Perspectives for biological control

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Summary

The recent invasion by common ragweed, *Ambrosia artemisiifolia,* has, like no other plant, raised the awareness of invasive plants in Europe. Recently, chemical and mechanical control methods have been developed and partially implemented in Europe, but sustainable control strategies to mitigate its spread into extensively managed land and to reduce its abundance in badly infested areas are lacking. One management tool - not yet implemented in Europe but successfully applied in Australia - is biological control. With the notable exception of the recently detected leaf beetle *Ophraella communa*, almost all natural enemies that have colonized *A. artemisiifolia* in Europe are polyphagous and impose only little damage, rendering them unsuitable for a system management approach. Two fungal pathogens have been reported to adversely impact *A. artemisiifolia* in the introduced range, but their biology makes them difficult for mass production and application as a mycoherbicide. In the native range of *A. artemisiifolia*, on the other hand, a number of herbivores and pathogens associated with this plant have a very narrow host-range and reduce pollen and seed production, the stage most sensitive for long-term population management of this winter annual. We discuss and propose a prioritisation of these biological control candidates for a classical or inundative biological control approach against common ragweed in Europe by considering past experiences from North America, Asia and Australia. We argue that the biological control approach should be considered as an integral part of an integrated management approach against common ragweed in Europe. Along these lines, the COST action 'SMARTER' (launched in 2012) aims at promoting biological control against common ragweed, integrating it with available chemical and physical control options, and developing habitat- and region-specific recommendations for a integrated management of common ragweed across Europe.

Introduction

Like no other plant, common ragweed, *Ambrosia artemisiifolia* L., has raised the awareness of invasive plants in Europe. First records of this plant species in western Europe date back to the mid 1800s and in eastern Europe to 1900, but it was only in the late 1920's that *A. artemisiifolia* became an increasing problem in Europe (Csontos *et al.,* 2010). The main concern regarding *A. artemisiifolia* is its large production of highly allergenic pollen that causes already rates of sensitisation among Europeans from 15% (e.g. Germany, Netherlands, Denmark) to 60% (Hungary; Rybnicek and Jäger, 2001; Taramarcaz *et al.,* 2005). This results in allergic rhinitis and severe asthma in over 20% of the population of affected areas (Kazinczi *et al.,* 2008).

The recent spread of *A. artemisiifolia* and the resulting increasing risk to human health and agriculture has resulted in a number of publications on the further invasion and potential danger of this invasive weed, its medical aspects, pollen monitoring across Europe, and control methods at a local scale (Buttenschøn *et al.,* 2009). Moreover, in 2006 the national authorities in Hungary and Switzerland established a legal basis for mandatory control of *A. artemisiifolia*. Yet, although chemical and mechanical control methods have been developed and partially implemented (Buttenschøn *et al.,* 2009), sustainable control strategies to mitigate its spread into areas not yet invaded and to reduce its abundance in badly infested areas are lacking in Europe.

One management tool that has received little attention in Europe so far is biological control. Three principal methods of biological weed control can be distinguished: (i) The classical approach aims to control naturalized weeds by a limited number of introductions of exotic control organisms from

the weed's native range; (ii) The inundative method uses periodic releases of an abundant supply of a native or exotic biological control agent over the entire weed population ; (iii) The system management approach, sometimes also referred to conservation biological control, aims to increase the impact of native antagonists (Müller-Schärer and Schaffner, 2008). Based on a prioritizing scheme developed by Sheppard *et al.* (2006), *A. artemisiifolia* was identified as one of the 20 most promising species for classical biological control in Europe.

Because *A. artemisiifolia* also causes problems in the northern parts of North America, Australia and large parts of Asia, there is a significant amount of information available on the biology of this plant and on the efficacy of various control measures from other parts of the invaded range. Up to date, *A. artemisiifolia* has been subjected to classical biological control programmes in Eastern Europe, Australia, and eastern Asia with variable success (Julien and Griffiths, 1998; Reznik *et al.,* 2007; Zhou *et al.,* 2009). The information gathered in these biological control programmes may act as a solid basis to develop a biological control program for Europe. Its integration into existing short-term control measures may then lead to a sustainable management strategy of *A. artemisiifolia* and other *Ambrosia* species invasive in Europe.

This report summarizes previous attempts to control *A. artemisiifolia* using biological control worldwide and explores prospects for its application in Europe.

Natural enemies of *Ambrosia artemisiifolia*

To assess whether any natural enemies (herbivores or fungal pathogens) attacking *A. artemisiifolia* could potentially be used for biological control of this weed in Europe, we conducted a literature review to compile a comprehensive list of natural enemies associated with *A. artemisiifolia* and other *Ambrosia* species in the native range in North America and in the introduced range in Europe, and of the biological control activities that have been conducted worldwide so far. The results of the review were published in Gerber *et al.* (2011) and are outlined below.

Herbivores and pathogens associated with *A. artemisiifolia* in Eurasia

About ten species of insects, mites and fungi were recorded in Eurasia by Kovalev (1971a), several generalist fungal pathogens and insect species found in Hungary (Bohar and Vajna, 1996; Kiss *et al.,* 2008), and 28 species of insects recorded in former Yugoslavia (Maceljski and Igrc, 1989). In total, some 60 insect species (including two unidentified geometrids) are reported to be associated with *A. artemisiifolia* in Europe (Essl et al. 2015). The insect complex revealed mainly polyphagous species, some of them even known as agricultural pests. In China, the moth *Ostrinia orientalis* Mutuura & Munroe (*Lepidoptera*: *Pyralidae*) attacks *A. artemisiifolia* and was found to significantly reduce biomass and plant height (Wan *et al.,* 2003); however, the species is also recorded from *Xanthium sibiricum* and *Rumex species* (*Polygonaceae*), hence has a relatively broad host-range (Ishikawa *et al.,* 1999).

In 2013, the ragweed leaf beetle *Ophraella communa* Lesage (Col.: Chrysomelidae) was detected in southern Switzerland and northern Italy (Müller-Schärer et al. 2014). At sites where *O. communa* was found in Switzerland and Italy in 2013, up to 100% of the plants were attacked, with attack levels high enough to completely defoliate and prevent flowering and seed set of most ragweed plants (Müller-Schärer *et al.*, 2014). This oligophagous beetle is used as a biological control agent against *A. artemisiifolia* in China, but despite extensive host specificity tests, the risk of attack and the level of damage of sunflower under field conditions remain unclear (see below).

