ENVIRONMENTAL RISK ASSESSMENT FOR *NEODRYINUS TYPHLOCYBAE*, BIOLOGICAL CONTROL AGENT AGAINST *METCALFA PRUINOSA*, FOR AUSTRIA

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ABSTRACT

The potential environmental risks of *Neodryinus typhlocybae*, a parasitic wasp from North America, were evaluated with regard to its safe use as an exotic biocontrol agent for the planthopper *Metcalfa pruinosa* in Austria. Following an earlier host range study of *N. typhlocybae* conducted in the laboratory, the present study assessed the potential for establishment and spread as well as negative indirect effects on non-target organisms. The potential release sites in Austria were analysed for matching of the climatic requirements for establishment of *N. typhlocybae*. The two proposed release locations, Vienna and Graz, have a predominantly similar climate to the parasitoid's region of origin, though the comparably cooler mean summer temperatures might result in a low emergence rate of the partial second generation. The natural spread potential of *N. typhlocybae* was reviewed and is considered to be sufficiently good for released individuals to reach nearby sites infested with *M. pruinosa*. However, a perceptible spreading of *N. typhlocybae* females only occurs a few years after release and seems to be strongly dependent on the host density. *Gelis areator*, a hyperparasitoid of *N. typhlocybae* known to occur in Austria, might have negative effects on the population of the beneficial organism. Advantages and disadvantages of chemical and biological control methods against *M. pruinosa* were evaluated. It is concluded that *N. typhlocybae* is very well suited as a biological control agent for *M. pruinosa* in Austria, as no adverse effects on non-target species are expected but its release offers advantages with regard to sustainable and environmentally friendly pest management.

Keywords: classical biological control, environmental impact, harmful effects, *Metcalfa pruinosa*, *Neodryinus typhlocybae*, parasitoid

Introduction

Since the North American planthopper *Metcalfa pruinosa* (Say 1830) (Hemiptera: Flatidae) was first reported from northern Italy in 1979, it has spread into new areas of the Mediterranean and is now widely distributed in several European countries (Nicoli 1997; Strauss 2009). This polyphagous planthopper species has become a serious pest in agriculture and public green space by forming dense populations which subsequently cause severe damage in orchards, vineyards, ornamentals and urban areas (Zangheri and Donadini 1980; Girolami and Camporese 1994). Large infestations of *M. pruinosa* may weaken the host plants by excessive phloem sucking and honeydew production which supports the growth of sooty moulds on affected plants, resulting in reduced fruit quality and leaf photosynthesis but also in quality damage in ornamentals.

Native natural enemies in the Mediterranean region have not effectively controlled *M. pruinosa* so far (Greatti et al. 1994). The import and release of an exotic natural enemy from the area of origin of *M. pruinosa* was considered a promising control strategy. The parasitic wasp *Neodryinus typhlocybae* (Ashmead 1893) (Hymenoptera: Dryinidae) has therefore been released in some European countries for the control of mass occurrences of *M. pruinosa* (Girolami and Camporese 1994; Ciglar et al. 1998; Tommasini et al. 1998; Malausa 2000; Žežlina et al. 2001; Anagnou-Veroniki et al. 2008; DAISIE 2008).

In Austria, the distribution and phenology of *M. pruinosa* in Vienna has been monitored since 2003, as populations of *M. pruinosa* have also established in urban areas, private gardens and public green spaces in Vienna and Graz. Based on this monitoring, it is assumed that this planthopper is likely to spread locally in the short term and occupy a great part of the area suitable for establishment if no plant protection measures are taken (Kahrer et al. 2009; Strauss 2010). Release of *N. typhlocybae* is therefore also being considered in Austria to prevent further spread and economic impact of the pest.

The authorization as a plant protection product (PPP) for the import and release of a biological control agent (BCA) is a compulsory requirement in Austria, in a similar way to conventional plant protection products (Pflanzenschutzmittelgesetz 2011). For the authorization of biological control agents as PPP, comprehensive information on the environmental effects of the specific organism is necessary.

