



# Contributions to improve current environmental risk assessment procedures of generalist arthropod biological control agents (GABCAs) in Argentina

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Received: 27 January 2020 / Accepted: 23 October 2020 / Published online: 6 November 2020  
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**Abstract** Argentina has over 100 years of experience in classical biological control, mostly based on the importation of biological control agents (BCAs) against arthropod pests. We present the state-of-the-art of the importation regulatory framework from the last 30 years to date. We also applied a part of a recent developed environmental risk assessment (ERA) methodology to analyze retrospectively the potential negative effects on non-target species of 15 BCAs (12 parasitoids and three predators) imported since 1996

in Argentina, supported by the published literature (Tier 1 Scoping Assessment and Tier 2 Screening Assessment). We demonstrated that the previously imported species could have negative effects on non-target species [Adverse Effect risk characterization > 5 for ERA categories 2 (Reduction of native natural enemies), 3 (Reduction in herbivory) and 4 (Reduction in valued species)], which would be worth evaluating with a Definitive Assessment (Tier 3) and field research to determine if any were actually occurring. We discuss some suggestions for government organizations, state officials and decision makers, scientific researchers, and biological control practitioners to improve the current evaluation for the introduction of new BCAs into Argentina.

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Handling Editor: Bob Pfannenstiel.

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**Electronic supplementary material** The online version of this article (<https://doi.org/10.1007/s10526-020-10063-6>) contains supplementary material, which is available to authorized users.

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**Keywords** Environmental risk assessment · Non-target species · Exotic species · Importation guidelines · Biological control · Decision-making

## Introduction

A thorough review by van Lenteren et al. (2006) showed that introductions of about 2000 species of exotic arthropod agents for control of arthropod pests, in 196 countries or islands during the past 120 years, rarely have resulted in negative environmental effects. However, modern classical biological control (CBC) requires evaluating the economic and environmental

benefits for society as well as the potential for non-target effects when exotic species are released (Kenis et al. 2017). Particularly, in the last three decades, there was a growing interest in determining the selection criteria and steps to implement biocontrol programmes that has resulted in an important body of research contributing to the security of introductions based in biological and ecological knowledge (van Driesche and Hoddle 2002; Louda et al. 2003). Five risk factors of natural enemies, such as host range, establishment, dispersal, and direct and indirect effects on non-target species, were identified and approaches for their quantification have been provided (van Lenteren et al. 2006).

There is a general agreement that environmental risk assessment (ERA) procedures must precede the release of exotic arthropod agents for control of arthropod pests (De Clercq et al. 2011) and pre-release risk assessment of these agents often involves tests with other related species in the agroecosystem. This information when added to post-release evaluation for efficacy and non-target effects would help to elaborate better ERA protocols and enables implementation of a trustworthy CBC. A main controversy has been about the non-target effects from the use of exotic arthropod biological control agents to control arthropod pests, specifically referring to the probabilities of attacking non-target organisms as well as the functional disturbance of native biotic communities and ecosystem services (van Driesche et al. 2010; Hajek et al. 2016). ERA methodologies developed in the past, and still in use in many countries, barely took into account those non-target effects (Parry 2008) because most biological control agents were assumed to be specialists that attack only the target pest. In addition to considering the safe introduction of exotic BCAs into a particular region or country and predicting their impact on local or established non-target species, it is important to distinguish specialists (mono- or oligophagous species) and generalists (consuming more than one genus of prey/host species). At least in principle, the latter appear to have a higher risk of producing non-target impacts. On the other hand, the use of generalist arthropod BCAs (GABCAs), both indigenous and exotic, has recently started to gain interest due to its proved efficacy and that they have rarely shown non-target effects in the agroecosystems for which their use was approved (van Lenteren 2012). Hence, biological control practitioners are facing the

challenge of developing new ERA methodologies for these generalist agents.

In agreement with van Lenteren et al. (2006), host range testing, although being a complicated task, usually provides clear evidences to make risk recommendations to protect non-target species, particularly for the entry of generalist BCAs species. Other natural enemy features, besides host/prey range, have been included for risk assessment in recent years and numerous projects are currently in progress to develop guidelines for ERA methodologies of GABCAs. International organizations, such as the Organization for Economic Cooperation and Development (OECD) (2004) and the IOBC-WPRS (International Organization for Biological Control—West Palearctic Regional Section) (2005), are among the most important in this sense. More recently, during the Fifth International Symposium on Biological Control of Arthropods (2017), conferences were held to discuss the importance of regulations and risk assessment methodologies for GABCAs. After this symposium, a year of six online remote multidisciplinary discussions (2018–2019) were carried out with an expert panel of specialists and stakeholders to seek consensus about ERA methodologies for GABCAs. The aim was to identify several criteria to support an improved tiered ERA for exotic GABCAs (Paula et al. 2021): Tier 1 Scoping, Tier 2 Screening and Tier 3 Definitive assessments, built on previous methods (van Lenteren et al. 2003; van Lenteren et al. 2006; Babendreier et al. 2005; Bigler et al. 2006).