Of the 20 fungal pathogens found associated with *Ambrosia* species in Eurasia (Gerber *et al.,* 2011), most are known to have a wide host range and were found to have little impact on the plant in the field (Kiss *et al.,* 2003). Outbreaks of disease epidemics caused by two biotrophic fungal pathogens, *Phyllachora ambrosiae* (Berk. & M.A. Curtis) Sacc. and *Plasmopara halstedii* (Farl.) Berl. & De Toni, did affect *A. artemisiifolia* in Hungary in the years 1999 and 2002 (Vajna *et al.,* 2000; Vajna ,2002), but not in other years (Kiss, 2007).

A newly described species associated with *A. artemisiifolia* in Hungary (Farr and Castlebury, 2001), *Septoria epambrosiae* D.F. Farr, is also known from *A. trifida* in North America. In China, the damaging microcyclic rust *Puccinia xanthii* Schwein. has been recorded from *A. trifida* as *P. xanthii* forma specialis *ambrosiae-trifidae* Batra (Lu *et al.,* 2004), following Batra's initial classification of a host-specific *P. xanthii* accession from the same host plant in North America (Batra, 1981). This rust species is considered to comprise a number of host-specific rust populations adapted to specific *Asteraceae* hosts (Batra, 1981; Morin *et al.,* 1993; Kiss, 2007; Seier *et al.,* 2009).

Herbivores and pathogens associated with *A. artemisiifolia* in the native range

Compared to the low number of phytophagous organisms associated with *Ambrosia* species in the introduced range in Eurasia, numerous species are known from their native range. Up to date, as many as 450 species of insects, mites and fungi have been identified to be associated with *Ambrosia* species in North and South America (Goeden and Andres, 1999). On individual *Ambrosia* species as many as 113 (on *A. psilostachya*) and 88 (on *A. confertifolia*) insect species were recorded in Southern California alone (Goeden and Ricker, 1975; Goeden and Ricker, 1976b). Many of these species are not specific as they also feed on other genera in the *Asteraceae* family or are known to develop on species in other plant families. However, our survey for species potentially specific at the subtribe level (i.e. associated with *Ambrosia* and for which no other host plant record has been found outside of the subtribe *Ambrosiinae*) revealed as many as 109 specialist invertebrate and 19 specialist fungal species (Gerber *et al.*, 2011). This amounts to approximately 36 and 25% of the total number of invertebrates and fungal species recorded from the native range, respectively. Within invertebrates, Lepidoptera (40 species) largely dominate, followed by Coleoptera (28 species), Diptera (19 species) and Hemiptera (18 species). In addition, four mite species have been recorded from members of the genus *Ambrosia*. The majority of herbivores with known feeding niche are leaf-feeders (50%), followed by stem-miners (28%), seed-feeders (12%) and flower- or pollen-feeders (9%).

As observed for the invertebrate fauna, numerous fungal pathogens known to be associated with *Ambrosia* species in the native range have a wide host range, either within the *Asteraceae* or across a number of different plant families. However, some fungal species are similarly restricted to the genus *Ambrosia*, e.g. *Septoria ambrosiicola* Speg. and *Passalora ambrosiae* (Chupp) Crous & U. Braun (synonym *Cercospora ambrosiae* Chupp; see Gerber *et al.*, 2011). Other pathogen species such as the white blister 'rust', *Pustula tragopogonis* (Pers.) Thines (synonym *Albugo tragopogonis* (D.C.) Gray), and the true rust *Puccinia xanthii* have been recorded from a number of different genera within the *Asteraceae*; however, *P. tragopogonis* and, as indicated above, *P. xanthii* have been shown to comprise different formae speciales with a highly restricted host range. The existence of formae speciales is also known for the powdery mildew species *Golovinomyces cichoracearum* var. *chichoracearum* (DC.) V.P. Heluta (synonym *Erisyphe cichoracearum* DC.), and a restricted host range of accessions of this pathogen associated with *A. artemisiifolia* cannot be ruled out (Ellison and Barreto, 2003). However, this hypothesis would need to be verified through cross-inoculations and molecular studies (Evans, 2000).

Biological control of Ambrosia species

Biological control of *Ambrosia* species in their native range

Ambrosia artemisiifolia and *A. trifida* are also noxious weeds in their native range, in particular in Canada (Cowbrough, 2006) and in the Northern United States (USDA-NRCS, 2009a; USDA-NRCS, 2009b), causing allergenic hay fever (Bassett and Crompton, 1975). As the highest densities of both species are found in the most densely populated part of Canada (southern Ontario and Quebec), the feasibility of the mycoherbicide approach, i.e. the periodic inundative application of high doses of indigenous pathogens over an entire weed population, was studied in both Canada and the USA. Protomyces gravidus Davis, which attacks *A. artemisiifolia*, *A. trifida*, *Xanthium strumarium* L. and members of the genus *Bidens* (tribe *Coreopsideae, Asteraceae*), was studied in the USA (Cartwright

and Templeton, 1988). The species causes stem gall disease and killed plants when these were infected systemically. However, the low rate of infection and lack of virulence when applied as a mycoherbicide strongly limits the use of this organism to control *Ambrosia* species. The project was therefore stopped. A forma specialis of *Pustula tragopogonis* has been described on *A. artemisiifolia* in Canada (Hartmann and Watson, 1980a). Host specificity tests on 59 species from 46 genera indicate that, other than *A. artemisiifolia*, disease symptoms developed only on sunflower cultivars (*Helianthus annuus* L.). Although a few pustules developed on the cultivars inoculated, the disease did not persist and sunflower is therefore considered a non-compatible host for the *P. tragopogonis* accession from *A. artemisiifolia* (Hartmann and Watson , 1980a). Attack by *P. tragopogonis* can be very damaging and significantly reduce pollen and seed production if systemic infection is achieved, as shown both in laboratory and in field trials. The rate of systemic infection obtained in the laboratory was however low (14%), and Hartmann and Watson (1980b) suggested that multi-cyclic applications of *P. tragopogonis* suspensions would be necessary to increase infection level in a field environment. Difficulties to mass produce this white blister 'rust' have so far limited its potential use (Teshler *et al.*, 2002). *Pustula tragopogonis* was accidentally introduced from Canada into the former USSR in the early 1960s where initially it caused heavy infection of *A. artemisiifolia* and reduction in biomass and seed production, but levels of damage have strongly declined since (Julien and Griffiths , 1998).