Consequently, with regard to the safe use of *N. typhlocybae* as a biocontrol agent in Austria an environmental risk assessment (ERA) was performed based on international standards (EPPO 2000; OECD 2004; FAO 2005). In a first step, the host specificity of *N. typhlocybae* was investigated in laboratory host range tests with respect to non-target plant- and leafhopper species in Austria (Strauss 2009). The results of these tests support the assumption that *N. typhlocybae* is host specific to species of the family Flatidae, of which only the introduced pest species occurs in Austria. *N. typhlocybae* was also used as a model organism to check if the existing international regulatory standards for the import and release of BCAs are useful for assessing the environmental risks of parasitoids.

The objectives of the present study were to assess (1) the potential for the establishment and (2) spread of *N. typhlocybae* in two locations in Austria with *M. pruinosa* infestation, as well as (3) its potential negative indirect impacts on native predators, (4) the negative effect of naturally occurring hyperparasitoids on *N. typhlocybae,* and finally (5) to provide the competent national authority with an overall recommendation as to whether the release of *N. typhlocybae* would pose an unacceptable risk to the environment.

Material and Methods

This environmental risk assessment was compiled on the basis of regulatory documents produced by international organizations on the import and release of candidate invertebrate biological control agents (IBCAs) (EPPO 2000; OECD 2004; FAO 2005). Data from scientific literature, meeting reports, records from insect collections, personal information from insect taxonomists and various internet databases on biogeography for the relevant species (Nearctica 1998; Fauna Europaea 2004; DAISIE 2008) and climate (World Climate 1996; Zentralanstalt für Meteorologie und Geodynamik 2002) were used to determine the following risk factors for *N. typhlocybae* in Austria, as proposed by van Lenteren et al. (2006): the potential of establishment and spread, the host range and direct and indirect effects on non-target organisms.

The objective of introducing *N. typhlocybae* into Austria is to establish self-sustaining and multiplying populations on *M. pruinosa* infestation sites in Vienna (48°14′N 16°21′E) and Graz (47°5′N 15°27′E). The release of *N. typhlocybae* in Austria would represent the northernmost release in the field in Europe. Therefore, the climate of the proposed release sites, Vienna and Graz, was compared to that of the original area of *N. typhlocybae* in North America. In Austria, the *N. typhlocybae* strain originally deriving from Connecticut, USA would be released. For this purpose, the progression of the mean daily temperature in the reference locations New Haven (Connecticut, USA) and Toronto (Ontario, Canada) was compared with that in Vienna and Graz to see if deviations exist between the locations. Toronto was chosen because it is one of the northernmost locations in North America where *N. typhlocybae* occurs. Distribution data for *N. typhlocybae* in North America and Europe were used to create distribution maps using ArcMAP (ESRI® ArcMAP™ Version 10.0).

Results

Probability of establishment

The purpose of this section is to analyse climatic similarities or differences between the native area and the area of intended release of *N. typhlocybae* that could affect its establishment. In North America, the geographical distribution of *N. typhlocybae* occurs across a broad climatic area, from southern Ontario, Canada, to almost all eastern states of the USA. The parasitoid has a local occurrence in Arizona (Cochise County only) and Mexico (Baja California Sur) (Fig. 1).