Argentina is a major global agricultural producer country and a top exporter. The country has over 100 years of history in classic biological control, mostly based on the introduction of arthropod agents against arthropod pests and weeds. Since the beginning of the twentieth century, Argentina has introduced 85 agents for the biological control of arthropod pests, among them, parasitoids, predators, nematodes, and pathogens. Approximately 88% of the introductions were with specialized or moderately specialized agents, and 80% of them were parasitoid species (Greco et al. 2020).

In this paper, we first summarized information on legislation and procedures aimed at regulating the importation of biocontrol agents in Argentina from 1996 to date, a period in which records of introductions are publicly available at the websites of the governmental administrative agencies and white paper

documents. Secondly, we re-evaluated the potential for non-target effects of 14 BCAs requested and approved for importation during the last two decades. Additionally, we included one other species not listed in official records but currently used in the country. We utilized part of the recent three-tiered ERA methodology for exotic GABCAs developed by Paula et al. (2021). We ranked the species according to the categories for Likelihood of Effect (LEf) and Magnitude of Effect (MEf) for the exotic agents to estimate their Adverse Effects (AEi) on non-target species, based on literature published for those species. Finally, we proposed suggestions aimed at improving current protocols, paying special attention to the risk assessment procedures for GABCAs in Argentina.

### Current legislation in Argentina

From the first decade of the 1900s until 1996, classical biological control programs were overseen by provincial or national ministries of agriculture and the National Institute for Agricultural Technology (INTA) (Greco et al. 2020). Introductions were made under national regulations until the 1990s when Argentina signed the International Standards for Phytosanitary Measures (ISPMs) of the International Plant Protection Convention (IPPC) of the Food and Agriculture Organization of the United Nations (FAO) for the importation, exportation, and release of biological control agents. The Southern Cone Plant Health Committee (COSAVE), a Regional Plant Protection Organization (RPPO), was created in 1989 through an agreement among the governments of Argentina, Bolivia, Brazil, Chile, Paraguay and Uruguay. ISPM No. 3 “Guidelines for the Export, Shipment, Import and Release of Biological Control Agents and other Beneficial Organisms” from IPPC (1996) was endorsed by COSAVE. Most recently, international and regional regulations were reviewed and amendments approved (IPPC 2005; COSAVE 2017).

In Argentina, at the national level, the National Animal and Plant Health Service (SENASA) is the institution responsible for authorizing the import request, quarantine monitoring and pre- and post-release monitoring of exotic biocontrol agents, through the Resolutions N°758/97. An applicant interested in introducing a BCA has to fill out an application form (See Supplementary Information) in

which information about the organism to be imported, the technical staff responsible for importation, conditions for safe packaging, the eventual method of disposal, a justification for the importation, and other requirements, are provided. The corresponding ERA is included in a dossier containing complementary information about the origin of the BCA, biological and ecological data and bibliographic references. SENASA will issue the importation permit after completing several procedures that are indicated in the next sections.

Interestingly, all COSAVE country members with the exception of Paraguay have adhered to the Nagoya Protocol on access to genetic resources and benefit sharing. The entry into force of the protocol in 2014 has triggered not only regulations on international exchange of biocontrol agents but also provincial regulations over collection and transportation of specimens for scientific or commercial purposes in Argentina (Acosta and Pérez González 2019). The first attempts to implement the protocol has impeded fieldwork and study within the country for scientists, especially taxonomists, and created obstacles for incipient biocontrol business to collect local BCA populations to supply biological control programs. Among most cited restrictions are that rules do not discriminate non-profit scientific activities from commercial ones, and create overwhelming bureaucratic requirements. Currently the National Environment and Sustainable Development Ministry and provincial environmental agencies are coordinating joint collection permits, to simplify access to biological materials in relation to field collections.

### Quarantine station procedures

The BCA intended to be introduced must be taxonomically identified, and colonies must be reared and cleaned of pathogens or parasites. Voucher specimens from each importation should be deposited in the official quarantine belonging to the Agricultural Microbiology and Zoology Institute (Instituto de Microbiología y Zoología Agrícola, IMyZA, INTA Castelar). A specificity assessment of the BCA should be carried out in relation to non-target organisms. If additional shipments of the same species are needed to be imported, the same procedures have to be followed.