A *Phoma* species, recorded on *A. artemisiifolia* in North America, was considered as a potential mycoherbicide candidate (Brière *et al.* , 1995). A combination of this *Phoma* species and a phytophagous insect, the leaf beetle *Ophraella communa* LaSage (Coleoptera: Chrysomelidae), were synergistic and resulted in high plant mortality (Teshler *et al.* , 1996). Unfortunately, the culture of *Phoma sp.* lost its virulence and attempts to revive or re-isolate the species from natural sites have failed (Teshler *et al.*, 2002). Two plurivorous pathogens, the soil borne fungus *Rhizoctonia solani* J.G. Kuehn and the gram-negative bacterium *Pseudomonas syringae* pv. *tagetis* (Hellmers) Young, Dye & Wilkie have also been preliminarily evaluated as potential biocontrol agents for a crop management strategy against A. grayi in the USA (Sheikh *et al.*, 1999; Sheikh *et al.*, 2001; Wheeler *et al.*, 1998). Under greenhouse conditions *R. solani* was shown to cause significant disease in inoculated *A. grayi* plants seen as an increase in root necrosis and a reduction in plant emergence as well as in fresh and dry leaf weight (Wheeler *et al.*, 1998). *Pseudomonas syringae* pv. *tagetis* proved to be pathogenic towards *A. grayi* causing systemic chlorosis in infected plants during greenhouse trials. Subsequent field trials conducted in Texas showed the bacterium to be effective against the weed at relatively low concentrations and following a single application (Sheik *et al.*, 1999; Sheikh *et al.*, 2001).

The beetles *Zygogramma suturalis* and *Ophraella communa* are natural enemies of *A. artemisiifolia* in Canada and were studied as inundative biological control agents (Teshler *et al.*, 2002). The reduction or cessation of *Z. suturalis* oviposition on extensively damaged plants (as observed in the former USSR; see below) and pupation in soil are, however, an important limitation for the mass-rearing of this species (Teshler *et al.*, 2002). *Ophraella communa* is considered more promising because it is easy to mass-rear and handle (Teshler *et al.*, 2002). Under favourable conditions the beetles can completely defoliate their host plants (Welch ,1978), but generally, population densities and impact of *O. communa* in North America are low, presumably because of strong attack by predators and parasitoids by the end of summer (Teshler *et al.*, 2002). If used in inundative biological control, it was therefore suggested that releases of beetles should occur early in the growing season (Teshler *et al.*, 1996).

Classical biological control of *Ambrosia* species worldwide

There is a long history of classical biological control attempts against exotic *Ambrosia*, mainly *A. artemisiifolia*, in different parts of the world, including eastern Europe (Russia, former Yugoslavia, Georgia, Ukraine), Australia and Asia (China and Kazakhstan), resulting in the release of numerous invertebrate biological control agents (see below). To date, no studies and therefore also no intentional introductions of fungal pathogens from the native range have been made in any of the introduced ranges of invasive *Ambrosia* species (Julien & Griffiths, 1998).

Eastern Europe

Classical biological control of exotic *Ambrosia* species started in the former Soviet Union in the 1960s, when more than 30 insect species from North America were introduced into quarantine (Goeden and Andres, 1999). Host-specificity testing of the candidate natural enemies were conducted in quarantine, involving eight varieties of sunflower (*Helianthus annuus*), 18 other *Helianthus* species, and 80 species representing 46 genera and 18 families of plants (Kovalev, 1971b). By 1990, five species of insects had been released with the aim to establish a complex of natural enemies. In 1969 the release of the noctuid moth *Tarachidia candefacta* (synonym: *Ponometia candefacta*) collected on *A. artemisiifolia* in Canada and California, was the first intentional introduction of a natural enemy for the biological control of an invasive exotic plant into Europe (Kovalev, 1971b). In 1972, a subspecies of *T. candefacta* collected on *A. psilostachya* (now *A. coronopifolia*) was also released (Julien and Griffiths, 1998; Kovalev, 1971b). The species established on both *A. artemisiifolia* and *A. psilostachya* (Kovalev, 1971b), but so far *T. candefacta* has been unsuccessful as a biological control agent. Predation of the exposed larvae (Goeden and Andres, 1999) and unsuitable climatic conditions (Poltavsky and Artokhin, 2006) have been stated as potential reason for its failure. While in the past, strong frosts might have limited population growth, Poltavsky and Artokhin (2006) observed increased numbers in their study region (Rostov-on-Don) from 2003 onwards after a series of mild winters.

In 1978, the leaf beetle *Zygogramma suturalis* from Canada and the USA was released and quickly established in the North Caucasus (Julien and Griffiths, 1998), and has since spread practically over the whole area heavily infested by *A. artemisiifolia* in Russia (Reznik *et al.*, 2007). In the same year, the species was also released in Kazakhstan, Georgia and Ukraine, but establishment is only confirmed from Kazakhstan (Julien and Griffiths, 1998). *Zygogramma suturalis* was further released in 1985 and again in 1990 in former Yugoslavia (now Croatia). Prior to its release in 1985, host specificity tests under no-choice condition were conducted on 128 plant species/varieties and no feeding was reported on any other plant than *A. artemisiifolia* (Igrc, 1987). The species has established in Croatia, but so far densities of beetles in the field are low (Igrc *et al.*, 1995). In Russia, one complete and a partial second generation are produced and both larvae and adults feed on leaves and flowers of *A. artemisiifolia* from April to mid September (Reznik, pers. comm.). At first, the results obtained with this beetle in Russia were very promising (Reznik, 1991). *Zygogramma suturalis* reached densities as high as 5,000 individuals per m^2 in one locality in southern Russia and completely destroyed all of the *A. artemisiifolia* as the beetle population moved across an infested field at a rate of 3 m per day (Goeden and Andres, 1999). Reduction of the weed increased crop yield by two- to threefold (Goeden and Andres, 1999). Further investigations have however shown that *Z. suturalis* is not able to control the weed sufficiently, in particular on arable land (Reznik, 1996). Serious damage of *A. artemisiifolia* plants over large areas provoke oviposition inhibition and can result in summer diapause in female *Z. suturalis* (Reznik, 1991). Population outbreaks and complete destruction of host plant populations as reported by Kovalev (1989) can only occur during the short period in spring when young adults emerge and lay eggs, since females of the first generation show little or no reaction to the degree of damage of their host plant (Reznik, 1991). Data from field surveys conducted between 2005 and 2006 indicate that average population densities in Russia are very low and, consequently, the impact on the target weed is negligible (Reznik *et al.*, 2007). Damage to ragweed was recorded mainly in undisturbed patches, where both *A. artemisiifolia* and beetle densities were higher (Reznik *et al.*, 2007).

Further releases of North American insects into the former Soviet Union included the seed feeding fly *Euaresta bella* from Canada and the USA in 1969 and again in 1990, the pollen-feeding beetle *Trigonorhinus tomentosus* from the USA in 1977 and the leaf feeding beetle *Zygogramma disrupta* from USA in 1978, but all three species failed to establish (Julien and Griffiths, 1998).