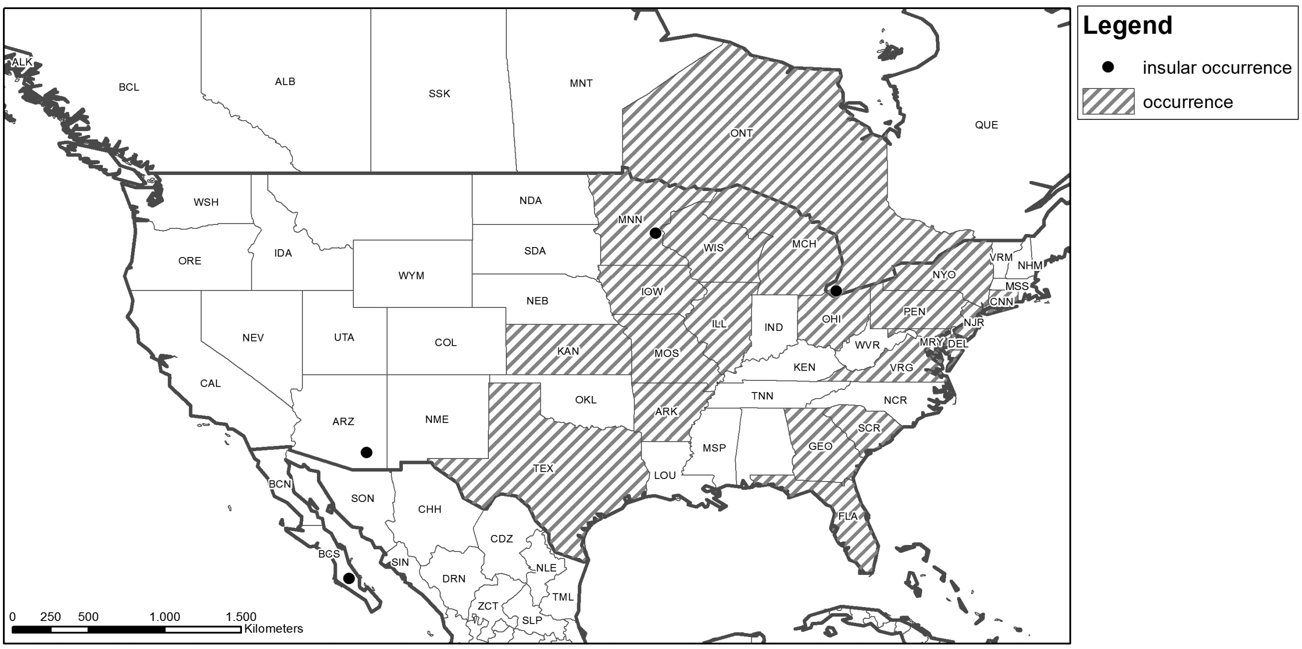

Fig. 1 Natural distribution of *Neodryinus typhlocybae* in North America. Shaded areas indicate occurrence on a state level and not actual territory occupied by this species.

