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Assessing the feasibility of pre-emptive biological control against the emerald ash borer, *Agrilus planipennis*, an imminent biosecurity threat to Europe

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HIGHLIGHTS

- Emerald ash borer is a key biosecurity threat to European ash forests.
- Pre-emptive biological control could improve preparedness for its arrival.
- Three parasitoids were deemed suitable for pre-emptive biological control in Europe.
- European countries should conduct pre-emptive risk assessment for these parasitoids.

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ABSTRACT

As the globalisation of trade increases, so does the spread of arthropod pests, leading to a growing focus on biosecurity preparedness. One approach to this is pre-emptive biological control, involving the importation of classical biological control (CBC) agents for risk assessments and acquiring approval for their release prior to the expected arrival of their target pests. This aims to mitigate the economic and/or environmental consequences of a delayed biological control response to pest invasions. Guidelines to assess the feasibility of pre-emptive biological control for high priority pests were recently developed. Emerald ash borer (EAB), *Agrilus planipennis*, is an invasive woodboring pest of ash (*Fraxinus* spp.) in North America, European Russia and Ukraine, and is spreading westward into Europe, threatening the future of European ash (*Fraxinus excelsior*). We applied the aforementioned guidelines to assess the feasibility of pre-emptive biological control in Europe using four EAB parasitoids, already released in North America for CBC. Three of the parasitoids; *Oobius agrili*, *Spathius galinae*, and *Tetrastichus planipennisi*, were found suitable for pre-emptive biological control. The fourth parasitoid, *Spathius agrili*, was found to have limited establishment in new environmental conditions, and was therefore deemed less suitable for pre-emptive biological control of EAB in Europe. This assessment can inform scientists and regulators in Europe on the most promising EAB parasitoids that should be considered for pre-emptive applications for importation and risk assessment to acquire pre-approval for immediate release should the target pest subsequently be discovered. In turn, this study contributes to the development of biosecurity preparedness against EAB's imminent spread throughout Europe.

1. Introduction

A key invasion pathway for invasive arthropod pests is international trade, and its increasing globalisation is causing a commensurate escalation in the spread of pests to new countries (Liebhold et al., 2016; Suckling et al., 2019; Tobin et al., 2014). Many countries are therefore

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adopting, and in some cases mandating, a biosecurity preparedness approach, involving, for example, proactive risk assessment and border surveillance for high priority pests (Caron et al., 2021; EU, 2016; Poland and Rassati, 2019). Scientists and administrative bodies can therefore develop management and/or eradication strategies before the arrival of a pest, to mitigate the long-term management costs of an increasing frequency of invasions (Brockerhoff et al., 2010; Suckling et al., 2012). Coinciding with this biosecurity challenge is a growing public awareness towards environmentally friendly, socially acceptable approaches to pest management (Mankad et al., 2017; Paterson et al., 2019; Suckling et al., 2017).

Classical biological control (CBC) is one such generally environmentally friendly method that comprises the importation of co-evolved natural enemies and their release in invaded areas for long-term control of invasive pest populations and their impact (DeBach and Rosen, 1991). The benefits of CBC include the rarity of ecologically relevant non-target impacts, its self-sustaining and cost-effective nature, and no evolved resistance in target pests (Bale et al., 2008; Collatz et al., 2021; van Lenteren et al., 2006). However, due to the purported irreversible risks to non-target organisms, governments generally stringently regulate the importation and release of CBC agents (Barratt et al., 2010; Barratt, 2011; Barratt et al., 2021), leading to the requirement of extensive and time consuming pre-release biosafety assessments (van Lenteren et al., 2003; van Lenteren et al., 2006). Logistical challenges, such as sourcing and shipping CBC agents, can also be burdensome (Avila et al., 2023). Procedures are conventionally initiated when the impact from an invasive pest has been realised, giving it enough time to spread and exert substantial damage before approval for CBC release is granted (Hoddle et al., 2018).