The eriophyid mite *Eriophyes boycei* collected on *A. psilostachya* was also considered as a potential agent of *A. artemisiifolia* and was shipped to the former Soviet Union but did not survive the transport (Goeden *et al.*, 1974). Eriophyid mites have repeatedly been used in classical biological control programmes, and have contributed to the successful management of alien invasive weeds (Briese

and Cullen, 2001). However, they tend to be highly host specific (Skoracka, 2006), raising doubt on whether *A. artemisiifolia* indeed belongs to the fundamental host-range of *E. boycei*.

Australia

Between 1980 and 1984, three biological control agents from Mexico were introduced into Australia for the biological control of *Parthenium hysterophorus* L., which is closely related to *A. artemisiifolia*, i.e. the leaf feeding chrysomelid beetle *Zygogramma bicolorata*, the sap sucking bug *Stobaera concinna* and the tip-galling moth *Epiblema strenuana* (McFadyen and Weggler-Beaton, 2000). All three insects also attack *A. artemisiifolia* and in particular *E. strenuana* is reported to reduce its size, abundance and pollen production. In 1990 *Z. suturalis* was introduced into Australia from the USA to increase *A. artemisiifolia* control, but the species failed to establish (Julien and Griffiths, 1998). Further, an undescribed *Liothrips* species collected on *A. elatior* (now an accepted synonym for *A. artemisiifolia*) in northern Argentina, was tested in quarantine (McFadyen and Weggler-Beaton, 2000). However, host specificity tests revealed that the species also develops on and severely damaged young sunflower seedlings. Even though this Liothrips species was not recorded to attack sunflowers in the field in Argentina, the species was rejected for field release. The species was also considered and rejected for introduction into Canada (McFadyen and Weggler-Beaton, 2000).

The gall midge *Asphondylia ambrosiae* was shipped to Australia several times, but could not be successfully reared (Goeden and Palmer, 1995). *Asphondylia* larvae feed on symbiontic fungi that line the walls of their galls, and not on the plant material directly. The host plant is inoculated with the fungi by ovipositing females. The release of *A. ambrosiae* and other fungus-feeding cecidomyid flies for classical biological control would therefore require the simultaneous importation of these symbiotic fungi, which makes the use of these cecidomyiid flies as biological control agents rather unrealistic. An alternative approach might consist of rearing *A. ambrosiae* using fungi from European gall midges; such an approach has been successfully adopted in the rearing of the fungus-feeding galling midge *Schizomyia cryptostegiae* Gagné, which was introduced in Australia as a biological control agent against rubber vine, *Cryptostegia grandiflora* R.Br. (McFadyen, pers. comm.).

Presently, the two agents *E. strenuana* and *Z. bicolorata* are known to be widespread and exerting a degree of control in most of the affected areas in eastern Australia. There has been no formal assessment of the impact of these biocontrol agents on *A. artemisiifolia*. However, according to Palmer and McFadyen (2012) there is much less *A. artemisiifolia* in southeastern Queensland and northern New South Wales than there was in the 1980s. The plant is now relatively rare and no longer causes significant allergenic symptoms in the flowering season (Palmer and McFadyen, 2012). From an economic point of view, biological control of *A. artemisiifolia* is regarded as an outstanding success in Australia (Palmer *et al.*, 2010).

Eastern Asia

Releases of *Zygogramma suturalis* in China in 1985, both from Canada and from the former Soviet Union, resulted in establishment in some locations, but failed in others (Wan *et al.*, 1995). Additional tests on 74 plant species/varieties were conducted prior to field releases and feeding was only recorded on *A. artemisiifolia*. Interestingly, the close relative *A. trifida*, a species also invasive in Europe, was not accepted as a host by the beetle (Wan *et al.*, 1989). *Euaresta bella* was introduced into China in the late 1980s, but as in Russia, this seed-feeding fly failed to establish (Wan *et al.*, 1993).

In 1991, *Epiblema strenuana* was introduced from Australia into China where additional host specificity tests were conducted (Ma *et al.*, 2003; Wan *et al.*, 1995). In contrast to results from host specificity tests conducted in Australia (McFadyen, 1985), *E. strenuana* was able to complete its development on a local sunflower variety tested (Wan *et al.*, 1995). In subsequent choice-tests (i.e. in the presence of the target weed *A. artemisiifolia*), acceptance and suitability as host varied according to test conditions: sunflowers were attacked and adults emerged from plants that were exposed under mul-

tiple choice conditions in a greenhouse (Wan *et al.*, 1995), while sunflowers were attacked but no development was found in a field cage test (Wan and Wang, 2000). Under open field condition, no eggs were laid on sunflowers but larvae moved from *A. artemisiifolia* that had died prematurely to sunflower and completed their development (Wan and Wang, 2000). Overall, Wan and Wang (2000) considered the risk of *E. strenuana* to cause economic damage to sunflowers to be low. To further avoid potential damage to sunflower, it was recommended to release the species only south of the Yangtze river, i.e. where sunflower is not a major crop (Wan and Wang, 2000). However, *E. strenuana* has also been recorded from members of the genera *Bidens* and *Chenopodium*, indicating that its host-range includes plant species outside the tribe Ambrosiinae.

In addition to the deliberate releases of biological control agents, *Ophraella communa*, a North American leaf beetle, was accidentally introduced into Japan in the late 1990s (Yamanaka *et al.*, 2007 and references therein). The beetles can cause complete defoliation and death of *A. artemisiifolia* (Dernovici *et al.*, 2006; Palmer and Goeden, 1991). In 2001 it was also found in Jiangsu province in China (Zhang *et al.*, 2005), from where good control of *A. artemisiifolia* populations is reported (Zhou *et al.*, 2009). Originally, the species was reported only from *A. artemisiifolia*, but more recently it also has been recorded in the field from several other species within the subtribe Ambrosiinae, including several *Ambrosia* and *Xanthium* species, *Parthenium hysterophorus*, *Iva axillaris* Pursh., *Ratibida pinnata* (Vent.) Barnhart (subtribe Rudbeckiinae), as well as from *Helianthus ciliaris* DC. (subtribe Helianthinae; Dernovici *et al.*, 2006; Futuyma and McCafferty, 1990; Goeden and Ricker, 1985; McFadyen and McClay, 1981; Palmer and Goeden, 1991; Watanabe and Hirai, 2004). Host-specificity tests revealed that *O. communa* can attack and complete its life-cycle on sunflower and the species was subsequently rejected as biological control agent for Australia (Palmer and Goeden, 1991). Recent studies indicate however only a low risk that *O. communa* would cause significant damage to sunflower plants in the field. *Ophraella communa* rarely lays eggs on sunflowers under choice conditions, larval mortality on sunflower is high and newly emerged adults leave the sunflower plants in search of *Ambrosia* (Dernovici *et al.*, 2006). Only if *Ambrosia* plants are completely defoliated, 1st instar larvae move to adjacent sunflower (Dernovici *et al.*, 2006). These results are in accordance with field observations from Japan where adults only occasionally feed on sunflowers and where reproduction has only been found on *A. trifida* and *A. artemisiifolia* (Watanabe and Hirai, 2004). The distribution of *O. communa* in China is predicted to only partially overlap with sunflower cultivation (Cao *et al.*, 2007). Recently, a mass rearing programme was established with *O. communa* in China with the aim to use this agent for inundative application in severely invaded habitats (Zhou *et al.*, 2009).