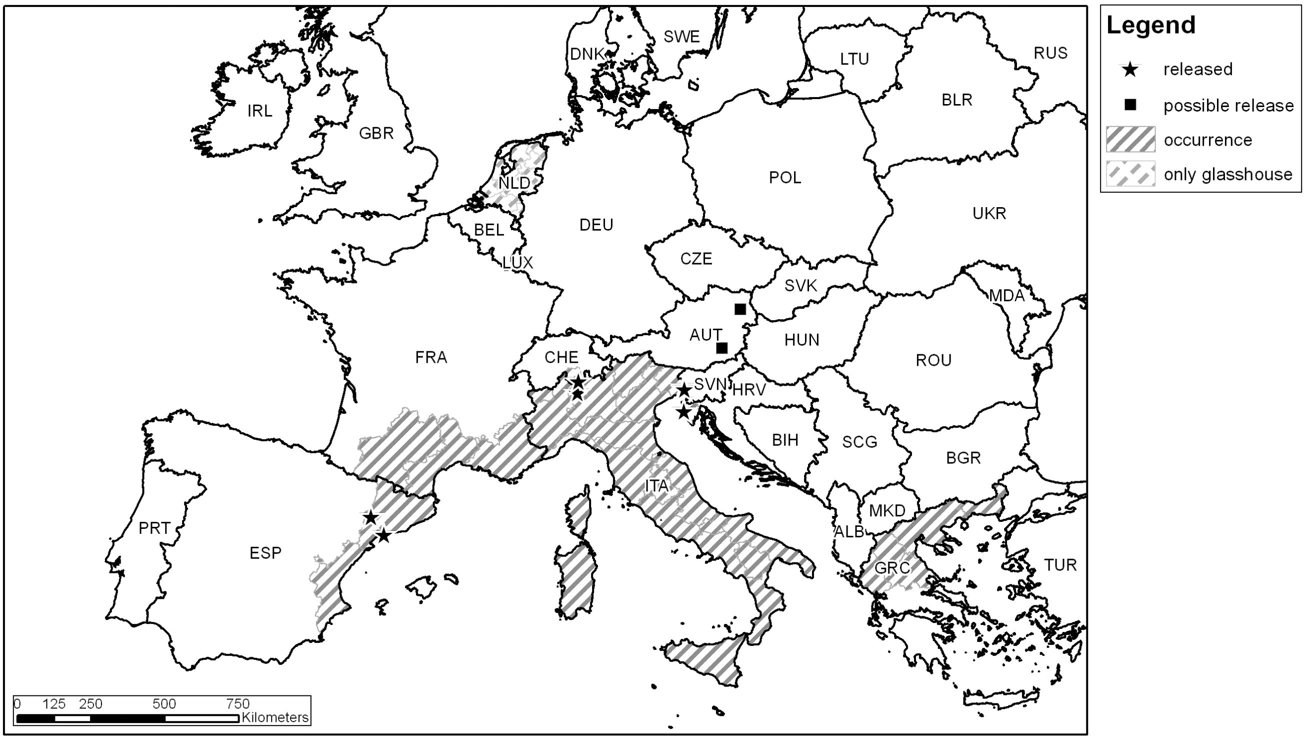

Fig. 2 European countries where *Neodryinus typhlocybae* has been released as a biocontrol agent for the introduced planthopper *Metcalfa pruinosa*. Shaded areas indicate occurrence on a national or regional level and not actual territory occupied by this species.

Since 1987, *N. typhlocybae* cocoons have repeatedly been collected in several states in the USA and introduced into Italy. *Neodryinus typhlocybae* populations released in Europe originate from Connecticut (Girolami and Mazzon 1999). Further releases have been undertaken in the canton of Ticino (Coldrerio and Carasso) in Switzerland (Jermini et al. 2000), in western Slovenia close to the Italian border (Nova Gorica and Volčja Draga) (Žežlina et al. 2001), in Istria in Croatia (Poreč) (Ciglar et al. 1998; B. Barić, pers. comm.), and in southern France (Malausa et al. 2003), as well as in Catalonia and Valencia in Spain (A. Soto Sanchez, pers. comm.) (Fig. 2).

In the classical biological control strategy, permanent establishment of the non-native organism in the new environment is an essential pre-requisite for a successful pest management (Bale 2011). In Austria, it is desired that a stable population of *N. typhlocybae* be established in the areas infested by *M. pruinosa* and reduce the latter's density. Information on the natural range of distribution of *N. typhlocybae* in North America and in the release areas in Europe was summarised to determine its climatic tolerance (Fig. 3).

The two Austrian release sites for *N. typhlocybae* are not only located further north than the previous release areas in Europe, they are even further north than the northernmost occurrence of *N. typhlocybae* in North America. In moderate climatic zones, *N. typhlocybae* adults emerge from the middle of June, parasitise *M. pruinosa* larvae and build cocoons by the end of June. The partial second generation emerges from these cocoons in July and August (Lucchi and Wilson 2003; Alma et al. 2005). Thus, the climate from June to August of these two novel sites was assessed to see whether it matches the climatic requirements of the exotic parasitoid for establishment, as it is known that temperature is an important factor influencing the efficacy of wasps as biological control agents (Wajnberg et al. 2007). The mean daily temperatures of Vienna, Graz, New Haven and Toronto were compared in order to analyse the climatic deviation with respect to the developmental season of *N. typhlocybae*. As far as is known from the literature, *N. typhlocybae* has its northernmost occurrence near Toronto (43°70′N, 79°40′W), Canada (Steve Paiero, pers. comm.).

Graz has the lowest mean daily temperature from the middle of June until the middle of October (Fig. 3), with 1.3 °C and 2.6 °C lower in July than Toronto and New Haven, respectively. This might have negative implications for the emergence rate of the second incomplete generation of *N. typhlocybae*. Because of the cooler summer temperatures, a greater proportion of the second generation could go into diapause and emerge only in the following year. The second generation, however, has an important role in the spreading of the beneficial insect (see probability of spread). In Vienna, the average daily temperature during the developmental period of the wasp is one to two degrees Celsius below the temperature of New Haven, but similar to the temperature of Toronto, where *N. typhlocybae* is established.