To mitigate this issue in CBC preparedness, a novel approach to CBC, termed pre-emptive biological control, was recently conceived. This approach aims to complete feasibility and biosafety assessments for promising candidate CBC agents, and obtain approval for their release, before the anticipated arrival of the target pest. This would allow CBC releases to commence immediately following the detection of pest incursions, thereby limiting their spread and alleviating the impact during the early stages of invasion (Caron et al., 2021; Charles et al., 2019; Hoddle et al., 2018). In a world first, New Zealand recently adopted this approach against *Halyomorpha halys* Stål (Hemiptera: Pentatomidae), a high-risk pest that is frequently intercepted at the border but not yet established (KVH, 2023). In response, the CBC agent *Trissolcus japonicus* Yang (Hymenoptera: Scelionidae) was imported into containment for biosafety assessment, and in 2018, release was approved conditional to a future *H. halys* incursion (Charles et al., 2019; Environmental Protection Authority, 2018). More recently, comprehensive guidelines were developed to define the requirements for pre-emptive biological control and provide a decision framework to assess its feasibility against high-risk pests (Avila et al., 2023).

Emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is a wood-boring beetle native to north-eastern China, Japan, Korea, and the Russian Far East that infests ash trees (*Fraxinus* spp.) (Orlova-Bienkowskaja and Volkovitsh, 2018). The larvae create boreholes to feed on the cambium and phloem, disrupting the movement of water and nutrients throughout the tree, which ultimately results in its death (Knight et al., 2013). Since the first detection of EAB outside its native range, in Michigan in 2002 (Cappaert et al., 2005), it has spread to 36 U.S. states and six Canadian provinces (CFIA, 2024; MapBioControl, 2023a), and has become the most destructive pest of American ash species (Klooster et al., 2014; Smith et al., 2015). The forecasted cost of managing EAB in the U.S. between 2010 and 2020, considering treatment, removal and replacement of infested trees, was USD$12.5 billion (Kovacs et al., 2011). In 2007, EAB was observed infesting American ash (*Fraxinus pennsylvanica* Marshall) throughout Moscow, Russia (Baranchikov et al., 2008). The insect spread further to St. Petersburg, the border with Belarus, Eastern Ukraine (Musolin et al., 2017; Musolin et al., 2021; Orlova-Bienkowskaja et al., 2020), and most recently it was also found in Kiev (EPPO, 2024). In the newly invaded areas it is causing substantial decline of European ash (*Fraxinus excelsior* L.) (Drogvalenko et al., 2019; Musolin et al., 2021; Orlova-Bienkowskaja & Bieńkowski, 2018, 2022a; Volkovitsh et al., 2021). The EAB-susceptible ash species are widespread throughout Europe, and the pest exhibits a flexible lifecycle across different climatic conditions throughout its native and invaded ranges (Baranchikov et al., 2008; Orlova-Bienkowskaja and Bieńkowski, 2016; Orlova-Bienkowskaja and Bieńkowski, 2022b; Valenta et al., 2015). The potential economic and environmental consequences of the anticipated incursion of EAB further into Europe is therefore generating significant concern (Evans et al., 2020; Musolin et al., 2021; Valenta et al., 2017; Volkovitsh et al., 2021), with administrative and advisory bodies considering it a high-risk priority pest (EPPO, 2023; Petter et al., 2020).

In North America, EAB is subject to an ongoing and successful CBC programme (Duan et al., 2018a, 2022a). Extensive natural enemy surveys in north-eastern China and subsequent biosafety assessments eventuated in approval to release three parasitoids in the US in 2007; *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae), *Spathius agrili* Yang (Hymenoptera: Braconidae), and *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae), with releases commencing that same year in Michigan (Bauer et al., 2008, 2015; Duan et al., 2015b; Yang et al., 2008). A fourth parasitoid native to the Russian Far East, *Spathius galinae* Belokobylskij and Strazanac (Hymenoptera: Braconidae), was later approved for release in 2015 (Duan et al., 2019a). Together, but with the exception of *S. agrili* due to a climate mismatch, these parasitoids have been released and successfully established in 16 states (MapBioControl, 2023b), where they are providing effective biological control of EAB and contributing to ash stand recovery (e.g. Duan et al., 2015a; Duan et al., 2017; Duan et al., 2022b; Margulies et al., 2017). Considering the impending threat of EAB in Europe and the wealth of available knowledge regarding effective CBC agents in North America, the scene is set to investigate the potential for pre-emptive biological control from the European perspective. In this paper, we summarise the pertinent information gathered through applying the aforementioned guidelines (Avila et al., 2023) to assess the feasibility of pre-emptive biological control, in the European context, for the four EAB CBC agents released in North America. We further provide comparative discussion on the relative feasibility of pre-emptive biological control amongst these parasitoids, and consider the implications of this for the outlook of pre-emptive biological control as a preparedness strategy against the anticipated invasion of EAB into Europe.