Prospects for biological control of *Ambrosia artemisiifolia* in Europe

While both the inundative and the system management approach (see above) are primarily aimed at crop weeds, the classical approach has traditionally and most successfully been used against invasive plants spreading over large areas of natural and semi-natural habitats, extensively managed agro-ecosystems or aquatic ecosystems (environmental weeds; Müller-Schärer and Schaffner, 2008). As outlined above, with the possible exception of the leaf beetle *Ophraella communa*, distinct virulent strains of the rust fungus *P. xanthii* as well as the two pathogens *Phyllachora ambrosiae* and *Plasmopara halstedii*, no natural enemy recorded on *A. artemisiifolia* and other exotic *Ambrosia* species in Eurasia so far appears to be sufficiently specific and/or damaging, particularly with regard to long-term and sustainable control. The apparent lack of a regular re-occurrence of epiphytotics by *P. ambrosiae* and *P. halstedii* (Kiss, 2007) raises the question whether they could be facilitated through artificial inundative application of these two fungal pathogens. However, neither of these fungi can be cultured in vitro; thus their biology makes them presently unsuitable for mass production and application as a mycoherbicide. This thus excludes a system management approach or an inundative application of European antagonists to control *A. artemisiifolia* in Europe, and leaves either classical biological control or an inundative application of exotic organisms for managing common ragweed in Europe by biological means.

When developing a biological control approach as part of an integrated management programme against *A. artemisiifolia* in Europe, priority should be given to organisms with a narrow host range and that have the potential to either negatively impact the population growth rate of ragweed, or to quickly reduce ragweed biomass. In terms of host specificity, one of the most critical issues is the close relatedness of the target to the commercially important sunflower, *Helianthus annuus*. As sunflower varieties might differ in their susceptibility to biological control candidates (Morin *et al.*, 1993), several varieties need to be included in biosafety studies, especially those that occur in the regions where *A. artemisiifolia* is abundant and specific control agents are planned to be released. Only one plant species of the subtribe Ambrosiinae is considered native to Europe, i.e. *Ambrosia maritima*, which is furthermore restricted to the Mediterranean. Such a low number of very closely related native species increases the chance of finding "safe" biological control agents (Pemberton, 2000). Thus, the occurrence and conservation status of *A. maritima* in the different parts of Europe and its susceptibility as host will be crucial in the evaluation process of potential biological control agents. On the other hand, due to the observed high within-population variation (Genton *et al.*, 2005) of *A. artemisiifolia* found in France, biological control agents should also be not too (genotype or host strain) specific in order to account for genetic differences among populations and to control all individuals in a population.

In terms of impact, flower-, pollen- and seed-feeding organisms or those that contribute to a reduction in seed output should be considered first when applying the classical biological control approach, as pollen production is the prime factor causing the high impact on human health of ragweed (see above), and a reduction in seed output is likely to translate into reduced population densities and dispersal of annuals (Ramula *et al.*, 2008). On the other hand, natural enemies that quickly reduce the biomass are expected to be especially suited for an inundative application to reduce crop losses due to competition with ragweed (Müller-Schärer *et al.*, 2000; Harrison *et al.*, 2001). There is generally a lack of information on whether ragweed specialists are able to quickly reduce biomass of *A. artemisiifolia*, but indirect evidence may come from congeneric species that are known to seriously damage their host plants (see below). Building on the information compiled above, we propose in the following an outline to tackle biological control of *A. artemisiifolia* in Europe, involving both pathogens and insects and different biological control strategies for different habitats. Our prioritization of potential biological control candidates for *A. artemisiifolia* is based on evidence of their narrow host range, their feeding niche and control efficacy, availability and suitability to rear, and past experience. This allowed us to identify 23 potential agents, seven of which were given first priority (Table 1).

1) Redistribute insects already established as biological control agents in Europe

The moth *T. candefacta* is well established in Russia but so far considered an ineffective agent in areas with harsh winters. In recent years, however, this moth has increased its distribution range and locally also in abundance (Poltavsky and Artokhin, 2006; Stojanovic et al, 2011), suggesting that *T. candefacta* might more readily establish and be more successful in controlling its host plant in regions with less severe winters. Based on the criteria listed above, we give this species high priority for further studies (Table 1). Prior to considering *T. candefacta* or any other insect tested in Russia (see below) for further relocation or for release in Europe, additional host-specificity tests need to be conducted, in particular with plant species in the family *Asteraceae*. At the time when these insects were released in Russia, the main emphasis of host-specificity tests was placed on crop plants, assuring that the species would not attack cultivated species. Because of its relatively broad host-range, *Ophraella communa* was originally not considered as a high-priority species for the biological control of common ragweed in Europe (Gerber et al. 2011). The accidental establishment of this species in northern Italy and southern Switzerland has, however, generated a lot of interest in better understanding the potential risks and benefits of using *O. communa* for the biological control of common ragweed also in Europe. Within the frame of the COST action SMARTER ("Sustainable management of *Ambrosia artemisiifolia* in Europe"), coordinated research has been initiated to address aspects such as the potential distribution and climate-dependent population dynamics of *O.*

communa in Europe, risks of non-target effects under field conditions and impact on the population dynamics of the target weed.

2) Re-evaluate insect species tested and released in Russia that failed to establish

Three insect species, i.e. *E. bella*, *T. tomentosus* and *Z. disrupta*, were found to be sufficiently specific in host-specificity tests conducted in Russia and were released, but did not establish (Julien and Griffiths, 1998). Additional releases of these insects should be attempted, in particular to establish *T. tomentosus* and *E. bella*, as these species occupy feeding niches neither exploited by native herbivores nor by the two established biological control agents *T. candefacta* and *Z. suturalis* in Russia. Larvae of *E. bella* develop in seeds, thereby directly reducing seed output. *Trigonorhinus tomentosus* feeds as adult and larva on pollen and could directly contribute to reduce pollen load in the air. The third species, *Z. disrupta*, occupies a similar feeding niche as *Z. suturalis*. Additional efforts to establish this species could be considered in case *Z. disrupta* does not display oviposition inhibition on damaged *A. artemisiifolia* as seen for *Z. suturalis*. We rank all these three species as first priority control agents (Table 1).

