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Testing a modified version of the EPPO decision-support scheme for release of classical biological control agents of plant pests using *Ganaspis* cf. *brasiliensis* and *Cleruchoides noackae* as case studies

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Abstract

The 6/04 standard of the European and Mediterranean Plant Protection Organisation (EPPO) on the safe use of biological control is a decision-support scheme (DSS) for the import and release of biological control agents in Europe. It was recently developed by the Joint EPPO/International Organisation of Biological Control (IOBC) Panel on Biological Control Agents. The DSS can be used to assess the potential environmental impacts of biological control agents. It is valid for different types of biological control: classical and augmentative biological control of invertebrates, pathogens and weeds. However, the DSS is not yet widely implemented in Europe and, during preliminary trials, it was found that its broad range of usages could lead to some confusion or misunderstandings, as well as requiring unnecessary information in some cases. Thus, the scheme was modified to specifically assess classical biological control against plant pests, i.e. the introduction of exotic natural enemies of plant pests for establishment and long-term control. The new version of the scheme was then tested on two parasitoids that are presently being released in Europe, the figitid *Ganaspis* cf. *brasiliensis* against the spotted wing drosophila *Drosophila suzukii* and the mymarid *Cleruchoides noackae* against the Eucalyptus bronze bug *Thaumastocoris peregrinus*. Both parasitoids were successfully assessed with the new version of the DSS. No major issues were encountered during the assessments and most questions were answered with low levels of uncertainty. Both assessments concluded that the parasitoids were safe to release in the impact assessment areas, with positive impacts exceeding negative impacts. Suggestions for potential improvements are provided.

Keywords

bronze bug, *Drosophila suzukii*, importation, invasive species, *Thaumastocoris peregrinus*

Introduction

The number of invasive plant pests, in particular herbivorous insects and plant pathogens, is increasing dramatically around the world, despite improved quarantine measures and border security (Santini et al. 2013; Roques et al. 2016; Seebens et al. 2017). This increase is largely due to the exponential growth of the transcontinental trade of fruits, vegetables, live plants and other plant products, such as wood packaging material, seeds and cut flowers, on which plant pests are travelling inconspicuously (Kenis et al. 2007; Santini et al. 2018). Once established, invasive plant pests are difficult to manage because of their uncontrolled development in regions where natural enemies are lacking and host plants have not developed resistance mechanisms against them (Desurmont and Pearse 2014; Garnas et al. 2016; Lovett et al. 2016; McLaughlin and Dearden 2019).

One method for long-term management of invasive plant pests is classical biological control (CBC), i.e. the introduction of natural enemies of exotic origin to control invasive pests, aiming at permanent control of the pest (Van Driesche and Bellows 1996). CBC has been implemented since the 19th century, mostly against invasive insects using parasitoids and predators. Over 700 insect pests have been targeted, with significant successes (Cock et al. 2015, 2016; Kenis et al. 2017). However, in some cases, negative effects have been observed on non-target species, especially when polyphagous parasitoids or predators were used (Hajek et al. 2016). Therefore, nowadays, the introduction of CBC agents usually follows strict procedures, including a rigorous assessment of their potential non-target effects (Van Driesche and Hoddle 2016). Even so, regulatory frameworks and procedures for the assessment and release of CBC agents vary significantly amongst countries and continents, according to their own legal and societal context and their history of using biological control practices (Afonso et al., in press).

In Europe, the use of classical biological control is hampered by the heterogeneity of national legislation (when existing) and practices and by the lack of common regulations. The European and Mediterranean Plant Protection Organisation (EPPO) has recently published the standard PM 6/04 on the safe use of biological control, presented as a decision-support scheme (DSS) for the import and release of biological control agents of plant pests (EPPO 2018). It was written mainly for decision-makers and assessors of biological control applications to harmonise assessment procedures within EPPO countries. It provides detailed instructions for environmental impact assessment for biological control agents of plant pests for augmentative biological control (ABC) and classical biological control. Although its title states it is for biological control agents of plant pests, it can also be applied to biological control agents of weeds.

However, the EPPO PM 6/04 standard is not yet widely implemented in Europe. In this paper, we tested it to assess the potential as well as safety of two exotic parasitoids, *Ganaspis* cf. *brasiliensis* (Ihering) (Hym., Figitidae) and *Cleruchoides noackae* L in and Huber (Hym., Mymaridae), for release against *Drosophila suzukii* Matsumura (Dipt., Drosophilidae) and *Thaumastocoris peregrinus* Carpintero and Dellapé (Hem., Thaumastocoridae), respectively. In preliminary trials, we noticed ambiguities due to the fact that the DDS was made to cover both augmentative and classical biological control agents (including native species) in addition to invertebrate and weed biological control. Therefore, we modified the scheme and retained only questions relevant for CBC against invertebrates using exotic natural enemies.