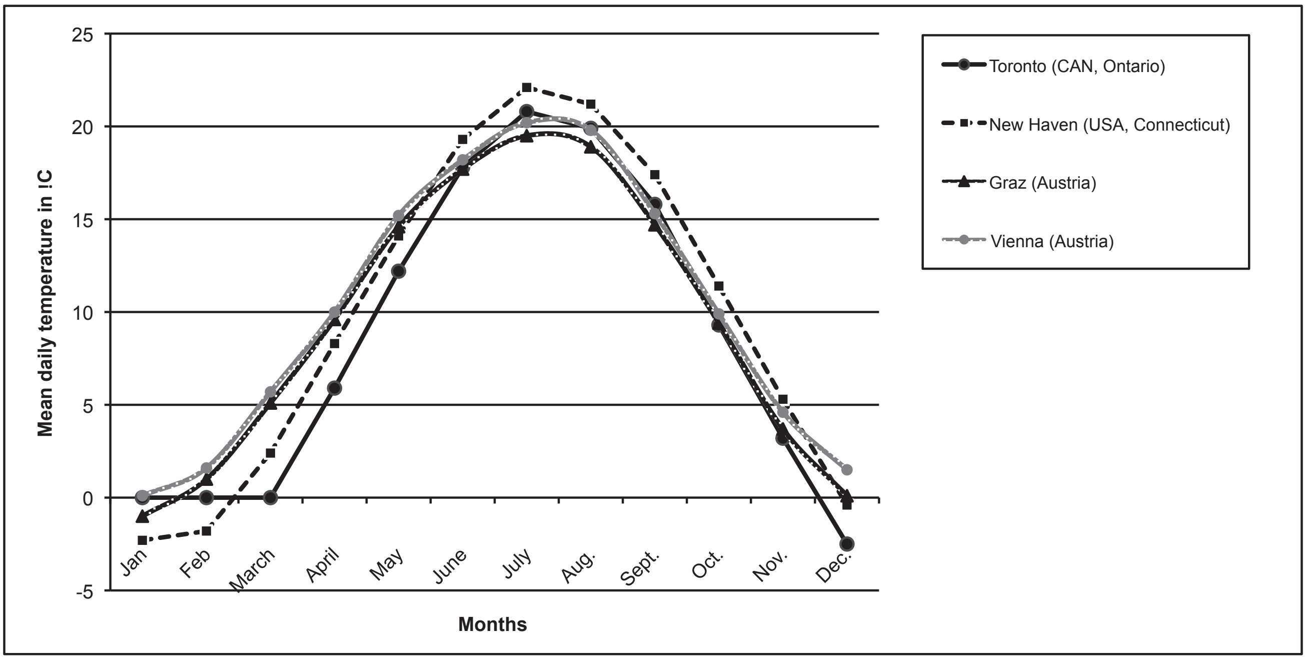

Fig. 3 Comparison of the mean daily temperature in Vienna and Graz, two prospective release sites in Austria, with locations in North America where *Neodryinus typhlocybae* is established. Vienna (latitude: 48°14´N, longitude: 16°21´E), Graz (latitude: 47°5´N, longitude: 15°27´E) (Source: ZAMG (2007), period 1971–2000), New Haven (latitude: 41°30´N, longitude: 72°90´W) (Source: www.worldclimate.com, period: 1781–1970) and Toronto (latitude: 43°70´N, longitude: 79°40´W) (Source: www.worldclimate.com, period: 1840–1990).

Probability of spread

For classical control agents high dispersal ability is important to ensure that the beneficial organism becomes well distributed within the release area, and to reduce the number of release points per area and hence costs (McDougall and Mills 1997; Wright et al. 2001). The scientific literature on the spread potential of *N. typhlocybae* in Italy, France and Slovenia was reviewed with regard to the dispersal rate per year and important influencing factors such as habitat structure and anthropogenic influence to make predictions for the dispersal of *N. typhlocybae* in the target regions in Austria. It is important to determine the potential for dispersal of the parasitoid in order to evaluate the probability of temporal and spatial encounter between the biocontrol agent and the pest species.

Generally, spread of *N. typhlocybae* occurs by flying and is facilitated by host population size, the occurrence of the two generations per year, contiguous vegetation and the longevity of females of ten days (Girolami and Mazzon 1999). Furthermore, *N. typhlocybae* could also be spread indirectly via parasitised larvae of *M. pruinosa*, by the transport of plants with cocoons by humans and via wind transport of leaves with cocoons.