3) Reconsider species that have been studied but, for different reasons, were never released

Zygogramma tortuosa, originally recorded from *Ambrosia eriocentra*, was introduced for testing in quarantine in Russia, but was rejected because adults also fed on sunflower (reviewed in Goeden and Ricker, 1979). Goeden and Ricker (1979) found however that *Z. tortuosa* did not feed and females did not oviposit on sunflower in open field tests. Furthermore, first instar larvae transferred onto sunflowers were not able to complete their development. *Zygogramma tortuosa* might therefore be reconsidered as a biological control agent, in particular if it does not show a similar oviposition inhibition on damaged *A. artemisiifolia* as *Z. suturalis*. Of the three *Zygogramma* species listed in Table 1, we consider *Z. disrupta* (see above) as the most promising biological control candidate and give *Z. tortuosa* second priority.

Besides the gall forming species *Asphondylia ambrosiae*, three additional cecidomyid flies, *Contarinia partheniicola* and *Rhopalomyia ambrosiae* and the stem mining *Neolasioptera ambrosiae*, have been proposed as potential biological control agents because they are likely to be host specific (Gagné, 1975). Similar to *Asphondylia* larvae, *Neolasioptera* larvae may also rely on symbiontic fungi, while *C. partheniicola* and *R. ambrosiae* are not considered to live in symbiosis with fungi (Skuhravá, pers. com.). However, *C. partheniicola* and *R. ambrosiae* appear to be difficult to collect; despite repeated, intensive surveys in Texas and Florida, *R. ambrosiae* could not be relocated and only small numbers of *C. partheniicola* were found (Goeden and Palmer, 1995). Nevertheless, these Dipteran species may have some potential as biological control agents against *A. artemisiifolia* in Europe (Table 1).

4) Assessment of additional phytophagous organisms recorded on *Ambrosia* species in the native range

The list of organisms recorded from *Ambrosia* species in their native range is long and several species appear to have a narrow host-range and are potentially of interest for biological control (Gerber *et al.*, 2011). However, Goeden and Palmer (1995) cautioned that the knowledge of the host range information on insects associated with *Ambrosiinae* might not prove to be reliable. Based on our prioritization criteria given above, we propose several species associated with *A. artemisiifolia* in its native range to be considered as potential biocontrol agents for *A. artemisiifolia* (Table 1) or potentially any of the other invasive *Ambrosia* species in Europe, such as *Ambrosia trifida*.

Evaluation of invertebrate organisms

The high number of species in the weevil genus *Smicronyx* and the moth genera *Schinia*, *Bucculatrix* and *Epiblema* recorded from *Ambrosia* species (Gerber *et al.*, 2011) may indicate that speciation

has occurred on common ragweed and consequently, narrow host associations can be expected. Furthermore, species in the genera *Epiblema* and *Smicronyx* have been reported to be successful biological control agents against *Parthenium hysterophorus* (McFadyen and Weggler-Beaton, 2000), indicating their potential as biological control agents for *Ambrosia* spp. Of particular interest is the seed-feeding weevil, *Smicronyx perpusillus*, which is only reported from *A. artemisiifolia*, and to which we therefore give first priority (Table 1). Also, the recent establishment of *Epiblema strenuana* in Israel (Yacooby and Seplyarsky, 2011) offers the opportunity to conduct field studies in Israel to assess its usefulness as a biological control agent against common ragweed in Europe.

Two additional presumably monophagous species are the leaf beetle *Ophraella slobodkini* and the moth *Bucculatrix agnella*, both of which feed on leaves. Provided that the European climate is suitable for *O. slobodkini* and that this species is as damaging as its congeneric *O. communa*, it could likely contribute to the control of *A. artemisiifolia* in Europe, using either the classical or the inundative approach (as with *O. communa* in China; see above). We therefore give this species first priority. Previous experiences in biological control of *A. artemisiifolia* indicate that defoliators can be effective in controlling plant populations in the invaded range (see above). *Ophraella slobodkini* is described only from *A. artemisiifolia* from northern Florida, but could also be reared on the closely related *Iva fructescens* L. in the laboratory (Futuyma, 1991). Larval survival was however lower and development time longer than on *A. artemisiifolia*, suggesting that this species is indeed more specific than *O. communa* that was accidentally introduced to China and Japan (see above).

In addition to these three species potentially monophagous on *A. artemisiifolia*, several other insect species are reported on *A. artemisiifolia* but also from other *Ambrosia* species in their native range, including the weevil *Smicronyx tesselatus*, the two dipteran flies *Callachna gibba* and *Euaresta toba* and the two moth species *Schinia rivulosa* and *Tischeria ambrosiaeella* (Table 1). Although not strictly monophagous, these species could possibly be considered as biological control agents against *A. artemisiifolia* if the risk of non-target attack on *A. maritima*, the only native congeneric species in Europe, turns out to be minimal.

Several insect and mite species listed in Gerber *et al.* (2011), including the above-mentioned *E. boyeci*, have been recorded on other *Ambrosia* species, but not on *A. artemisiifolia* under field conditions. For example, various invertebrates associated exclusively with the invasive *A. psilostachya* and *A. trifida* under field conditions might be considered as biological control agents specifically against these invasive species. Some of these herbivores may also have potential as biological control agents against *A. artemisiifolia*, provided that this plant species belongs to their fundamental host-range.

Evaluation of fungal pathogens

The potential of pathogens to impact adversely on A. artemisiifolia and its pollen production was documented during naturally occurring epiphytotics of *Phyllachora ambrosiae* and *Plasmophora halstedii* observed in Hungary in 1999 and 2002 (Kiss *et al.*, 2003; Vajna, 2002; Vajna *et al.*, 2000).