## Decision making process

As part of the approval process for the release of a BCA, the opinion of specialists from academia and scientific and technological research organizations is solicited about the ecological risk of the introduction. This consultation is open and non-binding, and is based mainly on the experience of the participating professionals, who must submit a report. The competent institution makes the final decision of accepting or rejecting the introduction by analyzing the information contained in the dossier, the report of the specialists, and the quarantine procedures, aimed at certifying that the BCA does not cause damages to non-target organisms. A panel of experts is available to mediate when conflicts of interests emerge among commercial, scientific or agricultural production sectors.

## Release of BCAs in the environment

After the completion of the steps described previously, the permit to release the BCA into the environment is delivered to the applicant, including detailed plans for post-release monitoring and evaluation. Applicants must inform the competent institution annually about the program progression, specifying the regions where the organism were released, the total number of organisms released, the availability of organisms in the breeding laboratory and data related to the establishment, efficiency and possible effects not foreseen in the field.

## Review of available information about BCAs entry applications in Argentina and potential effects on non-target species

Argentina has received importation requests for over 20 exotic BCAs since 1996, when ISPM Nro. 3 was endorsed. It is useful to evaluate retrospectively the possible negative effects that such introductions could have caused using the recent three-tiered ERA methodology for exotic BCAs proposed by Paula et al. (2021). To cope with that we reviewed information involving the importation of 12 parasitoid and two predatory species to be applied for biocontrol programs of agricultural pests. The list of imported species is publicly available on the SENASA and

COSAVE websites <https://www.argentina.gob.ar/senasa/programas-sanitarios/cadenavegetal/aromaticas/aromaticas-produccion-primaria/control-biologico/listado-de-agentes-evaluados> (cited September 12 2020). A third predatory species, the Mediterranean mite *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae), a well-known biocontrol agent commercially and widely used in Europe, was included in the analysis. The unforeseen presence of this exotic generalist mite was registered in horticultural crops in Argentina ca. ten years ago and it is currently used although its introduction does not appear on the SENASA websites.

Applications for the importation of biocontrol agents came from state agencies such as INTA, and a national private company, which is a subsidiary of Biobest. Although some of the introduced parasitoid BCAs are considered in the literature to be specialists, we included them in our analysis because most of these species were selected to be used in CBC programs in other geographic regions or under bioecological conditions different from those prevalent in Argentinian crops. We wanted to see if there was a difference between specialists and generalists.

To perform the Scoping Assessment (Tier 1) indicated in the ERA methodology, we firstly sorted out the BCA species into “specialists and generalists”, and then summarized existing biological information, including intended use, level of polyphagy and qualitative non-target species assessment. Information of host/prey range, biogeographical origin, target crop, and pest and natural enemies in its original and introduced region was collected from Google Scholar, CABI and other biological databases, as well as from published primary literature sources and non-peer reviewed papers (technical reports, thesis works, etc.) (Table 1). The main target crops were fruit orchards (citrus, apple, pear and peach), sugarcane, forest plantations (*Pinus* sp. and *Eucalyptus* sp.), and horticultural crops (sweet pepper, tomato and strawberry). Four species, *Ageniaspis citricola* Logvinovskaya (Hymenoptera: Encyrtidae), *Megarhyssa nortoni* (Cresson) (Hymenoptera: Ichneumonidae), *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) and *Selitrichodes neseri* Kelly and La Salle (Hymenoptera: Eulophidae) were re-introduced (Table 1). All species were considered to be established based on reports and highly likely to have adverse effects on non-target organisms.

**Table 1** Arthropod biological control agents approved for importation in Argentina from the year 1996 to date, as published by SENASA (<https://www.argentina.gov.ar/senasa/programas-sanitarios/cadenavegetal/aromaticas/produccion-primaria/control-biologico/listado-de-agentes-evaluados>). Biological information in relation to the host/prey range of target and potential non-target species in Argentinian environments is provided. BCAs list is given in alphabetical order

