

FOUNDATION FOR REGULATIONS OF INVERTEBRATE BIOCONTROL AGENTS IN SERBIA: FIRST OVERVIEW OF DATA AND RECOMMENDATIONS

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ABSTRACT

The European and Mediterranean Plant Protection Organization (EPPO) is an international organization responsible for cooperation and harmonization in plant protection in the European and Mediterranean region. As part of Yugoslavia, Serbia was a member of the organization from 1951 until it withdrew its membership in 1992. Serbia rejoined in 2004. The EPPO's first activities on biological control agents (BCAs) took place in 1997 and the first "positive list" was drawn up in 2001. It contains a list of BCAs that can be safely used in Europe and the Mediterranean region. The standard PM 6/3 - Biocontrol agents safely used in the EPPO region is the latest document with a list of BCAs and their short biology, which does not require a complete procedure for import and release and contains three appendices: Appendix I - Commercially or officially used biological control agents, Appendix II - Classical BCAs successfully established in the EPPO region, Appendix III - List of biological control agents removed from Appendices I or II. We searched the literature and databases for species listed in Appendices I and II that have ever been detected in Serbia. Of the 112 species listed in Appendix I, 45 have been recorded in Serbia, and of the 42 species listed in Appendix II, six have been recorded in Serbia. In comparison, 38 species listed as indigenous biological control agents in Slovenia: 34 from Appendix I, two from Appendix II and two others.

1 INTRODUCTION

The European and Mediterranean Plant Protection Organization (EPPO) is an international organization responsible for cooperation and harmonization in plant protection in the European and Mediterranean region. It was founded in 1951 by 15

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countries. As part of Yugoslavia, Serbia was a member of the organization from its foundation until it withdrew its membership in 1992. Serbia rejoined in 2004.

The EPPO's first activities on biological control agents (BCAs) took place in 1997 with the establishment of the Panel for the 'Introduction of Exotic Biological Control Agents'. Over several years, panel developed standards of the PM 6 series (Orlinski, 2016) and in 2001 the standard PM 6/3 - Biocontrol agents (BCA) safely used in the EPPO region was created. It contains a list of BCAs that can be safely used in Europe and the Mediterranean region. The organism is considered safe for the EPPO region in several cases (indigenous and widespread, established and widespread, used for at least 5 years in at least 5 EPPO countries without adverse effects) for augmentative biocontrol or found at least 5 years after release without adverse effects for classical biocontrol PM, E. (2021). The latest version of the standard PM6/3 is from 2021 and contains a list of BCAs and their short biology, which does not require a full procedure for import and release and contains three appendices: Appendix I - Commercially or officially used biological control agents, Appendix II - Classical BCAs successfully established in the EPPO region, Appendix III - List of biological control agents removed from Appendices I or II.

Serbia has no regulations for invertebrate biological control agents (IBCA), while Slovenia has had them since 2006. The Slovenian regulation divides biological control agents into two groups: native and nonnative, so that only organisms from native list may be used for biological control. This approach is much stricter than that of most EPPO countries (Trdan et al., 2023). Slovenia is the closest country with a well-regulated IBCA application, so we use their approach as a basis for proposing regulation for IBCA in Serbia.

2 MATERIAL AND METHODS

We performed an extensive literature survey for species listed in EPPO Appendices I and II, that have ever been detected in Serbia. In addition we searched all available databases and compiled first list of EPPO positive biocontrol agents present in Serbia. We also analysed species lists of IBCA recorded in countries surrounding Serbia (Bosnia and Herzegovina, Bulgaria, Croatia, Hungary, North Macedonia, Montenegro and Romania) and compile a list of species which are potentially present in Serbia, but not recorded yet.

3 RESULTS

In total we found that 51 species listed in EPPO Appendices I and II were recorded in Serbia (Tables 1 and 2). Of the 112 species listed in Appendix I, 45 have been recorded in Serbia (Table 1), and of the 42 species listed in Appendix II, six have been recorded in Serbia (Table 2). Also 13 species from Appendix I and II are present in countries close to Serbia and could be possibly present in Serbia (Table 3).

Table 1. List of invertebrate species from Appendix I recorded in Serbia (*also present in Slovenia [Pravilnik o biotičnem varstvu rastlin, 2006]).

