a licence for the release of the rust from quarantine. This was an understandable position because with rusts there can be no eradication plan once they are released into the wild since spores cannot be contained and could potentially cross the Channel into Europe carried on wind currents. In response, the PRA was redrafted to include data relevant to the new area of introduction, the whole of Europe, after which SCOPH endorsed the release of the rust.

The UK Minister for the Environment approved release on the 27th July 2014, making this the first fungal BCA to be released against a weed in the EU. Since then, the rust has been released at 25 sites across England and southern Wales and readily spread onto naturalised Himalayan balsam, reaching significant levels of infection at some sites. The rust was also found to complete its life cycle under UK climatic conditions. However, field observation and inoculation studies showed variation in the susceptibility of different plant populations to the Indian rust strain, suggesting that the plant could have been introduced on more than one occasion into the UK and Europe (Nagy and Korpelainen 2015). This could mean that the rust will only be effective against a subset of the Himalayan balsam populations in the UK, dependent on their origin. Additionally, it has highlighted the potential need to release additional strains of the rust in order to have impact on other plant genetic forms of the target plant in its invasive range (Varia et al. 2016). A new strain of the rust from Pakistan (previously collected and stored in liquid nitrogen at CABI) has now been checked for safety by testing it on the most closely related plant species to Himalayan balsam and, as expected, has the same level of specificity as the Indian strain. Initial screening suggests that the strain can heavily infect some populations of the plant that are not significantly infected by the Indian strain. Permission to release the new rust strain from quarantine was granted by Defra in early 2017 after internal consultation concerning any risk posed by the new strain. A molecular analysis of UK and native-range populations of Himalayan balsam is underway at CABI to ascertain how many genetic types there are in the country and if necessary to help target future surveys to collect new rust strains in the native range. Further development of this tool, specifically targeting the genes governing plant resistance/susceptibility to the rust could also allow site-specific assessment of likely success and inform other countries of the susceptibility of their invasive balsam populations to this promising biological control agent.

## Discussion

Historically, Europe has, and still is benefiting from the unplanned spread of some weed biological control agents. Although mostly successful, these serendipitous

cases of biological control carry the risk of encouraging future illegal and inadequately researched releases and are clearly not procedurally or ethically correct, nor safe. The positive outcomes of such releases have served an inadvertent purpose in that people in Europe are becoming more aware of the potential of natural enemies to control large scale weed invasions. In parallel, more and more research towards intentional releases in the EU is being undertaken as the result of strategic national funding. It is expected that the European Invasive Species Regulation (1143/2014) will further benefit this discipline since promising biological control target species are included on the initial list of 37 species published by the EU Commission in its implementing regulation 2016/1141, for which Member States are obliged to publish a management plan. There are now 23 plants on this list, after its recent expansion, and the scale of some of these invasions leave few alternatives to biological control.

For example, Spain cannot continue indefinitely spending tens of millions of Euros (Anonymous 2010) on the mechanical removal of water hyacinth (*Eichhornia crassipes* (Mart.) Solms) (Pontederiaceae) from the Guadiana river. The favoured BCA, a weevil (*Neochetina eichhorniae* Warner) (Coleoptera: Curculionidae), should be able to establish and control the weed as it has done on many occasions elsewhere in ecoclimatic ranges that are similar to those of the Mediterranean basin. In addition, the invasion of *Ludwigia* spp. (Onagraceae) in France has such significant environmental and economic impacts over such a large area (Muller 2004) that land managers often give up management attempts. Work is in progress in Argentina where potential BCAs have already been identified such as *Liothrips ludwigi* Zamar et al. (Thysanoptera: Phlaeothripidae) (Zamar et al. 2013), and an as yet unidentified *Puccinia* rust (C. Ellison, unpublished data). Furthermore, there is ongoing research into the potential for biological control of *Hydrocotyle ranunculoides* L.f. (Apiaceae) (Cabrera-Walsh et al. 2013) which is invasive in both the UK and the Netherlands, as well as of *Lagarosiphon major* (Ridley) Moss (Hydrocharitaceae) where an ephydrid fly (Diptera: Ephydridae) may have potential as a BCA (Mangan and Baars 2013). In addition to those species on the EU list, there are other aquatic and riparian weeds for which there is ongoing biological control research, including *Crassula helmsii* (T. Kirk) Cockayne (Crassulaceae), for which an

eriphyid mite (Acari: Eriophyidae) is a highly promising candidate agent (S. Varia, unpublished data). Some terrestrial species such as *Solanum elaeagnifolium* Cavanilles (Solanaceae) and *Ailanthus altissima* (Mill.) Swingle (Simaroubaceae) are also potential targets for biological control in Europe.