The results on the dispersal potential of *N. typhlocybae* in the literature differ and range from 30 m to 20 km per year (Girolami et al. 1996; Girolami and Mazzon 1999). In northern Italy, *N. typhlocybae* spread 750–2500 m in 6–18 months after release (Cenderello 2006). In general, the spread potential of *N. typhlocybae* is sufficiently good to reach and colonize other locations with *M. pruinosa*, but host density apparently influences the movement of *N. typhlocybae*. In Italy, Girolami and Mazzon (1999) reported a sudden and rapid increase of *N. typhlocybae* in the area of Padova-Legnaro (Veneto region, Northern Italy), which spread up to 10 km in one year. In the years before, the rate of spread was very low, reaching just a few hundred meters in 3–4 years. These authors and also Malausa et al. (2003) concluded that *N. typhlocybae* females stayed at the release sites as long as the host density was sufficiently high, but spread actively when host density was declining and were then found several hundred meters away from the release points.

Neodryinus typhlocybae has a partial second generation, from adults emerging in July/August and parasitizing the remaining *M. pruinosa* larvae. Wasp larvae of this generation overwinter in the cocoon (Girolami et al. 1996). By the beginning of August, however, hardly any larvae of *M. pruinosa* remain, but mainly the adults are present. In the USA, *N. typhlocybae* reproduces on three flatid species that have somewhat different development times and phenologies (Wilson and McPherson 1981). Thus, the appropriate host stages for oviposition are present for female wasps of the second generation and the life cycle is not interrupted. It is assumed that the adults of the second generation of *N. typhlocybae* spread over greater distances because females need to search for new host patches with the appropriate larval stages for reproduction, the host larvae numbers already having decreased by then. In North America, the percentage of the emerging second generation varies from 20–80%, depending on the climatic region. Parasitoid populations originating from Texas have a higher percentage of bivoltism than populations descending from Connecticut (Tommasini et al. 1998). The average percentage of bivoltism of *N. typhlocybae* in Italy and France, which have a warmer climate than Austria, is about 30–40% (Malausa 2000; Girolami and Mazzon 2001). For example, the mean daily temperature in Udine/Campoformido (latitude: 46°N, longitude: 13°10′E, source: World Climate, period: 1803–1991) in June and July is 2.8 °C and 3.1 °C higher than in Graz. For Austria, it is expected that a smaller portion of the second generation of *N. typhlocybae* will emerge due to the cooler climate, and that spread will be slower.

For the successful spread of *N. typhlocybae* the habitat structure plays an important role. The widespread presence of green areas, gardens and hedges aids the expansion of population of *N. typhlocybae* (Girolami and Mazzon 1999). Spread of *N. typhlocybae* to host-free places has no relevance, since establishment would be impossible as no other host species occur in Austria (Strauss 2009).

Negative indirect effects on the native fauna

Introduced exotic natural enemies may negatively affect the abundance of native predators or parasitoids that use the same prey or host (Messing et al. 2006).

Various naturally occurring generalist predators have been observed to prey upon *M. pruinosa*, particularly Coccinellidae (Coleoptera) e.g. *Coccinella septempunctata* (L., 1758), Miridae (Hemiptera) and Chrysopidae (Neuroptera), as well as various bird species (Barbattini et al. 1991; Greatti et al. 1994). As these native predators consume small numbers of *M. pruinosa* individuals and do not depend on them as their only source of prey, competition with or displacement of these species through the introduction of *N. typhlocybae* is very unlikely (Zandigiacomo and Villani 2003).

Risk/benefit analysis of the use of *N. typhlocybae*

The last step of a comprehensive environmental risk assessment is the comparison of the risk/benefit of the biocontrol method with other pest management methods (van Lenteren et al. 2008). Small populations of young instars of *M. pruinosa* can be controlled with pesticides in production sites as well as in public and private green spaces, but several pesticide treatments per season are required because of the prolonged hatching period (Malumphy et al. 1994). For the control of sucking insects and leafhoppers in viticulture and orchards in Austria in 2011, Imidacloprid (Confidor 70 WG, Reg. No. 2602), Fenpyroximat (Samba K, Reg. No. 2762), Chlorpyrifos-methyl (Reldan 2E, Reg. No. 2225) and Indoxacarb (Steward, Reg. No. 2737) are approved. In Vienna, two bigger infestation sites (51,000 m^2 and 3500 m^2) and three smaller sites (~200–500 m^2) have been detected since 2003 (Kahrer et al. 2009). Costs of insecticide treatment were considered low and amounted to a maximum of 70 Euro/ha for one application. In the long term, however, more working hours would be needed for chemical control than for biological control due to the necessity of repeated applications, and the costs of equipment and amortisation also have to be considered. Furthermore, no pesticide application is permitted for containment in other sites of potential occurrence of *M. pruinosa*, such as natural areas, which may serve as over-wintering sites from which it may infest nearby cultivated land. Thus, a major disadvantage of insecticide treatment is that pesticide treatment would not control the pest completely in all infestation areas and no long-term control would be achieved.