Description of the original EPPO standard PM 6/4

The EPPO standard PM 6/04 "Decision-support scheme for import and release of biological control agents of plant pests" (EPPO 2018) was prepared by the Joint EPPO/IOBC Panel on Biological Control Agents. It follows the recommendations of the International Standard for Phytosanitary measures 3 (ISPM 3) "Guidelines for the export, shipment, import and release of biological control agents and other beneficial organisms" (FAO 2017) and complements the earlier EPPO Standard PM 6/2 "Import and release of non-indigenous biological control agents" (EPPO 2014) by converting the guidelines into a question-based decision-support scheme.

The DSS is based on a sequence of questions that aim at deciding whether the introduction of a biological control agent (BCA) could cause unwanted environmental impacts and to compare the likelihood and impact of such negative effects with potential positive environmental effects. The DSS is a comprehensive document and rather unique amongst protocols and standards to assess the potential environmental impacts of biological control agents. To the best of our knowledge, no other DSS exist for environmental impact assessments (EIA) of biological control agents. It is valid for different types of biological control: classical and augmentative biological control of invertebrates, pathogens and weeds.

The document consists of two main parts: (I) an express scheme which may produce a rapid result and (II) a comprehensive scheme for certain cases of biological control, for which the express scheme does not lead to sufficiently clear recommendations. Within part I, the following steps are included: Step 1 Initiation; Step 2 BCA categorisation; Step 3 Impact assessment (four questions); Step 4 Decision taking. Part II consists of: Step 1 Pre-assessment (18 questions); Step 2 Assessment of probability of establishment (20 questions); Step 3 Assessment of probability of spread (three questions); Step 4 Assessment of potential environmental consequences (37 questions); Step 5 Recording the degree and types of uncertainty (one part); Step 6 Conclusion of the EIA. The latter is subdivided into the categories: establishment; spread; environmental impact; overall conclusion.

Case study 1: *Drosophila suzukii* and its parasitoid *Ganaspis* cf. *brasiliensis*

Drosophila suzukii is an East Asian fruit fly that is particularly damaging for small fruits such as cherry, strawberry, raspberry, blueberry and blackberry. In contrast to most other drosophilids, females of *D. suzukii* have a strongly serrated ovipositor and can lay eggs through the skin of mature, undamaged fruits (Asplen et al. 2015). It was first found in Europe and North America in 2008 and it is now present in most European countries, as well as in numerous regions of North and South America, western Asia and Africa (Rossi-Stacconi 2022). *Drosophila suzukii* is particularly difficult to control in invaded regions (Mazzi et al. 2017; Santoiemma et al. 2020; Tait et al. 2021). Current control methods are restrictive and expensive, especially because *D. suzukii* has many generations per year in a very large number of cultivated and wild fruits (Kenis et al. 2016). As a result, crops are constantly invaded from the surroundings. Against this type of invasive pest, large-scale control strategies, such as CBC, are recommended, to lower pest populations both in cultivated and wild habitats (Haye et al. 2016; Wang et al. 2020).

Extensive surveys for parasitoids of *D. suzukii* have been conducted in the area of origin of the pest, viz. Japan, China and South Korea (Kasuya et al. 2013; Daane et al. 2016; Girod et al. 2018a; Matsuura et al. 2018; Fang et al. 2019; Giorgini et al. 2019; Kimura and Mitsui 2020). In most surveys, the figitid wasp *Ganaspis brasiliensis* was the most abundant parasitoid. Subsequent studies in the laboratory also showed that this species was more specific than other common parasitoids of *D. suzukii*, such as *Asobara japonica* and *Leptopilina japonica* (Girod et al. 2018b; Giorgini et al. 2019; Daane et al. 2021). However, they also showed marked differences in host specificity amongst populations of *G. brasiliensis* (Girod et al. 2018b; Seehausen et al. 2020). Previous molecular studies in Japan by Nomano et al. (2017) had already shown that *G. brasiliensis* was composed of five genetic groups (corresponding to biotypes or possibly cryptic species), with different host preferences and geographic distributions. Surveys in Asia and subsequent molecular analyses revealed that at least two or three genetically distinct groups (G1, G3 and possibly G4) attack *D. suzukii* (Giorgini et al. 2019; Seehausen et al. 2020). Under laboratory conditions, one genetic group (G3–4) readily parasitised some closely-related *Drosophila* species, such as *D. melanogaster* and *D. simulans* regardless of their food source. In contrast, the other genetic group (G1) was almost entirely specific to larvae feeding in ripening fruits (Seehausen et al. 2020). Further studies showed marked genetic differences between those populations (Seehausen et al. 2020) and their expressed proteins (Reeve and Seehausen 2019). In addition, these two groups did not interbreed and it is plausible that these are two cryptic species. The discovery that *G.* cf. *brasiliensis* is likely a species complex of at least two congeneric species and that one of them can clearly be associated with a higher habitat specificity to fresh fruits, shows the suitability of *G.* cf. *brasiliensis* G1 to control *D. suzukii*. This allows research to focus on that species to assess its potential as a classical biological control agent. The difference in natural habitat preference between immature *D. suzukii* developing in fresh ripe fruits and other frugivorous *Drosophila* species in decomposing fruits supports the hypothesis that *G.* cf. *brasiliensis* G1 is host-specific. This specialisation would then support the conclusion that *G.* cf. *brasiliensis* G1 wasps will only parasitise *D. suzukii* in its invaded range.