INSECTA		
Coleoptera		
species	family	reference
<i>Adalia bipunctata</i> *	Coccinellidae	Damjanović, 2024
<i>Chilocorus bipustulatus</i> *	Coccinellidae	Graora et al., 2009
<i>Coccinella septempunctata</i> *	Coccinellidae	Damjanović, 2024
<i>Exochomus quadripustulatus</i> *	Coccinellidae	Graora et al., 2012
<i>Propylea quatuordecimpunctata</i> *	Coccinellidae	Jovičić et al., 2020
<i>Rodolia cardinalis (Novius cardinalis)</i>	Coccinellidae	Gerber and Schaffner, 2016
<i>Scymnus rubromaculatus</i>	Coccinellidae	Damjanović, 2024
<i>Stethorus punctillum</i>	Coccinellidae	Milenković, 1992
Diptera		
species	family	reference
<i>Aphidoletes aphidimyza</i> *	Cecidomyiidae	Simova-Tošić et al., 2000
<i>Episyrphus balteatus</i> *	Syrphidae	Janković et al., 2019
<i>Eupeodes corolla</i> *	Syrphidae	Janković et al., 2019
<i>Sphaerophoria rueppellii</i> *	Syrphidae	Van Steenis, 2015
Hemiptera		
species	family	reference
<i>Anthocoris nemoralis</i> *	Anthocoridae	Jerinić-Prodanović and Protić, 2013
<i>Anthocoris nemorum</i> *	Anthocoridae	Jerinić-Prodanović and Protić, 2013
<i>Macrolophus pygmaeus</i> *	Miridae	Protić, 2011
<i>Orius laevigatus</i>	Anthocoridae	Protić, 2011
<i>Orius majuscules</i> *	Anthocoridae	Protić, 2011
<i>Picromerus bidens</i> *	Pentatomidae	Protić, 2011
<i>Podisus maculiventris</i>	Pentatomidae	Gerber and Schaffner, 2016
Hymenoptera		
species	family	reference
<i>Anagrus atomus</i>	Mymaridae	Donev, 1998
<i>Anastatus bifasciatus</i> *	Eupelmidae	Boucek, 1977
<i>Aphelinus abdominalis</i>	Aphelinidae	Boucek, 1977

<i>Aphidius ervi</i> *	Braconidae	Tomanović et al., 2021
<i>Aphidius matricariae</i> *	Braconidae	Tomanović et al., 2021
<i>Bracon hebetor</i>	Braconidae	Žikić et al., 2012
<i>Coccophagus lycimnia</i>	Aphelinidae	Graora et al., 2012
<i>Coccophagus scutellaris</i>	Aphelinidae	Dervišević et al., 2023
<i>Diglyphus isaea</i> *	Eulophidae	Boucek, 1977 Graora et al., 1994 ; Graora et al., 2009
<i>Encarsia citrine</i>	Aphelinidae	
<i>Encarsia formosa</i> *	Aphelinidae	Drobnjaković et al., 2016
<i>Ephedrus cerasicola</i>	Braconidae	Tomanović et al., 2021
<i>Leptomastix epona</i>	Encyrtidae	Boucek, 1977
<i>Opius pallipes</i>	Braconidae	Žikić et al., 2013
<i>Praon volucre</i> *	Braconidae	Tomanović et al., 2021
<i>Trichogramma brassicae</i> *	Trichogrammatida e	Ivezić et al., 2018
<i>Trichogramma cacoeciae</i>	Trichogrammatida e	Mihajlović, 2008
<i>Trichogramma evanescens</i>	Trichogrammatida e	Ivezić et al., 2021
Neuroptera		
species	family	reference
<i>Chrysoperla carnea</i> *	Chrysopidae	Graora et al., 2009
<i>Micromus angulatus</i> *	Hemeroibiidae	Devetak et al., 2023
ARACHNIDA		
Acarina		
species	family	reference
<i>Amblyseius andersoni</i> *	Phytoseiidae	Stojnić et al., 2014
<i>Amblyseius barkeri</i> *	Phytoseiidae	Szabó et al., 2018
<i>Neoseiulus californicus</i> *	Phytoseiidae	Stojnić et al., 2002
<i>Neoseiulus cucumeris</i> *	Phytoseiidae	Szabó et al., 2018
<i>Phytoseiulus persimilis</i>	Phytoseiidae	Stojnić et al., 2002
<i>Typhlodromus pyri</i> *	Phytoseiidae	Szabó et al., 2018

Table 2. List of invertebrate species from Appendix II recorded in Serbia (*also present in Slovenia [Pravilnik o biotičnem varstvu rastlin, 2006]).

INSECTA		
Coleoptera		
species	family	reference
<i>Adalia bipunctata*</i>	Coccinellidae	Damjanović, 2024
<i>Rodolia cardinalis (Novius cardinalis)</i>	Coccinellidae	Gerber and Schaffner, 2016
Hymenoptera		
<i>Aphelinus mali</i>	Aphelinidae	Petrović-Obradović, 2022
<i>Aphytis proclia</i>	Aphelinidae	Graora et al., 1994; Graora et al., 2009
<i>Encarsia berleseii</i>	Aphelinidae	Graora et al., 1994; Graora et al., 2009
<i>Encarsia perniciosi</i>	Aphelinidae	Graora et al., 1994; Graora et al., 2009
<i>Neodryinus typhlocybae</i>	Dryinidae	Milosavljević et al., 2017

Table 3. List of invertebrate species from List of biocontrol agents safely used in EPPO region, present in surrounding countries, potentially present in Serbia.