Biological control agent	Feeding habit <sup>a</sup>	Target pest and crop	BCAs host/prey range and other insect associates	Year	Area of origin of the imported strain	Experimental/commercial aims	References
<b>BCA classical agents</b>							
<i>Agonaspis citricola</i> (Hymenoptera: Encyrtidae)	Specialist PAR	Citrus leafminer <i>Phyllocnistis citrella</i> in citrus	Reports of other hosts unavailable. Other <i>P. citrella</i> native natural enemies: the parasitoids <i>Cirrospilus</i> sp. and <i>Elasmus</i> sp., and predatory chrysopids	1996 1997	Peru and Spain USA	Experimental/commercial Experimental/commercial	Díez et al (2006)
<i>Ascogaster quadridentata</i> (Hymenoptera: Braconidae)	Generalist PAR	Codling moth <i>Cydia pomonella</i> in fruit orchards	Reports of other hosts unavailable. Other <i>C. pomonella</i> native and imported natural enemies: parasitoids <i>Trichogramma nerudai</i> , <i>T. cacoeciae</i> , <i>Mastrus ridens</i> (see below), <i>Goniozus legneri</i> , the hyperparasitoid <i>Dibrachys microgastri</i> and predator <i>Chrysoperla externa</i>	2003	Chile	Experimental/commercial	D'Hervé and Aquino (2015), Hernández (2015)
<i>Cleruchoides noackae</i> (Hymenoptera: Mymaridae)	Specialist? PAR	Australian bug or bronze bug <i>Thaumastocoris peregrinus</i> in <i>Eucalyptus</i> plantations	Reports of other hosts unavailable. Other <i>T. peregrinus</i> natural enemies: chrysopids, a predatory bug and two entomopathogenic fungi	2014 Released in Entre Ríos province, Argentina (2018)	Australia	?	Mutitu et al. (2013), Noyes (2019) and references therein
<i>Cotesia flavipes</i> (Hymenoptera: Braconidae)	Generalist PAR	Sugarcane borer <i>Diatraea saccharalis</i> in sugarcane	Reports of other hosts unavailable. Other <i>D. saccharalis</i> natural enemies: at least 20 parasitoid species. Two tachinids imported in earlier biological control projects	2000, 2005	Brazil	Experimental/commercial	De Santis (1967, 1979), De Santis and Fidalgo (1994), Greco et al. (2020)
<i>Diachasmimorpha longicaudata</i> and <i>D. tryoni</i> (Hymenoptera: Braconidae)	Generalist PAR	Fruit or medfly <i>Ceratitis capitata</i> in citrus orchards	Other host reported: South American fruit fly <i>Anastrepha fraterculus</i> . Other <i>C. capitata</i> natural enemies: five parasitoid species	1998	Mexico	Experimental/commercial	Ovruski et al. (2000), Sánchez et al. (2016), Viscarret et al. (2006)

Table 1 continued

Biological control agent	Feeding habit <sup>a</sup>	Target pest and crop	BCAs host/prey range and other insect associates	Year	Area of origin of the imported strain	Experimental/commercial aims	References
<i>Eretmocerus mundus</i> (Hymenoptera: Encyrtidae)	Generalist PAR	Whiteflies <i>Bemisia tabaci</i> and <i>Trialeurodes vaporariorum</i> in horticultural crops	Other hosts reported: whiteflies. Other whitefly natural enemies: <i>Encarsia</i> spp. and <i>Eretmocerus corni</i>	2007	The Netherlands	Experimental/commercial	CABI.org, Urbaneja et al. (2006), De Santis (1979), De Santis and Fidalgo (1994)
<i>Macrocentrus ancylivorus</i> (Hymenoptera: Braconidae)	Generalist PAR	Oriental fruit moth <i>Grapholitha molesta</i> in apple orchards	Previously imported in Argentina in 1936 and 1946. Displaced by <i>M. delicatulus</i> . Other <i>G. molesta</i> natural enemies: at least 20 parasitoid species belonging to different guilds	2008	USA	Commercial	De Santis (1967, 1979), De Santis and Fidalgo (1994)
<i>Mastrus ridens</i> <sup>b</sup> (Hymenoptera: Ichneumonidae)	Specialist PAR	<i>C. pomonella</i> , in apple, pear, and walnut orchards	Other insect host: <i>G. molesta</i> . Other <i>C. pomonella</i> natural enemies; idem as for <i>A. quadridentata</i> . Interactions with natural enemies of <i>G. molesta</i>	2005	USA	Experimental/commercial	Tortosa et al. (2014), Charles et al. (2013), Hernández (2015)
<i>Megarhyssa nortoni</i> (Hymenoptera: Ichneumonidae)	Generalist PAR	Woodwasp <i>Sirex noctilio</i> in <i>Pinus</i> spp. plantations	Other siricids as hosts. Other <i>S. noctilio</i> natural enemies: established or imported parasitoids, such as <i>Ibalia leucospoides</i> and <i>Rhyssa persuasoria</i> (see below)	1999, 2004, 2008	Tasmania, N. Zealand, Chile	Experimental/commercial	Villacide and Corley (2008)
<i>Rhyssa persuasoria</i> (Hymenoptera: Ichneumonidae)	Generalist PAR	<i>S. noctilio</i>	Other siricids and cerambycids as hosts. Other <i>S. noctilio</i> natural enemies: Idem for <i>M. nortoni</i>	1999		Experimental/commercial	
<i>Selitrichodes neseri</i> (Hymenoptera: Eulophidae)	Specialist PAR	Blue gum chalcid or gall wasp <i>Leptocybe invasa</i> in <i>Eucalyptus</i> spp plantations	No other hosts known. Other <i>L. invasa</i> natural enemies: A complex trophic web formed by several generalist predators and two parasitoid species, <i>Megastigmus zebrinus</i> and <i>Quadrastichus mendeli</i>	2016, 2017 Released in <i>Eucalyptus</i> plantations in Entre Rios and Corrientes provinces, Argentina. Exported to Laos	Chile	Experimental/commercial	Aquino et al. (2019), Hernández et al. (2015)