In 2021, large-arena field cage releases of *G.* cf. *brasiliensis* G1 conducted in Switzerland confirmed that this species has a very high preference for *D. suzukii* in fresh fruits (Seehausen et al. 2022). At the same time, *G.* cf. *brasiliensis* G1 was found to be adventively present in western Canada (Abram et al. 2020) and the western USA (Beers et al. 2022).

The modified EPPO DSS for *G.* cf. *brasiliensis* G1 included an impact assessment area (IAA) comprising all European countries west of Russia (excluding EU and UK Overseas Countries and Territories). For the purpose of testing the DSS, the risk assessment was carried out, based on the assumption that no release has been made in Europe at the time of the assessment, although *G.* cf. *brasiliensis* G1 was released in 2021 in Italy after a risk assessment was conducted at the national level (Lisi et al. 2022; Fellin et al. 2023).

Case study 2: *Thaumastocoris peregrinus* and its parasitoid *Cleruchoides noackae*

The Eucalyptus bronze bug *Thaumastocoris peregrinus* Carpintero and Dellapé, (Hem., Thaumastocoridae) is native to Australia where it is distributed across several climatic regions (Nadel et al. 2012). This hemipteran is a sap sucker that feeds on the foliage of a broad range of *Eucalyptus* species. Its feeding activity causes necrosis and discolouration of the tree canopy. Attacked leaves exhibit a characteristic bronze colour, followed by drying. The intensive damage in the canopy results in tree growth losses and weakens the trees. Both in its native and invaded range, the bronze bug is found in forest plantations as well as on ornamental trees in urban areas, such as in streets and gardens (Nadel et al. 2012; Laudonia and Sasso 2012; Garcia et al. 2013). Extensive damage has been reported in the countries where this species is established, with significant economic losses. For example, the loss in wood production caused by *T. peregrinus* was estimated to exceed US$ 380 per hectare in Brazil (Junqueira et al. 2018). Many ecosystem services provided by *Eucalyptus* trees are also disrupted by the *Eucalyptus* defoliation (Branco et al. 2015).

The bronze bug was first found outside Australia in South Africa in 2003 (Jacobs and Neser 2005) and in Argentina in 2005 (Noack and Coviella 2006). These two separate introductions correspond to two different Cytochrome Oxidase subunit 1 (COI) haplotypes (Machado et al. 2020), but probably both originate from a population in Sydney (Montagu et al. 2020). From Argentina, the bronze bug spread rapidly to nearby countries in South America and, similarly, from South Africa, it spread to neighbouring African countries (Hurley et al. 2011). In Europe, the species was first found in Italy in 2011 (Laudonia and Sasso 2012) and later in Portugal (Garcia et al. 2013) and Spain (Nascimento-Machado et al. 2019). European populations are the same haplotype as that present in South America (Machado et al. 2020). Molecular data support the hypothesis that the bronze bug was introduced from South America to Europe and then spread to Israel (Montagu et al. 2020).

Insecticides such as imidacloprid, thiamethoxam and acephate were tested and shown to be effective against the bronze bug (Noack et al. 2009; Wilcken et al. 2019). Biopesticides *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin (Cordycipitaceae) and *Metarhizium anisopliae* (Metchnikoff) Sorokin (Clavicipitaceae) also showed good results (Wilcken et al. 2019). However, insecticides are not economically nor ecologically sustainable, especially as a long-term strategy and their application in forest plantations and urban areas is highly restricted by national regulations. The search for biopesticides provided promising results with *B. bassiana* (Corallo et al. 2019). However, there is a high variability amongst strains and the biopesticide's efficiency is highly dependent on environmental conditions, such as temperature and humidity. Moreover, the need for continuous applications renders this management strategy costly. Selection for host resistance was not considered a viable management option, due to the broad host range of *T. peregrinus* (Nadel and Noack 2012). Biological control using the egg parasitoid *Cleruchoides noackae* Lin and Huber (Hym., Mymaridae) is currently the main control strategy in the invaded areas with effective and sustainable long-lasting results.