INSECTA		
Diptera		
species	family	contry/reference
<i>Feltiella acarisuga</i>	Cecidomyiidae	Bosnia and Herzegovina - Simova-Tošić et al., 2007 Slovenia - Trdan et al., 2023
Hymenoptera		
species	family	contry/reference
<i>Aprostocetus hagenowii</i>	Eulophidae	Croatia - Noyes, 2019
<i>Comperiella bifasciata</i>	Encyrtidae	Hungary, Montenegro - Noyes, 2019
<i>Ooencyrtus kuvanae</i>	Encyrtidae	Bosnia and Herzegovina, Croatia, North Macedonia - Boucek, 1977
<i>Trichogramma dendrolimi</i>	Trichogrammatidae	Hungary, Romania - Polaszek, 2009
<i>Trichogramma pintoi</i>	Trichogrammatidae	Bulgaria, Romania - Polaszek, 2009
<i>Trichopria drosophilae</i>	Diapriidae	Slovenia - Trdan et al., 2023
<i>Trissolcus basalis</i>	Scelionidae	Slovenia - Trdan et al., 2023
NEMATODA		
species	family	contry/reference

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<i>Heterorhabditis bacteriophora</i>	Heterorhabditidae	Bosnia and Herzegovina - Grujić et al., 2021 Slovenia - Trdan et al., 2023
<i>Steinernema carpocapsae</i>	Steinernematidae	Slovenia - Trdan et al., 2023
<i>Steinernema feltiae</i>	Steinernematidae	Croatia - Majić et al., 2018 Slovenia - Trdan et al., 2023
<i>Steinernema kraussei</i>	Steinernematidae	Slovenia - Trdan et al., 2023

4 DISCUSSION

Some countries in the EPPO organization have regulations for invertebrate biocontrol agents that sometimes do not match those of other participating countries, or they have no regulations at all (Mason et al., 2017). Despite some regulations, there is no directive proposed by the European Commission to regulate IBCA that would force countries to adopt regulations in this area. This situation leaves a gap between the regulations of different countries, making regulations pointless due to the mobility of IBCAs. Some invasive IBCAs can cross national borders from a country with no or less stringent regulations to a country with regulations, as in the case of the release of *Harmonia axyridis* in France in the 1990s (Mason et al., 2017). For this reason, regulations should be harmonized across all regions to allow efficient introduction of biocontrol agents, especially for highly invasive pests (Mason et al., 2017). The global cost of damage caused by invasive pest species is estimated at over US\$ 70 billion per year (Bradshaw et al., 2016). Unfortunately, many invasive species go unnoticed upon introduction and remain undetected until their populations are well established and causing significant damage to crops and other cultures. Once established, it is often difficult to control these species effectively. Due to raising concerns about use of chemical insecticides (because of health and environmental risks) (Ansari et al. 2014) as well as increasing resistance of pest species to various chemical treatments (Siddiqui et al. 2023), an integrated pest management using biological control agents is increasingly being adopted and has the potential to effectively manage pest species.

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Current study is a part of collective effort of several researchers from western Balkans to harmonize IBCA regulations across the region. Although, there were no systematic research efforts to determine field presence and distribution of invertebrate species from EPPO Appendices I and II in Serbia, provided lists should be used as a foundation for official legislative of Serbian government.

There are two more steps which will considerably improve the IBCA regulative in Republic of Serbia, beside the document based on this study. First, there is a need for governmental founded project which will evaluate field presence and distribution of IBCAs in Serbia, and a lists should be updated according to results. Furthermore, the project should also identify the genetic variability of most important IBCAs because there could be different control potential in different strains of BCA.

The second step is to introduce pre-emptive biological control in official legislative. The pre-emptive biological control is a novel approach which represents a potentially more effective way of pest control, where natural enemies can be selected, assessed and

ready for release when the pest species arrive (Gonzalo et al. 2023). For example, a fall army worm, *Spodoptera frugiperda* (Lepidoptera, Noctuidae), is a major polyphagous pest native to tropical Americas and spread throughout the world, but still not reported from Serbia. In Europe, it is detected in Cyprus, Greece, Portugal, Spain and Turkey (www.eppo.int). In the region where this species is present it can cause up to 50% of yield loss of maize, and is also a serious economic threat to the production of over 80 different crops (Bakry and Abdel-Bakry, 2023). Several different agents from the genera *Trichogramma* and *Telenomus* are effective in biological control (Jin et al., 2021, Colmenarez et al. 2022). On the EPPO list Biological control agents safely used in the EPPO region (PM6/3(5)), the genus *Trichogramma* is represented by 6 species, while *Telenomus* is still absent. Of these species, two are reported to parasitize the egg masses of *Spodoptera* (*T. evanescens* and *T. dendrolimi*). Currently, only *T. evanescens* is recorded in Serbia, however, we suspect that *T. dendrolimi* is also present, as it has been reported from neighboring countries (Hungary, Bulgaria, Romania) (Noyes, 2019). According to current predictions (Gilioli et al. 2023), transient populations of *Spodoptera frugiperda* could make local damage to maize production in Serbia. *Trichogramma evanescens* could be a prospective candidate for pre-emptive biological control of *S. frugiperda* in Serbia. However, thorough field surveys, screening and assessment in laboratory conditions are needed in order to establish its potential.

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