Table 1 continued

Biological control agent	Feeding habit <sup>a</sup>	Target pest and crop	BCAs host/prey range and other insect associates	Year	Area of origin of the imported strain	Experimental/commercial aims	References
<i>Neoseiulus californicus</i> (Acari: Phytoseiidae)	Generalist PRE	Two-spotted spider mite <i>Tetranychus urticae</i> in strawberry crops	Preys on tetranychid mites, thrips, predatory bugs, etc	2007	The Netherlands	Commercial	Crop Protection Compendium (CABI.org), Pascua et al. (2018) ( <a href="https://www.cabi.org/cpc/">https://www.cabi.org/cpc/</a> )
<i>Orius insidiosus</i> (Hemiptera: Anthocoridae)	Generalist PRE	<i>F. occidentalis</i>	Preys on phytophagous and predatory arthropods. Commercial strains of unknown geographic origin were imported	2007, 2009, 2011	The Netherlands	Commercial	Crop Protection Compendium ( <a href="https://www.cabi.org/cpc/">https://www.cabi.org/cpc/</a> ); Pascua et al. (2018)

<sup>a</sup>PAR, parasitoid; PRE, predator<sup>b</sup>misidentified as *M. ridibundus* (D'Hervé and Aquino 2015)

We would like to point out some issues associated with the introductions summarized in Table 1 that deserve attention. Despite prior knowledge of the wide host range of some of the parasitoid species, they were nevertheless imported. For example, Stiling (2004) reported 16 host species for *Ascogaster quadridentata* Wesmael (Hymenoptera: Braconidae), a parasitoid of the codling moth *Cydia pomonella* L. (Lepidoptera: Tortricidae) imported and reared at the INTA Alto Valle del Río Negro Experimental Station for a CBC program in northern Patagonia. *Megarhyssa nortoni*, introduced in a joint biocontrol program against the siren woodwasp *Sirex noctilio* Fabricius (Hymenoptera: Siricidae) by SENASA (Argentina) and the Livestock Agricultural Service (SAG, Chile), parasitizes hosts of several species belonging to three different genera (Stiling 2004). In addition, five other generalist parasitoid species were introduced (Table 1) Another issue that merits reflection is the importation of exotic enemies when several native or established species could play the same role in providing biocontrol programs. The scarcity of studies on existing beneficial arthropods in Argentina may have led to importing BCAs from other regions before studying those already present in the country. This was the case for the introduction of *Diachasmimorpha longicaudata* (Ashmead) and *D. tryoni* (Cameron) (Hymenoptera: Braconidae), parasitoids of fruit flies. After their introduction, studies revealed at least five indigenous parasitoid species attacking the pests *Anastrepha fraterculus* (Wiedemann) and *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) on seven host plant species. In particular, the native parasitoid *Doryctobracon areolatus* (Szépligeti) (Hymenoptera: Braconidae), alone, reached 62% parasitism (Ovruski Alderete et al. 2004). Another striking case is the importation of *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae) individuals in 2007 to control *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae). A report indicated that this parasitoid species was already widely present in pepper, tomato and cantaloupe crops in northeastern, western and central Argentina since 2002 (López and Evans 2008). In addition, other parasitoids of whiteflies in the same genus (*Eretmocerus*) attacked same whitefly hosts, such as *E. corni* Haldeman, and coexist in these regions (De Santis 1967; Viscarret et al. 2000). A third case is the classical biocontrol program to manage *Leptocybe invasa* Fisher and La Salle in eucalyptus