Cleruchoides noackae is a tiny wasp, < 0.5 mm in length, which was described by Lin et al. (2007) from parasitised eggs of *T. peregrinus* collected in Australia. The species can be unambiguously identified by morphological traits. Molecular analysis, using COI confirmed that *C. noackae* is widespread in Australia (Nadel et al. 2012).

The host range of *C. noackae* consists of species within the Thaumastocorinae subfamily, in particular, in the genera *Thaumastocoris* and *Baclozygum* (Lin et al. 2007; Cross 2009). The family Thaumastocoridae is native to Australasian regions and there are no species native to Europe. Therefore, non-target parasitism is unlikely in the introduced range. An unpublished study in Portugal (CA and MB) used no-choice tests to assess potential use of 19 non-target host species within eight Hemiptera families (Anthocoridae, Miridae, Tingidae, Coreidae, Pentatomidae, Pyrrhocoridae, Rhopalidae and Scutelleridae). Parasitism occurred only on *T. peregrinus*, suggesting that *C. noackae* will exclusively attack only the target host in Europe.

Cleruchoides noackae was released and became established in South Africa, South America (Chile, Brazil and Uruguay) (Nadel and Noack 2012; Barbosa et al. 2017) and Israel (Mendel and Protasov 2019). In a post-release study conducted in Brazil, Barbosa et al. (2017) showed that one year after the release, the parasitoid could be found up to 10 km from the release site. Given the small size of *C. noackae*, its capacity for active dispersal is possibly limited. Still, there is a high possibility of natural dispersal by wind.

Due to the specialised trophic relationships amongst *C. noackae*, *T. peregrinus* and *Eucalyptus* spp., in which all are non-native to Europe, indirect effects on non-target species are highly unlikely, as well as other negative environmental effects in native flora and fauna.

Release of *C. noackae* in Portugal was authorised in 2021 by the Portuguese National authorities, ICNF – Instituto de Conservação da Natureza e Florestas with the condition of a no release buffer zone of 20 km on the border with Spain. Initial releases were made in October 2021 in Central Portugal and post-release monitoring data are not yet available. *Cleruchoides noackae* is not present in other European countries where it could have a beneficial impact.

The modified EPPO DSS was tested for release of *C. noackae* in southern Europe. The IAA includes all European countries where *Eucalyptus* trees are planted and used for production or amenity objectives. Once again, for the purpose of testing the DSS, the risk assessment was carried out with the assumption that no release has been made in Europe at the time of the assessment.

Critical evaluation of the EPPO DSS

The DSS includes questions about any potential earlier assessments (section 1.2 in the Express assessment and 1.04 in the Full assessment), which may save resources. If the BCA, or a very similar organism (e.g. another population of the same species), may have been subjected to an EIA process before, nationally or internationally, this may partly or entirely replace the need for a new assessment. These questions could be enhanced by requesting information about previous releases of the same BCA, for example, in similar regions/conditions (if yes, how long ago?) and what was the outcome in terms of establishment, impact on target species and non-target species? For both case studies, *G.* cf. *brasiliensis* and *C. noackae*, this would be necessary and useful information to render the decision process for or against releases in additional countries more efficient. For example, information of the outcome of prior releases of *C. noackae* in South Africa, South America and Israel were instrumental for the release application in Portugal. Similarly, for the *G.* cf. *brasiliensis* release application in Switzerland, results from studies on releases or adventive distributions in North America and Italy were considered important information for the risk assessment.

The DSS provides the opportunity to include undescribed cryptic species as potential BCAs, as it includes consideration of taxonomic levels higher or lower than species, including those characterised using molecular methods (Step 2 BCA categorisation of the Express assessment and step 1.02 of the Full assessment). This is important in high priority cases like the CBC of *Drosophila suzukii*, where only one genetic group of the parasitoid *G.* cf. *brasiliensis* (a probable cryptic species) is specific to *D. suzukii* larvae feeding in fresh fruits.

The DSS starts with the Express assessment for invertebrates and pathogens whereas, for weed biocontrol, it is recommended to start directly with the Full assessment. This is based on the higher risk of weed BCAs becoming pests of important crops. While the Express assessment may be convenient in some cases and save resources such as time and money, especially for augmentative BCA, few countries, if any, in the EPPO region would allow an Express assessment for new CBC agents of plant pests. This is especially the case in countries like France, Israel, Italy or Switzerland, where national authorities require comprehensive risk assessments for the release of any non-native species. Therefore, any new CBC, whether it is introduced against weeds or plant pests, should only be evaluated using the Full assessment.