Additionally, insecticides could negatively affect non-target arthropods such as honeybees which collect honeydew excretions of *M. pruinosa* (Greatti and Barbattini 2003). Imidacloprid and Chlorpyrifos-methyl are toxic to honeybees and their application is not allowed if honeybees are present; Fenpyroximat and Indoxacarb are toxic to beneficials, e.g. Ichneumonidae (Suchail et al 2000; Bundesamt für Ernährungssicherheit 2012).

Releases of *N. typhlocybae* in Vienna could be considered for three of the five infested sites, because the other two sites are close enough (up to 3 km away) for the biocontrol agent to colonize them by natural spread. A relatively small population of about 200 *N. typhlocybae* cocoons per site is assumed to be sufficient for the release and control of *M. pruinosa*, based on the experience in Slovenia where *N. typhlocybae* established successfully (Žežlina et al. 2001).

Classical biological control of *M. pruinosa* with *N. typhlocybae* is considered to be economically feasible in terms of material costs, application and working hours in the long term. Additionally an evaluation of effectiveness for chemical and biological control would be needed. Alma et al. (2005) developed a sampling plan to estimate *N. typhlocybae* cocoon density and the impact on *M. pruinosa*, which can be applied easily in the field. The cost for a post-release evaluation of *N. typhlocybae*'s parasitisation rate and of assessing efficacy of the insecticide treatment is considered to be comparable, therefore. In Italy, France and Slovenia, the release of *N. typhlocybae* led to a high parasitisation rate (from below 50% to up to 80%) and thus to a considerable reduction of the pest and damage (Visentini 1998; Girolami and Mazzon 1999). Different authors frequently report that crop damage has declined to below the economic damage threshold after release of the beneficial insect (Malausa 2000; Žežlina et al. 2001; Chapelle et al. 2002; A. Soto Sánchez pers. comm.), albeit without stating exact figures about the size of the reduction.

Negative effects on *N. typhlocybae*

The main interaction factors resulting in negative effects in biological control programmes are hyperparasitism, predation of cocoons and cultivation practices. High levels of hyperparasitism can lead to a strong decrease in the population of the primary parasitoid and thus to a reduced level of pest control (Höller et al. 1993). In Europe, *N. typhlocybae* is known to be parasitised by the following four parasitic wasp species: *Cheiloneurus boldyrevi* (Trjapitzin and Agekyan 1978) (Hymenoptera: Encyrtidae), *Gelis areator* (Panzer 1804) (Hymenoptera: Ichneumonidae), *Callitula bicolor* (Spinola 1811) and *Pachyneuron muscarum* (L., 1758) (Hymenoptera: Pteromalidae) (Girolami et al. 1996; Villani and Zandigiacomo 1999; Olmi 2000; Viggiani et al. 2004). Of these, only *G. areator* occurs in Austria (Fauna Europaea 2004). As *G. areator* also parasitises the key grapevine pest *Lobesia botrana* (Denis and Schiffermüller 1776) (Lepidoptera: Tortricidae), biological control of *M. pruinosa* with *N. typhlocybae* in vineyards could be negatively influenced through the hyperparasitisation of *N. typhlocybae* by *G. areator*. Furthermore, the cocoons of *N. typhlocybae* on autumn foliage on the ground are particularly at risk of being eaten by insectivorous birds, rodents and entomophagous insects. Tommasini et al. (1998) reported that 48–60% of the cocoons on the ground were eaten. Also human interaction such as chemical weed control or removal of autumn leaves with the overwintering cocoons of *N. typhlocybae* on them could negatively affect the population of *N. typhlocybae* and reduce the biocontrol effect on *M. pruinosa*.