Among the range of fungal pathogens known to attack *Ambrosia* species in their native range (Gerber *et al.*, 2011), the highly damaging rust fungus *Puccinia xanthii* is the most promising candidate for biological control of *A. artemisiifolia*. The rust completes its life cycle on one single host species and while recorded from numerous Asteraceous genera (Hennen *et al.*, 2005), individual rust populations or accessions within *P. xanthii* have shown a high degree of host specialization. For example an accession of *P. xanthii* collected on *A. trifida* in North America showed high specificity to its original host but failed to infect *A. artemisiifolia* and *X. strumarium*; this accession was therefore named *P. xanthii* forma specialis *ambrosiae-trifidae* (Batra, 1981). Similarly, accessions of the rust originating from *Xanthium* species were shown to be non-infectious to *A. artemisiifolia* (Morin *et al.*, 1993, Kiss, 2007). Accessions of *P. xanthii* from *A. artemisiifolia* collected in Texas (USA) in 1989 showed evidence of an equally high host specialization; they proved to be highly pathogenic to an *A. artemisiifolia* biotype from Australia during initial evaluations, while failing to infect *P. hysterophorus* and *Xan-*

thium species (pers. comm. H.C. Evans). The significant impact *Puccinia xanthii* can have on its hosts has been documented from China when a sudden outbreak of *P. xanthii* f. sp. *ambrosiae-trifidae* on *A. trifida* caused serious die-back of infected plants in 2003 (Lu *et al.*, 2004), as well as from Australia where a strain of *P. xanthii* successfully controls a number of highly invasive *Xanthium* species of the Noogoora burr complex (Morin *et al.*, 1996). Based on the documented host specificity of individual *P. xanthii* accessions and their damaging impact we give this rust first priority. Doubts have been cast on the potential of *P. xanthii* as a biocontrol agent for *A. artemisiifolia* based on a lack of disease incidence following unsuccessful attempts to collect the rust on this host in North America in 2002 and 2003. However, these latest surveys included neither the region in Texas where the most recent collections of this rust strain were made nor the majority of other sites where previous herbarium material had been collected (Kiss, 2007). Moreover, scarcity in the native range does not preclude a fungal pathogen from becoming a successful biocontrol agent (e.g. Trujillo, 2005).

The documented host range of *Septoria ambrosiicola* and *S. epambrosiae* as well as of *Passalora ambrosiae* (synonym *Cercospora ambrosiae*) and *Passalora trifidae* (Chupp) U. Braun & Crous (synonym *Cercospora trifidae* Chupp, 1949) is restricted to the genus *Ambrosia* (Gerber *et al.*, 2011). As stated for the invertebrate candidates, these fungal pathogens could be considered for biological control if the risk of damage to *A. maritima*, the only European native congeneric species, was assessed as minimal. Based on this uncertainty as well as a lack of data about the impact of the two *Septoria* and *Passalora* species on their *Ambrosia* hosts in the native range we give them second priority. However, *Septoria* as well as *Cercospora* species have previously been evaluated and used against a number of invasive weed species and, in the case of *Septoria passiflorae*, applied inundatively to control Banana Poka Vine, y tripartita var. y, in Hawaii (Charudattan *et al.*1985; Julien and Griffiths, 1998; Trujillo *et al.*, 2001).

5) New surveys in source regions matching specific European conditions

We expect that further explorations of the natural enemy complexes associated with *A. artemisiifolia* or closely related species will reveal new candidate species, or biotypes of known species (Gerber *et al.*, 2011), for the biological control of *A. artemisiifolia* in Europe.

Most biological control agents for *A. artemisiifolia* and *A. trifida* have so far been collected in the eastern United States and Canada, where both ragweed species occur. However, the genus *Ambrosia* covers a much larger geographical area, including different climatic zones. Targeting regions with climatic conditions comparable to those in the invaded range in Europe increases the chances that biological control agents will establish and persist. The richest source of natural enemies is probably the Sonoran desert region (i.e. in the south-western United States and northern Mexico), the centre of origin and diversification of the genus *Ambrosia* (Harris and Piper, 1970). Surveys for phytophagous or pathogenic organisms in the Sonoran Desert have so far mainly been restricted to the state of California and large areas remain unexplored (Goeden and Palmer, 1995). Natural enemies from the Sonoran desert itself might be well pre-adapted to warmer climates in Sub-Mediterranean Europe, e.g. the Rhone Valley, Northern Italy and some parts of the Balkans. These organisms are, however, unlikely to become adapted to more temperate or continental areas, except if they are collected at high elevations. The most likely regions to harbour cold adapted specialized herbivore species are the mountains of Mexico adjacent to the Sonoran desert (Harris and Piper, 1970) and/or areas at higher elevation in the northern part of Mexico (Bohar and Vajna, 1996). Due to their eco-geographical separation from the southern parts of the United States because of the Sonoran desert, different organisms are likely to have evolved in these mountain ranges.

Early on in the history of biological control of *Ambrosia* species, mountain regions of South America were also highlighted as a potential source for climatically adapted phytophagous species for Canada and Europe (Harris and Piper, 1970). These regions are likely to have different natural enemy complexes because they are isolated from the Mexican mountain range by a tropical region. The presence of several *Ambrosia* species in mountain regions of South America originates from a phy-

logenetically early invasion, indicating that the genus might have been present there long enough to acquire specialist phytophages originating from the local fauna (Harris and Piper, 1970). Despite these recommendations by Harris and Piper (1970), few surveys have been conducted and only little information is available on species associated with *Ambrosia* in South America. In 1975-76, McFadyen (1976) conducted limited surveys on insects associated with *A. tenuifolia* (later attributed to *A. eliator*, an accepted synonym of *A. artemisiifolia*) in northern Argentina and reported several potentially specific insect species from this area. Besides the *Liothrips* species mentioned above, two stem mining beetles (*Curculionidae* and *Cerambycidae*) were sent to a quarantine facility in Canada, but the species entered diapause from which they failed to emerge and no host-specificity tests could be conducted (Maw, 1981). The weevil *Conotrachelus albocinereus* Fiedler (*Coleoptera*, *Curculionidae*) which was collected from *A. elatior* in Argentina, was released in Australia as a biological control agent of *Parthenium hysterophorus* and has proven to be highly damaging to his weed (R. McFadyen, pers. comm.). Recent collections in warm temperate, mountainous areas of southern Brazil have revealed new pathogen records on *A. artemisiifolia* (H.C. Evans, pers. comm.), confirming the recommendations made by Harris and Piper (1970).

Outlook

Herbicides and mechanical control (uprooting, cutting, ploughing) are well suited as local and short-term measures to eradicate initial and small populations and to reduce yield losses in crops. However, these control methods largely remain limited to well-managed habitat types with the main focus to protect crop yield. Yet, a large part of land infested by common ragweed in Europe is non-crop land such as riverbeds, roadsides and field borders, on which eradication of ragweed using herbicides is too expensive and/or prohibited. Additionally, the need to protect the accompanying vegetation, especially in sensitive ecosystems, does not allow large-scale application of herbicides. We therefore propose that sustainable control strategies to mitigate *Ambrosia's* further spread into areas not yet invaded and to reduce its abundance in badly infested areas in Europe need to be based on a wider combination of methods, including biological control.