plantations by means of the parasitoid *Selitrichodes neseri* Kelly and La Salle (Hymenoptera: Eulophidae: Tetrastichinae), which was recently imported from Australia. Field releases of *S. neseri* are being conducted in Entre Ríos and Corrientes provinces. Further studies found a complex of predatory insects and two native parasitoids attacking the pest in Argentina (Hernández 2015; Hernandez et al. 2015). A fourth issue is that some of the introduced BCAs were already widely distributed in Argentina and commonly present. In addition to *E. mundus*, mentioned above, the two predatory species were already established in Argentina and were common in sweet pepper and strawberry crops, where they had been proposed for use. These are the predatory mite *Neoseiulus californicus* (Mc Gregor) (Acari: Phytoseiidae) and the pirate bug *O. insidiosus*, with the latter being re-introduced three times. The mite *N. californicus* is the most frequent species associated with tetranychid mites, especially *Tetranychus urticae* Koch (Acari: Tetranychidae) in vegetable crops, fruit and ornamental plants. Both pest and predator populations display a high spatio-temporal synchrony, even at low densities of the pest. For that reason, conservation biological control program of *T. urticae* in strawberry based on this predator was developed (Greco et al. 2004). *Orius insidiosus* is also commonly present in several South American agroecosystems, particularly in Argentina (Bueno et al. 2006; Pascua et al. 2018), where it has a wide distribution particularly in the northern and central provinces. After the petition approval, experiments in strawberry crops to assess the biocontrol of flower thrips were started in the province of Tucumán by releasing individuals of imported strains, which entered three times from Belgium after request of a national private company (Lefebvre et al. 2013). A possible reason for importing exotic strains could be the lack of commercial mass production of these well-known effective biocontrol agents in Argentina. Since the company did not provide information on the geographical origin of the founder populations of *N. californicus* and *O. insidiosus* colonies reared in Belgium, a concern arises about possible effects on non-target native indigenous populations of those species, via reproductive interference or hybridization within strains (effects of non-target species category 2, as in Paula et al. (2021). Finally, as mentioned above, the exotic mite *A. swirskii* is now released in Argentinian



**Table 2** Partial environmental risk analysis (ERA) for non-target species after the introduction and release of arthropod biocontrol agents (BCAs) in Argentina for the control of several agricultural pests during the period 1996–2017. Scores were assigned based on biological information summarized in Table 1 and the authors' expert judgements

Category of ERA	<i>Agoniaspis citricola</i>			<i>Amblyseius swirskii</i>			<i>Ascogaster quadridentata, Macrocentrus ancyliivorus, Mastrus ridens</i>			<i>Cleruchoides noackae</i>			<i>Cotesia flavipes</i>			<i>Diachasmimorpha longicaudata, D. tryoni</i>				
	LEf	Mef	AE/risk	LEf	Mef	AE/risk	LEf	Mef	AE/risk	LEf	Mef	AE/risk	LEf	Mef	AE/risk	LEf	Mef	AE/risk		
2	2a	5	2	10	5	3	15	5	2	10	5	2	10	5	2	10	5	2	10	
	2b	5	2	10	5	3	15	5	2	10	5	2	10	5	2	10	5	2	10	
	2c	4	3	12	5	3	15	4	3	12	4	2	8	4	2	8	5	2	10	
	2d	3	1	3	3	1	3	3	1	3	3	1	3	3	1	3	3	1	3	3
	2e	3	1	3	3	1	3	3	1	3	3	1	3	3	1	3	3	1	3	3
	2f	0	0	0	5	2	10	0	0	0	0	0	0	4	2	8	2	1	2	2
	2g	0	0	0	5	2	10	0	0	0	0	0	0	4	2	8	2	1	2	2
	2h	4	1	4	4	3	12	4	1	4	4	1	4	4	1	4	4	1	4	4
3	3a	5	-3	-15	4	-1	-4	4	-1	-4	4	-1	-4	4	-1	-4	4	-1	-4	
	3b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	3c	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
4	3d	5	-3	-15	5	-3	-15	5	-3	-15	5	-3	-15	5	-3	-15	5	-3	-15	
	4a	2	0	0	2	0	0	2	0	0	2	0	0	2	0	0	2	0	0	
	4b	2	0	0	2	0	0	2	0	0	2	0	0	2	0	0	2	0	0	
	4c	2	0	0	4	2	8 <sup>a</sup>	2	0	0	2	0	0	3	2	6	1	0	0	
Category of ERA	<i>Eretmocerus mundus</i>			<i>Megarhyssa nortoni, Rhyssa persuasoria</i>			<i>Neoseiulus californicus</i>			<i>Orius insidiosus</i>			<i>Seltrichodes neseri</i>							
	LEf	Mef	AE/risk	LEf	Mef	AE/risk	LEf	Mef	AE/risk	LEf	Mef	AE/risk	LEf	Mef	AE/risk	LEf	Mef	AE/risk		
	2a	5	2	10	5	2	10	4	3	12	4	3	12	3	12	3	2	6	6	
	2b	5	2	10	5	2	10	4	3	12	4	3	12	3	12	3	2	6	6	
	2c	5	2	10	4	2	8	4	3	12	4	3	12	3	12	3	2	6	6	
	2d	3	1	3	3	1	3	3	1	3	3	1	3	3	1	3	3	1	3	
	2e	3	1	3	3	1	3	3	1	3	3	1	3	3	1	3	3	1	3	
	2f	4	2	8	0	0	0	4	2	8	3	2	6	0	0	0	0	0	0	
	2g	4	2	8	0	0	0	6	2	12	6	2	12	0	0	0	0	0	0	
	2h	4	1	4	4	1	4	4	2	8	4	2	8	4	2	8	4	1	4	4
	3a	4	-1	-4	4	-1	-4	4	-1	-4	4	-1	-4	4	-1	-4	4	-1	-4	-4
	3b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3c	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