The sections about agent establishment (Step 2) and spread (Step 3) in the Full assessment are very detailed and comprehensive. While for the assessment of agent establishment, a distinction between different types of biological control (ABC vs. CBC) is possible, this is not the case for the assessment of spread. In ABC, agents are released in the field or in greenhouses to augment the numbers of a species that are native or already established. In the case of ABC for protected crops, an agent is regularly released for temporary control. Establishment in the wild and spread of the released populations of an agent are, in most cases, undesired in ABC, because the aim is to minimise unwanted effects on the environment and non-target species. In contrast, CBC is the intentional introduction of a species into an area where it is not indigenous and, to be successful, it must establish and spread on its own after release. Therefore, for CBC, the section dealing with establishment and spread of a BCA is relevant to assess the feasibility, efficiency and economic risk of a CBC programme, but not for an EIA because the establishment and spread to all areas where the host/prey is present has to be assumed. However, this means that the EIA of CBC agents must very thoroughly assess possible non-target impacts (especially including host specificity) in the entire geographic area to which the impact assessment applies. In contrast, ABC is applied in a defined place such as a cropland and it frequently involves generalist agents. The spread of released BCAs to areas outside the IAA can, therefore, have a detrimental effect on native species and their populations. In the current DSS, the same questions about establishment and dispersal are asked for both ABC and CBC and it remains unclear from the document if the answer is actually a benefit (e.g. high probability of spread and establishment for CBC agents) or a risk (e.g. high probability of spread and establishment for ABC agents). A solution to this problem would be to have a separate DSS for the different types of BC. In a DSS for CBC, many questions about establishment and dispersal would then not be necessary and could be simplified into just a few questions to ensure that establishment is likely. As for the spread, the questions could be totally suppressed, since an established CBC agent will spread anyway, no matter the rapidity of spread. If the natural spread is too slow, the BCA can be distributed through a release programme. Additionally, other questions, for example, whether the BCA is already present or indigenous in the IAA or may cause transient effects, would then become obsolete. Applied to the case studies, a first introduction of the CBC agents into a European country would imply that establishment and spread into neighbouring countries, where the target and its host plant are present and where climate is suitable has to be assumed and thus, the IAA needs to include these countries. In the case of *C. noackae*, all southern European countries where *Eucalyptus* trees are planted would be included and, for G. cf. *brasiliensis*, all European countries.

Several questions are strictly related to CBC of weeds. While it is clearly mentioned in the DSS that such questions have to be answered only in the case of CBC of weeds, these questions needlessly lengthen the scheme under which CBC agents of invertebrates are assessed. Furthermore, the potential impact of weed BC agents (herbivores and plant pathogens) are different from those caused by invertebrate BC agents (parasitoids, predators and entomopathogens), so that a full separation from CBC of weeds and pathogens would allow more precise responses to questions on impact mechanisms, establishment and spread.

One of the clear strengths of the DSS is the consideration of positive environmental impacts of BCA releases. This is a great step forward compared to most processes that solely consider risks. While the selection of the risks and benefits that are included in the evaluation provide an objective baseline, the decision whether the positive impacts exceed the negative ones, remains a subjective expert opinion. In CBC, decision-making processes should weigh both the risks and potential benefits. These benefits should include environmental and socio-economic considerations, such as direct and indirect benefits for farmers/producers and the general public. For example, in the case of *G.* cf. *brasiliensis* releases against *D. suzukii*, environmental benefits (e.g. reduction of pesticide use and reduction of infestation of wild fruits), as well as socio-economic benefits (e.g. reduction of costs for protecting crops), would be expected and these reduce the high losses fruit growers have through this invasive species (Burrack 2015; De Ros et al. 2015; Knapp et al. 2020). However, if the goal of the DSS is to be purely an environmental risk-benefit assessment, this latter suggestion might not be applicable.

Another strength of the DSS is the recording of the degree and types of uncertainty (Step 5 in the Full assessment) when it comes to potential environmental consequences. As some aspects of the risks and benefits may be based on subjective expert opinions, limited data or only on available data from closely-related species, the level and type of uncertainty helps to identify weaknesses in the assessment, research needs or even unacceptable risks for approving releases. What is unclear in this step is the definition of the consequences if the uncertainty is high. A probable consequence in the case of high uncertainty could be the requirement to gather more data through literature reviews or additional tests. Thus, there should be clear directions on what steps should be taken if uncertainties are high.