Discussion

In the course of the literature study for the environmental risk assessment for *N. typhlocybae* it was noticed that, with just a few exceptions, potential risks of the release of exotic invertebrates for the control of pests have not been routinely assessed in pre-release evaluations, and that screening information on the impact on non-target organisms is required in only a few countries (van Lenteren et al. 2006; Stewart et al. 2007; Bale 2011). To mitigate the risk associated with the introduction of exotic natural enemies of plant pests in a new geographic region, the threat to the native fauna, in particular, needs to be assessed before a new species is released (Hoddle 2004; Bélanger and Lucas 2011; Maisonhaute and Lucas 2011).

In Europe, the regulation of import and release of invertebrate biological control agents (IBCA) has not yet been harmonised (Bigler et al. 2005; Bale 2011). Indeed, although environmental risk assessment is an emerging issue for both pest and biological control organisms, the new EC regulation concerning the placing of plant protection products on the market (Regulation (EC) No. 1107/2009) contain no advice on conducting environmental risk assessments for biological control agents.

The regulation of introduction and release of IBCA is within the remit of national authorities and differs between European countries, some of which have yet to establish guidelines and procedures (Bigler et al. 2005; van Lenteren and Loomans 2006).

The international standards (EPPO, FAO, OECD) concerning the safe import and release of IBCAs, which are currently used as a basis for conducting the environmental risk assessment for *N. typhlocybae*, are in agreement on the key information necessary to assess the risk of IBCAs. Some differences exist with regard to the recommendations to carry out a pest risk analysis of the biological control agent prior to release and develop emergency action plans in case the BCA displays adverse properties, as well as concerning the detailed description of the required information.

For the environmental risk assessment for *N. typhlocybae* it was first necessary to determine the host range of the parasitoid to corroborate the existing information on its biology. In Europe, *N. typhlocybae* depends on the presence of its host species *M. pruinosa*, whereas three other flatid host species are known in North America (Guglielmino and Olmi 1997). *Neodryinus typhlocybae* has a narrow host range and will only attack the target species, as this is the sole host species for the parasitoid present in Austria (Strauss 2009). In Austria, several inoculative releases of *N. typhlocybae* would initially be necessary at sites heavily infested with *M. pruinosa* in Vienna and Graz. It is expected that *N. typhlocybae* would spread to surrounding infestation sites by natural means after a few years. As a consequence of the comparably cooler summer temperatures in Vienna and Graz, however, it is assumed that a smaller portion of the second generation of *N. typhlocybae* will emerge and that natural spread will be slow. At a new infestation site of *M. pruinosa* far away from the existing release sites, the beneficial insect would have to be released actively by humans because its natural rate of spread would probably be too low. The influence of the known hyperparasitoid *G. areator* on the population of *N. typhlocybae* should be considered, because the parasitisation performance of the latter species could be negatively influenced. In other biological control programmes, the introduction of two other dryinids, *Gonatopus hospes* Perkins and *Haplogonatopus vitiensis* Perkins, which were introduced against the sugarcane planthopper *Perkinsiella saccharicida* Kirkaldy, failed because of hyperparasitoids (Williams 1931). From the European countries where *N. typhlocybae* has been released there are no known reports of disrupted control of *M. pruinosa* due to hyperparasitoids.

Conclusion

Based on the degree of climate matching between its original location in North America and the potential release areas in Austria, establishment of *N. typhlocybae* in the target areas in Vienna and Graz is likely. It is assumed that *N. typhlocybae* will provide satisfactory long-term suppression of *M. pruinosa*, as it does in Slovenia, which has a similar climate to Austria. Biological control of *M. pruinosa* with *N. typhlocybae* in Austria is assumed to be advantageous compared to chemical treatments because sustainable management of the pest would be achieved and the use of large amounts of insecticides, which are often broad spectrum insecticides killing natural beneficial species, could be avoided (Nicoli et al. 1995; Debras et al. 1998). The environmental risk of the release of *N. typhlocybae* for the biological control of *M. pruinosa* in Austria is therefore considered very low.

Acknowledgements

The author thanks Univ. Doz. DI Dr. Sylvia Blümel (AGES, Institute for Sustainable Plant Production) for her valuable comments on the manuscript and Mag. (FH) Michael Schwarz (AGES, Institute for Data, Statistics and Risk Assessment) for assistance in creating the distribution maps. A special thanks to Angela Parker and Natascha Wickramasinghe for correcting the English writing. The author would like to thank the anonymous reviewers for their useful comments.

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