**Table 2** continued

Category of ERA	<i>Eretmocerus mundus</i>			<i>Megarhyssa nortoni</i> , <i>Rhyssa persusaria</i>			<i>Neoseiulus californicus</i>			<i>Orius insidiosus</i>			<i>Selitrichodes neseri</i>		
	LEf	MEf	AE/risk	LEf	MEf	AE/risk	LEf	MEf	AE/risk	LEf	MEf	AE/risk	LEf	MEf	AE/risk
3d	5	-3	-15	5	-3	-15	5	-3	-15	5	-3	-15	5	-3	-15
4a	2	0	0	2	0	0	2	0	0	2	0	0	2	0	0
4b	1	0	0	2	0	0	2	0	0	2	0	0	2	0	0
4c	1	0	0	2	0	0	2	0	0	2	0	0	2	0	0

References: Categories of potential effects on non-target species: 2. Reduction of a native natural enemies via: 2a. Exploitative competition. 2b. Asymmetrical competition. 2c. Intraguild predation. 2d. Immunity from shared natural enemies with native species. 2e. Co-introduction of new pathogens that infect native species. 2f. Reproductive interference with native species. 2 g. Hybridization with another strain. 2 h. Reduced biological control. 3. Reduction in herbivory via: 3a. Improved biological control. 3b. Release of undesired plant (weed) population from herbivory. 3c. Competitive suppression of a plant by a released plant. 3d. Reduced insecticide use. 4. Reduction in valued species. 4a. Suppression or take of a rare or endangered herbivore. 4b. Reduction in a pollinator. 4c. Reduction in some other valued species. Biological interpretation of the categories is provided in Paula et al. (2021). Likelihood of Effect (LEf) scales: 0 = Very highly unlikely (~ 0); 1 = Highly unlikely; 2 = Unlikely; 3 = Neither unlikely or likely (~ 0.5); 4 = Likely; 5 = Highly likely, and 6 = Very highly likely (~ 1). *Magnitude of Effect (MEf) scales*: -4 = Massively beneficial: widespread, large, consistent; -3 = Highly beneficial: widespread, large, variable; -2 = Beneficial: local or small and variable; -1 = Slightly beneficial = local and small; 0 = Neutral, neither beneficial or adverse; 1 = Slightly adverse: local and small; 2 = Adverse: local or small and variable; 3 = Highly adverse: widespread and large but variable; 4 = Massively adverse = widespread, large and consistent. Adverse Effect (AE): LEf × MEf from the corresponding effect category. Negative AE are benefits. Risk (R): LE × AE. Since all species are assumed as established, LE, which is the Likelihood of Establishment is considered equal to 1, thus AE equals the Risk

<sup>a</sup> *Amblyseius swirskii* will compete (and probably outcompetes) *N. californicus* (see text)

greenhouses under biological control management. This species was firstly detected in protected horticultural crops of the province of Buenos Aires during 2011 (Cédola and Polack 2011) and later recorded in other provinces, such as Corrientes (Carrizo et al. 2017). For the Screening Assessment (Tier 2) (Table 2), species were ranked based on biological information gathered in Table 1 by assigning them to three of the six main categories of effects on non-target species and their corresponding scales following Paula et al. (2021): category 2 (reduction of native natural enemies), category 3 (reduction in herbivory) and category 4 (reduction in valued species). Categories 1 (reduction of a native top predator) and 5 (increase in herbivory) were not considered in the analysis because specific data on the trophic webs in which the BCA was involved were not available. Category 6 (increase in a damaging organism vectored by the exotic GABCA) was also dismissed because Argentina avoids this risk with quarantine procedures. To summarize, all of the BCA species showed Adverse Effects (AEi) effects > 5 (very highly likely) for category 2, specifically related to exploitative and asymmetrical competition and intra-guild predation (Table 2). In addition, the three generalist predators, and two of the generalist parasitoids (*A. swirskii*, *C. flavipes*, *E. mundus*, *N. californicus* and *O. insidiosus*) had AEi scores > 5 for reproductive interference with native species and reduced biological control, and for the last two predator species, for hybridization with another strain (categories 2f, g and h). For all species, AEi effects considered unlikely or highly unlikely for improved biological control and reduced insecticide use (categories 3a and 3d). Reduction of valued species or the use of commercial BCAs (category 4) was also determined as important for *A. swirskii*, *C. flavipes*, *N. californicus* and *O. insidiosus*. The exotic phytoseiid will compete (and probably outcompetes) *N. californicus*, a native predatory mite with potential to be used under conservation and augmentative biological control strategies in strawberry crops (Greco et al. 2004). Meanwhile *C. flavipes* could negatively affect biocontrol exerted by two tachinids imported previously in sugarcane biological control projects (Greco et al. 2020). Lastly, by applying the ERA methodology we demonstrated that several non-target species for each imported BCA were worth selecting to continuing the next Tier 3 (Definitive Assessment).