The overall conclusion of the DSS (Step 4 Decision taking in the Express assessment, Step 6 of the Full assessment) should be a clear acceptance or rejection of the application to import and release a BCA. While this is sufficiently clear in the Express assessment through the binary outcome in sections 4.3 (recommendation for releases) and 4.4 (no recommendation for releases), it is not clear in the Full assessment. However, the Full assessment allows the decision-maker to comment on and justify the conclusion in each of the three major sections: establishment, spread and environmental impact.

Modifications of the DSS for classical biological control of plant pests

Based on the considerations above, we have modified the EPPO Standard PM 6/04 (1) Decision-support scheme for import and release of biological control agents of plant pests (EPPO 2018) for specifically assessing the potential for releases of CBC agents of plant pests (Suppl. material 1). Only modifications essential for this specific type of assessment were made. Other changes, in the format and the phrasing, to be precise, have been kept to a minimum. As for the original standard, the decisions are based only on environmental impacts (negative and positive), not on socio-economic considerations.

The main modifications are as follows:

- The express scheme has been deleted.
- All questions specifically related to weed biological control have been deleted.
- All questions are now unambiguously concerned with classical biological control, i.e. the introduction of an exotic BCA (usually parasitoid or predator) against a plant pest, for a permanent establishment of the BCA leading to long-lasting control of the target pest. Some questions have been modified and all questions referring more specifically to augmentative biological control have been suppressed.
- The section on the assessment of probability of spread has been deleted.

The new scheme is presented in Suppl. material 1. The modified DSS now starts with a short pre-assessment (Step 1) to present the BCA and to ensure that it is a priori suitable for classical biological control in an IAA. Step 2 involves assessing the probability of establishment in the IAA. Step 3 consists of an assessment of potential environmental consequences. The degree and types of uncertainty are considered in Step 4 and Step 5 provides the conclusion of the EIA.

Assessment of *Ganaspis* cf. *brasiliensis* using the modified DSS

The Full assessment is presented in Suppl. material 2. For the most part, the assessment was straightforward because the parasitoid has been extensively studied. Thus, most questions were answered indicating low levels of uncertainty. The questions for which answers were given with medium or high uncertainty were particularly those on climate suitability and positive environmental impacts that may result from controlling *D. suzukii*. In some cases, answers were more complex due to the data on the parasitoid coming mostly from laboratory rearing and, to a much lower extent, from the field in its native range, whereas hardly any data are available from other regions of introduction. However, typically, baseline information from the field in the native range and from the quarantine laboratory in the area of introduction is used to predict the potential impact in the area where releases are planned.

The DSS suggested that *G.* cf. *brasiliensis* G1 is a safe biological control agent that can be released with minimal risk in Europe. Establishment of *G.* cf. *brasiliensis* G1 was considered very likely in a large part of the IAA because of the widespread distribution of its host, as well as climate conditions that are largely similar to its native range and other invaded areas in North America. However, a precise mapping of its establishment potential in the IAA could not be made because data on its climate requirements are lacking and the precise distribution of *G.* cf. *brasiliensis* G1 in Asia is still poorly known.

The negative environmental impact was considered minimal, mostly because the parasitoid is specific to *Drosophila* spp. infesting fresh fruits. In the laboratory, *G.* cf. *brasiliensis* G1 can occasionally attack closely-related species, such as *D. melanogaster*, a species only rarely found in fresh fruits (Girod et al. 2018b; Seehausen et al. 2020). In addition, during surveys in Asia, *G.* cf. *brasiliensis* were only obtained from *Drosophila* spp. in fresh ripe fruits that were collected directly from the plant and were never obtained from fruit baits (typically sliced banana pieces), indicating a high degree of habitat specificity under field conditions (Kasuya et al. 2013; Daane et al. 2016; Girod et al. 2018a; Giorgini et al. 2019). In Europe, the only *Drosophila* sp. occurring in fresh fruits is *D. suzukii*; therefore, field parasitism of other *Drosophila* spp. is likely to be very low and without negative impacts on their populations and their role in the ecosystem. The positive environmental impact is considered moderately important, mostly because of the expected reduction in pesticide applications, use of protective netting and mass trapping. Thus, it is clear that the positive environmental impacts largely outweigh the risk of causing negative environmental impacts on native biodiversity and ecosystem patterns and processes. This does not include the socio-economic impacts, which would presumably be noteworthy if the parasitoid successfully controls *D. suzukii* in fruit production. Nevertheless, as in all classical biological control programmes, non-target effects should be carefully monitored through post-release studies.