2. Assessing the feasibility of pre-emptive biological control for each candidate parasitoid

The guidelines developed to assess the feasibility of pre-emptive biological control (Avila et al., 2023) provide a robust set of key criteria that should be considered for both the selection of appropriate target pests for applying pre-emptive biological control, and to assess the feasibility of pre-emptive biological control using candidate CBC agents. These key considerations fall within the following overarching categories related to candidate agents: availability of information; taxonomic status; distribution and climatic suitability; biology and ecology; known effectiveness against the target host; availability of resident natural enemies as alternatives; challenges related to non-target risk assessment; and logistical challenges. Detailed explanations pertaining to the inclusion of the key criteria in the decision framework can be found in Avila et al. (2023). In this section, we summarise our findings on the performance of each candidate EAB parasitoid for each key consideration provided in the guidelines, and discuss the implications for the feasibility of pre-emptive biological control against EAB. The results are summarized in Table S1 and the full details surrounding the information gathered for each key consideration for each parasitoid, can be found within the supplementary material published alongside this paper.

2.1. *Availability of information about suitable biological control agents*

Are any natural enemies of the target pest identified as suitable BCA(s) elsewhere, which could be considered for CBC in the potential area of introduction?

Since the discovery of EAB in Michigan in 2002 (Cappaert et al., 2005), natural enemy explorations have been conducted in north-eastern China (Yang et al., 2006, 2005; Zhang et al., 2005), and later in the Russian Far East (Belokobylskij et al., 2012). From these surveys, four effective hymenopteran parasitoids were discovered, described, subject to non-target risk assessment, and released in North America, eventuating in a successful CBC programme (Duan et al., 2022a). These are the egg parasitoid *O. agrili*, and the larval parasitoids *S. agrili*, *S. galinae*, and *T. planipennisi*. There is a wealth of studies related to these species as CBC agents in North America (Duan et al., 2018a; Duan et al., 2022a; Duan et al., 2023), which justifies selection of these CBC agents as suitable candidates for pre-emptive biological control feasibility assessments for Europe. A more recently discovered egg parasitoid of EAB in the Russian Far East and South Korea, *Oobius primorskyensis* Yao and Duan (Hymenoptera: Encyrtidae), is currently being investigated for its potential contribution to the existing CBC programme in North America (Duan et al., 2019b; Yao et al., 2016). However, there remains a dearth of information on this species, and until more studies become available, it is not suitable for pre-emptive biological control feasibility assessment.

2.2. *Background information for the selected biological control agents*

2.2.1. *Taxonomic status and synonyms*

Has the taxonomic status of the selected BCA been defined?

All four of the candidate CBC agents for EAB have been described (Belokobylskij et al., 2012; Yang et al., 2005; Yang et al., 2006; Zhang et al., 2005). However, *S. galinae* was originally misidentified as *Spathius depressithorax* Belokobylski (Hymenoptera: Braconidae), but its distinction as a different species has since been resolved (Belokobylskij et al., 2012). The published descriptions for each of these parasitoid species additionally provide morphological details that clarify how they can be distinguished from closely related, or superficially similar, species in their native range that also target *Agrilus* spp. and/or other buprestids. Furthermore, the descriptions of *S. galinae* (Belokobylskij et al., 2012) and *O. primorskyensis* (Yao et al., 2016) provide morphological details to distinguish them from *S. agrili* and *O. agrili*, respectively, which is important given that all of these species attack EAB in its native range. No synonymy is reported for any of the candidate CBC agents. The taxonomies of the four candidate CBC agents for EAB are therefore well-defined, indicating that identifying the correct species for information-gathering and potential future introduction can be readily achieved. The existence of well-established colonies of these parasitoid species, and consistent reporting of monitoring studies, as part of the ongoing CBC programme against EAB in North America (Duan et al., 2022b; Gould et al., 2021), would also likely streamline the process of ensuring taxonomic accuracy prior to importation to European countries.