With regard to biological control interventions, we see a two-forked strategy. Firstly, a classical approach for the widespread and highly infested non-crop areas such as grassland, wasteland, roadsides and riverbanks using mainly agents that reduce flowering, pollen production and seed set. A number of herbivores and pathogens associated with *A. artemisiifolia* in its native range are likely to have a very narrow host-range that is either restricted to the target species itself or to a few species within the genus *Ambrosia*. Gerber *et al.* (2011) have identified 18 insect and 5 fungal pathogens to be promising candidates for a classical biological control approach (Table 1), and the recent establishment of *O. communa* in southern Europe warrants detailed investigations also of this species, although it was originally not prioritized for classical biological control of common ragweed in Europe. Secondly, an inundative approach will be necessary for crop fields that suffer from ragweed infestations. Candidate biological control agents for mass-rearing and repeated releases against ragweed in Europe are, similar to *O. communa* in China (Zhou *et al.*, 2009), the defoliator *Ophraella slobodkini* or the fungus *S. epambrosiae* (Table 1).

Based on its history of at least partially successful biological control attempts against exotic *Ambrosia*, we argue that biological control as part of an integrated management approach (Müller-Schärer *et al.*, 2000; Müller-Schärer, 2002) will likely be needed to produce acceptable levels of overall ragweed control across different habitats in Europe. To promote such a European-wide integrated management of common ragweed, a COST action named 'SMARTER' was recently launched. The objectives of the COST Action are to: (a) make available a forum for discussing innovative long-term options for managing and monitoring ragweed; (b) train, educate and motivate skilled young scientists to work on invasive species management to meet increased demands of the society for experts on this issue; (c) to identify knowledge gaps hindering the sustainable integrated management of ragweed and promote new research to fill these gaps, (d) to develop site- and country-specific recommendations for ragweed management and promote their implementation, and (e) develop a

common vision for interdisciplinary collaboration in research and monitoring of IAS, especially ragweed. Numerous scientists collaborating in the HALT Ambrosia project are also actively involved in this new action, and the findings generated in the HALT Ambrosia project will be of key relevance when developing habitat-specific recommendations for ragweed management in Europe.

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Table 1   Host-range, prioritization and management approach suggested for biological control candidates against Ambrosia artemisiifolia in Europe (see text for details; adapted from Gerber et al. 2011).

Taxon	Host range [a]		Biosafety / Feasibility	Priority for Europe	Management Approach
	Field observations	Experimental studies			
INSECTA					
COLEOPTERA					
Ophraella communa LeSage	*Ambrosia, Iva, Parthenium, Xanthium, Ratibida*	Heliantheae	Attack of sunflower/ Jerusalem artichoke?	2	Classical / inundative?
Ophraella slobodkini Futuyma	AMBEL	AMBEL, Ivaf		1	Classical / Inundative?
Smicronyx perpusillus Casey	AMBEL	?		1	Classical
Smicronyx tesselatus Dietz	AMBEL, *Ambrosia*	?	attack of *A. maritima*?	2	Classical
Trigonorhinus tomentosus (Say)[b]	*Acha*, FRSCO, *Ache*, AMBDU, AMBER	AMBEL[d]	attack of *A. maritima*? establishment?	1	Classical
Zygogramma bicolorata Pallister[c]	AMBEL, *Parthenium*	?	attack of *A. maritima*?	2	Classical
Zygogramma disrupta Rogers[b]	AMBEL	AMBEL[d]	establishment?	1	Classical
Zygogramma tortuosa Rogers[b]	AMBER	*Ambrosia*	attack of *A. maritima*?	2	Classical
DIPTERA					
Callachna gibba (Loew)	AMBEL, AMBPS	?	attack of *A. maritima*?	2	Classical
Contarinia partheniicola (Cockerell)	*Acha*, FRSCO, AMBDU, AMBER, AMBPS, Pinc	?	rare in native range?	2	Classical
Euaresta bella (Loew)[b]	AMBEL	AMBEL[d]	establishment?	1	Classical
Euaresta toba (Lindner)	AMBEL, AMBCU, AMBTE	?	attack of *A. maritima*?	2	Classical
Rhopalomyia ambrosiae Gagne	AMBEL, AMBPS	?	rare in native range?	2	Classical
HEMIPTERA					
Stobaera concinna Stal[c]	AMBEL, *Parthenium*	?	attack of *A. maritima*?	2	Classical
LEPIDOPTERA					
Adania ambrosiae Murtfeldt	FRSAC, AMBEL, *Acha*, AMBER, AMBPS	?	attack of *A. maritima*?	2	Classical
Bucculatrix agnella Chambers	AMBEL	?	attack of *A. maritima*?	2	Classical
Schinia rivulosa Guenée	AMBEL, AMBPS, *Ambrosia*	?	attack of *A. maritima*?	2	Classical
Tarachidia candefacta[b] (*Ponometia candefacta* Hübner)	AMBEL, FRSCO, AMBPS	AMBEL[e]	attack of *A. maritima*?	1	Classical
Tischeria ambrosiaeella Chambers	AMBEL, AMBTE	?	attack of *A. maritima*?	2	Classical

FUNGI

ASCOMYCOTA

DOTHIDEOMYCETES

CAPNODIALES

Mycosphaer-ellaceae				
Septoria ambrosiicola Speg. 1910	*Ambrosia*	attack of *A. maritima?*	2	Classical / Inundative?
S. epambro-siae D.F. Farr 2001	*Ambrosia*	attack of *A. maritima?*	2	Classical / Inundative?
Passalora ambrosiae (Chupp) Crous & U. Braun 2001	*Ambrosia*	attack of *A. maritima?*	2	Classical
Passalora tri-fidae (Chupp) U. Braun & Crous 2003	*Ambrosia*	attack of *A. maritima?*	2	Classical

BASIDIOMYCOTA

PUCCINIOMYCETES

PUCCINIALES

P u c c i n i - aceae

Puccinia xan-thii Schwein. 1822			1	Classical

^a Plant species: **EPPO (Bayer) codes used when available (see** http://eppt.eppo.org/index.php**);** FRSAC: *A. acanthicarpa*; AMBEL: *A. artemisiifolia*; Acha: *A. chamissonis*; FRSCO: *A. confertiflora*; Ache: *A. chenopodiifolia*; AMBCU: *A. cuma-nensis*; AMBDU: *A. dumosa*; AMBDE: *A. deltoideae*; AMBER: *A. eriocentra*; AMBPS: *A. psilos-tachya* (now *A. coronopifolia*); AMBTE: *A. tenuifolia;* Ivaf: *Iva frutescens*; Pinc: *Parthenium incanum.*

^b tested as classical biological control agent against *A. artemisiifolia*.

^c released as classical biological control agent against *P. hysterophorus*.

^d according to tests conducted in Russia but no access to data.

^e according to tests conducted in Russia (Kovalev 1971b)