Of particular interest was the fact that the DSS gave the possibility to consider undescribed cryptic species as potential BCAs, provided they can be unambiguously categorised. The fact that the different genetic groups (or cryptic species) of the *G.* cf. *brasiliensis* complex can be separated using molecular analyses and that only one genetic group of the parasitoid, *G.* cf. *brasiliensis* G1 is specific to *D. suzukii* larvae feeding in fresh fruits (Girod et al. 2018b; Seehausen et al. 2020) strongly supports justification for release of this biological control agent. Furthermore, considering that the introduction of the other genetic groups, *G.* cf. *brasiliensis* G3–G4, has a higher risk of non-target effects, because they also attack indigenous *Drosophila* spp. larvae in decomposing fruits in the region of potential introduction (e.g. North America or Europe) clearly demonstrates the value of DNA analysis to tease out genetic groups that cannot be separated through morphological characteristics (Nomano et al. 2017; Seehausen et al. 2020).

In conclusion, the DSS has demonstrated that *G.* cf. *brasiliensis* G1 is a safe biocontrol agent that can be released with minimal risk in Europe, while meaningful non-target risks were identified for other genetic groups of *G.* cf. *brasiliensis* (see Suppl. material 2).

Assessment of *Cleruchoides noackae* using the modified DSS

The Full assessment is presented in Suppl. material 3. The assessment was easy to follow. *Cleruchoides noackae* was used in other biological control programmes in South Africa, South America and Israel and was the object of several studies, which facilitated its assessment for release in Europe (Nadel and Noack 2012; FABI 2013; Barbosa et al. 2017). The specificity of *C. noackae* to the Thaumastocorinae subfamily, native and restricted to Australia (Lin et al. 2007; Cross 2009), minimises uncertainties related to its impacts on non-target species and native communities in Europe. The host specificity study from Portugal supports the conclusion of minimum uncertainty of impacts on non-targets. This study was included in the official application for releases, but the data are still unpublished. Although *C. noackae* was also assessed in other non-European countries (South Africa, Israel and Brazil) to our knowledge, the results have not yet been published.

An important point to note is that the three trophic levels involved in this system; the host plants, the insect pest and the parasitoid are all non-native to Europe and considering the specificity of the parasitoid, detrimental impacts of the releases on native species, native communities and conservation areas, are expected to be negligible. Based on this and on previous field and laboratory data, most questions regarding environmental risks were answered with minimal risk and low uncertainty. In this regard, the DSS provided strong arguments related to the minimal risk of releasing *C. noackae*. The questions for which answers were given with medium certainty were those on climate suitability and establishment probability. Data on the life cycle are available from laboratory studies (e.g. Barbosa et al. (2018)), but there is not much field data, specifically on the ability of the parasitoid for overwintering in cold winters. This might limit its establishment or efficiency in the colder areas of southern Europe. Additionally, there are no studies on climate distribution models applied to this species. Still, its current distribution in the native and introduced range includes several regions climatically similar to southern Europe.

There are socio-economic arguments in support of biological control of *T. peregrinus* that were not considered in the assessment. *Eucalyptus* plantations are highly valued for the fast-growing nature of the trees and their production of foliage, essential oils, wood, biomass and raw material for the pulp industry. Currently, *Eucalyptus* plantations in Europe occupy an area of more than 1.8 million ha. Although the expansion of *Eucalyptus* plantation in the Mediterranean Region has sometimes been associated with negative environmental impacts (Tomé et al. 2021), *Eucalyptus* trees also have many positive environmental impacts related to soil conservation, climate amenity, carbon sequestration and provision of nectar and pollen for pollinators, amongst others (Branco et al. 2015; Tomé et al. 2021). In urban areas, these trees are often used in streets and gardens for amenity purposes. The control of *T. peregrinus* or of other pests affecting *Eucalyptus*, will thus have significant positive impacts. The alternative method of applying insecticides would be costlier and would imply negative environmental impacts. In contrast, no negative environmental impacts are expected with the release of *C. noackae*. Therefore, there is low uncertainty when considering the positive environmental impacts related to the reduction of *T. peregrinus*. It should be noted that the distribution of *Eucalyptus* plantations in Europe is mainly concentrated in Portugal and Spain, so overall, we consider the expected impact will be moderate.

The DSS demonstrated that *C. noackae* is a safe biological control agent that can be released with minimal risk in Europe and that the positive environmental impacts largely outweigh the risks or uncertainties surrounding its establishment. Still, the establishment should be carefully monitored through post-release studies.