The EU is moving from a period of serendipity to a period of strategy regarding weed biological control and this should be to the benefit of the economies and environments in those Member States affected by the worst invasive plants. More non-native species are being regulated than ever before and the regulatory pathways for the licensing of exotic weed biological control agents have been made clear by recent projects that have culminated in the release of agents from multiple taxa within the EU. There is no shortage of target weeds and ten of the species highlighted in the reviews by Gassmann et al. (2006) and Sheppard et al. (2006) are included as species of European concern in the recent EU Regulation on Invasive Species and more are likely to be added at each revision of the list. In the section of the regulation covering the management of invasive species that are widely spread, there is a requirement for Member States to have in place effective management measures within 18 months of their inclusion on the list, and that these measures should be proportionate and prioritised based on a risk evaluation and their cost effectiveness. Though that timescale is too short for a candidate biological control agent to be developed, adopting a proactive strategy is likely the best approach as some of the targets in question have previously proven BCAs available.

In Europe, at present, weed biological control is very much a concern at the national level and there is a lack of coordination when it comes to any regional work. Research is currently carried out by teams on behalf of their host nations in some countries that have the necessary quarantine facilities and experience to do the work safely, such as the UK, Portugal, Ireland, Switzerland, France and, to some extent, Italy and Greece. A sensible next step would be for work to commence on those species highlighted above with the affected countries sharing the costs and conducting the research in collaboration with experienced research groups that have established quarantine facilities. Going forward, one key need in any collective EU or European strategy would be the application of a prioritisation tool, such as that developed by Paynter et al. (2009) which would allow

any resources secured as a result of the regulation on invasive species, to be expended on the most appropriate and important weeds in the EU and in the rest of Europe. At present, however, the biggest challenge is ensuring that classical weed biological control is given due consideration by decision makers who are inherently risk averse or unaware of the technique. The biological control community in Europe needs to continue to engage in raising awareness so that classical biological control can gain the confidence of regulators and politicians in Europe as well as some of their advisors in the ecology and conservation communities who may lack the necessary balance to consider relative risk of the agent and that of its target weed (Downey and Paterson 2016). Classical biological control is a highly successful, cost-effective and environmentally sound management strategy to deploy against weeds, as it has been for well over a century in many other non-EU countries across the world and is currently under-utilised.

**Acknowledgements** Many sincere thanks are given to the reviewers and in particular the special edition editorial team for providing such thorough reviews and guidance which have resulted in a much improved paper. For the prickly pear-*Dactylopius* monitoring we are grateful to Patricia Pérez Rovira, Cristóbal Torres Ródenas, Miguel Ángel Gómez Serrano, Jose Miguel Aguilar Serrano, Valentín Tena Lázaro and the Natura-2000 squads of Castellón, for their involvement and help throughout the process. For the common ragweed project support is acknowledged from EU COST Action FA1203 “Sustainable management of *Ambrosia artemisiifolia* in Europe (SMARTER)” (<http://internationalragweedsociety.org/smarter>). The Acacia research (from 2003 to 2016) was supported by Portuguese Foundation for Science and Technology (FCT) and European funds POCTI/POCTI/COMPETE/FEDER, through projects INVADER (POCTI/BSE/42335/2001), INVADER-II (POCi/AMB/61387/2004), INVADER-B (PTDC/AAG-REC/4607/2012) and INVADER-IV (PTDC/AAG-REC/4896/2014). López-Núñez FA, Freitas H, Hoffmann JH, Impson F, Marchante E are acknowledge by their significant contribution along several parts of the all process. A. Torrinha, S. Ribeiro, N. César de Sá, I. Seça, O. Ferreira, J. Carlos Filipe, C. O’Connor, S. Quaresma, C. Gonçalves, K. Dix, L. Barrico, R. Miranda, P. Duarte, R. Eusébio, A. Sofia Nunes and R. Vaz are acknowledged for help in field and laboratory work. For the Himalayan balsam research work thanks are given to Rob Tanner, Harry Evans, Sonal Varia, Kate Pollard and Marion Seier. Many donors have provided resources to this project with the main funding from Defra, Natural Resources Wales and Natural England. We are also very grateful to participating local action groups, River Trusts, Water Boards, the Environment Agency and local authorities that have provided invaluable support with the rust release programme. The knotweed project was first funded by the Welsh Development Agency (now Welsh Assembly Government) and the USDA

Forest Service before being picked up by a consortium of funders adding Defra, Network Rail, Environment Agency, South West Regional Development Agency, British Waterways (now Canals and Rivers Trust), all coordinated by Cornwall Council. It is now funded mainly by Defra with support from the Welsh Assembly Government and AAFC.

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