Information on post-releasing studies addressing these potential effects on non-target species is still lacking or not publicly available, thus the consequences of such introductions on other species remain unknown. It is expected that if the new ERA methodology is included as online appendix to the ISPMs, native beneficial organisms could be monitored and eventually environmental risks minimized. Notably, some of the imported BCAs are reported as providing some degree of biocontrol in pine and sugarcane plantations, fruit production, and citrus groves, when implemented along with cultural control, trapping and Sterile Insect Technique (SIT) technology (Greco et al. 2020).

## Conclusions

Results of this work indicate that the new ERA-GABCA methodology (Paula et al. 2021) could be helpful in examining the effects on non-target species when exotic BCAs are intended to be used for biological control. Current legislation in Argentina regarding importation of BCAs should be reviewed and protocols for ERA methodologies included to improve decision-making and to guarantee the safe introduction of exotic BCAs. Specifically, applicants should be asked to provide in the dossier information about the host range of the BCA (including direct and indirect effects on non-target species), and a comprehensive review of the role of other native and exotic natural enemies already attacking the target species in the receiving agroecosystem. This information, when added to the expert analysis by specialists, will improve decision-support tools available to SENASA, the institution in charge of regulating CBC programs.

Because in recent years, increased attention has been given to non-target impacts, ERAs should precede the release of exotic arthropod agents for arthropod pest control. If information is lacking, testing with non-target species should be performed prior to the release of these agents. Current regulatory legislation represents the challenge of finding a balance between a system that ensures safer and more reliable exotic BCAs and, at the same time, this is realistic and feasible enough, avoiding unneeded bureaucracy and unjustified restrictions for the introduction of BCAs. We consider that Argentina meets all the conditions to face this challenge, and we would

like to provide some suggestions to satisfy regulatory biosafety standards for importing BCAs:

- The state agencies could adapt their own guidelines and protocols to evaluate and carry out an ERA to assess the effects on non-target species by an exotic BCA considered for importation. Legislation and protocols should harmonize the introduction guidelines among the different provinces of the country.
- The risk factors of natural enemies should include the ERA methodologies recommended by van Lenteren et al. (2003, 2006) and Paula et al. (2021) concerning host/prey range, establishment, dispersal, and direct and indirect effects on non-targets, as well as the evaluation of the risk/benefit ratio from environmental, economics and a social point of view. Until the new ERA methodology comes into force in the competent national organizations, the evaluation of possible adverse effects should be included in the dossier and, if lacking, developed by specialists in their reports.
- A formal opinion by experts from universities and organizations of science and technology about the risks and benefits of the proposed introduction should be mandatory before reaching a final decision.
- It is highly recommended, before approving the release of an exotic agent, to confirm that there is no native or previously established exotic enemy(ies) in the country that can fulfill the same role and with similar efficiency as the proposed exotic species.
- Post-release evaluations should be promoted to determine if the predicted risks and benefits are being realized.
- The state agencies responsible for the importation should avoid the strong business lobby involvement in decision-making, which is sometimes carried out by stakeholders and private companies.
- We highlight the challenge of carrying out these regulatory changes for legislators and for those who make decisions on this complex issue, which requires a strong commitment to the environment and society in general.

**Acknowledgements** We thank Debora Pires Paula, David Andow and Joop van Lenteren for kindly inviting us to contribute to this Special Issue. David Andow and one

anonymous reviewer kindly helped us to improve the draft version of the manuscript

**Funding** The research was funded by PICT 2015 1427 (ANPCyT, Argentina), PUE CEPAVE 2016 (CONICET, Argentina), PIDUNLP N829 2017–2020 (Director: MG Luna), and PIDUNLP N854 2018–2021 (Co-Director: CV Cédola).

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** The manuscript is original and none of the material has been published or is under consideration elsewhere, including the Internet. We have no related manuscripts submitted to other journals.

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