Conclusions and recommendations

Both *G.* cf. *brasiliensis* G1 and *C. noackae* were successfully assessed with the modified version of the DSS for CBC of plant pests. No major issues were encountered during the assessments, most questions being answered with low levels of uncertainty. For both species, the outcome of the assessment was that the parasitoids were safe to release in the IAA, with the positive impacts exceeding the negative ones. The scheme would need to be further tested on agents that are less specific; however, there is evidence from unpublished assessments with polyphagous natural enemies where no major issues were apparent and the important risks were clearly highlighted.

The two parasitoids assessed in this study were well known due to previous releases in other regions in the case of *C. noackae* and because of extensive studies in Europe in the case of *G.* cf. *brasiliensis* G1. Potential CBC agents that are less studied would be more difficult to assess, resulting in higher uncertainties. The DSS could mention that species that have been well studied and are ready to be released are better suited to this assessment. On the other hand, the scheme could also be used at an earlier stage of a CBC programme to identify priorities for research, such as host range testing or climate matching models.

The goal of modifying the DSS was to build a version that is specifically and unambiguously made for assessing classical biological control agents of plant pests, but without changing the general aim and structure of the scheme and keeping other changes, for example, in the wording, to a minimum. However, the scheme could be further developed in various ways, outlined below.

1. Step 2. Assessment of probability of establishment could be further shortened. Several criteria for assessing establishment of the agent are still considered in our revised version in an effort to find a compromise, but most of them are more relevant for ABC or for CBC of weeds. In CBC of plant pests, the most relevant criteria are factor 1 about the presence of the hosts or prey and factor 3 about a suitable climate. Questions on other abiotic factors, on competition and natural enemies preventing establishment or on the adaptability of the BCA agent may be important in specific cases, but are often difficult to answer. Questions on management practices and control measures that may prevent establishment may be relevant, but all CBC practitioners will first release CBC agents for establishment at sites that are not managed with pesticides. The question on the presence or need of alternative hosts and other essential species is ambiguous and could be rephrased. For CBC, attacks of alternative hosts, i.e. those that occur at the same time as the target species, would be undesired, unless it is another pest that needs to be controlled. For some agents, the presence of an alternate host, i.e. a host that can be attacked before or after the occurrence of the target pest, may be crucial for the establishment in a new area and, thus, important for the success of a CBC programme. On the other hand, if the alternate host is native, it implies that non-target effects will occur. In most CBC programmes, no alternative nor alternate host is required for its success.
2. Step 3. Assessment of potential environmental consequences is based on a comparison and balancing between the negative (risks) and the positive (benefits) environment impacts. However, they are not assessed equally. In the positive impact assessment, only environmental criteria are included. In contrast, in the negative impact assessments, there are also four questions on negative impact on ecosystem services (3.02.12–3.02.15), based on the EFSA (2016) criteria. Impacts on ecosystem services implies socio-economic impacts. For example, provisioning ecosystem services includes provision of food, raw materials etc. In addition, impacts on cultural ecosystem services also clearly imply socio-economic impacts. Often, a decision to release a CBC agent takes into consideration the balance between environmental risks and socio-economic benefits. Thus, the questions of the DSS should explore both the negative and positive impacts for the environment, as well as from the socio-economic point of view. If possible, a positive socio-economic impact component should be included in

the DSS, for example, a set of questions determining the socio-economic benefits of BCA releases and the expected reduction in pest impact. If the scheme has to remain purely an environmental risk assessment, the questions on negative impacts on ecosystem services (3.02.12–3.02.15) should be removed, since the negative environmental impacts are sufficiently covered by other questions.

3. Finally, a well-defined rating system could be developed for possible negative and positive impacts by the CBC agent to facilitate the overall conclusion and make the final decision transparent and clear. In such a rating system, the level of uncertainties could be used to facilitate the overall conclusion derived from the DSS. For example, if uncertainty about potential non-target effects is high, the rating system could suggest declining the release application until the uncertainties are resolved. We acknowledge that this is a controversial subject, which has previously been tackled by using risks versus benefits matrices (e.g. Moeed et al. (2006); van Lenteren and Loomans (2006)). The main difficulty with such scoring systems is that the assignment of values is subjective and required total scores for decision-making may vary between jurisdictions.

Besides the use of the DSS by decision-makers, other proponents of a biological control agent release, researchers, for example, can use the document to identify and gather the information that needs to be provided in an application for releases to regulatory authorities. It is important to point out here that the DSS is not a substitute for a release application, but it could be used to develop a standard template for preparing an application for CBC of a plant pest.

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Supplementary material 1

Decision-support scheme for release of classical biological control agents of plant pests – Environmental impact assessment (EIA)

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Supplementary material 2

Assessment of *Ganaspis* cf. *brasiliensis*

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Supplementary material 3

Assessment of *Cleruchoides noackae*

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