Are there any biotypes, strains, subspecies, or cryptic species that need to be considered?

Upon the discovery of *O. primorskyensis*, it was postulated that together with *O. agrili* these two species constitute a cryptic species complex as it is unclear whether specimens could always be distinguished from morphology alone, despite differences being described (Yao et al., 2016). It has since been shown that *O. agrili* and *O. primorskyensis* exhibit distinct reproductive biology's under identical rearing conditions, which may suggest differences in their potential contribution to biological control of EAB both phenologically and geographically (Larson and Duan, 2016). This crypticity may therefore have important implications for CBC programmes, highlighting the need to ensure that the correct species is considered for any potential future implementation of pre-emptive biological control involving *O. agrili* in European countries. No different strains or biotypes have been identified for *O. agrili*.

For the remaining EAB pre-emptive biological control candidates, no biotypes, strains, subspecies, or cryptic species are known to exist. However, exploratory natural enemy surveys throughout the native range of EAB have not directly assessed the possible occurrence of these taxonomic nuances (Duan et al., 2020). For European countries considering pre-emptive biological control against EAB, caution must therefore be exercised during pre-release assessments to ensure the taxonomic accuracy of imported parasitoids.

Are there any DNA barcoding studies/databases available to help resolve the identity of the potential BCA we want to import?

DNA sequencing approaches have been used to further resolve the taxonomic identities of *O. agrili*, *S. agrili*, and *S. galinae*. Yao et al. (2018) confirmed through DNA divergence that *O. primorskyensis* is a distinct species from *O. agrili*, despite their morphological crypticity. Furthermore, a next-generation genome sequencing method has been developed that enables the rapid identification of both *S. agrili* and *S. galinae* (Kuhn et al., 2013). These advancements in molecular approaches for identification of EAB parasitoids provide both taxonomic clarity and tools that would be useful for ensuring taxonomic accuracy in potential future pre-emptive biological control programmes against EAB in Europe. To our knowledge, no DNA sequencing studies have been conducted to distinguish *T. planipennisi* from closely related congeners, though this should not exclude this parasitoid for consideration in pre-emptive biological control because sufficient morphological identification approaches exist (Yang et al., 2006).

2.2.2. *Geographic distribution, climatic suitability/similarity, and potential distribution overlap with target pest*

Is information available about the BCA's current geographic distribution (i.e. native, naturalised, adventive, and introduced range)?

The native distributions of the four pre-emptive biological control candidates considered in this assessment are reasonably well-defined, though records are mostly limited to sites selected for the exploratory surveys for natural enemies of EAB (Fig. 1). Individually, these parasitoid species occupy different latitudinal portions of the known native range of EAB (Wang et al., 2016). For instance, *S. agrili* is by far the most prevalent of these parasitoids within the southern portion of EAB's native range around Tianjin and Beijing, with much lower prevalence in Liaoning and Jilin (Wang et al., 2016), and no occurrences further north (Belokobylskij et al., 2012; Liu et al., 2003; Yang et al., 2005). Contrarily, *O. agrili* and *T. planipennisi* are the most prevalent species within the middle portion of EAB's native range around Liaoning, Jilin, and Heilongjiang, with lower prevalence in Beijing and absence in Tianjin (Duan et al., 2012; Liu et al., 2003; Liu et al., 2007; Wang et al., 2016; Yang et al., 2006; Zhang et al., 2005). Finally, *S. galinae* occupies the northern portion of EAB's native range, predominantly restricted to the Russian Far East, but has also been recovered in Daejeon, South Korea (Belokobylskij et al., 2012). In North America, *O. agrili*, *S. galinae*, and *T. planipennisi* have successfully established, particularly in the northern and north-eastern states (Duan et al., 2023; Duan et al., 2022b) (Fig. 2). Despite recoveries of small numbers, *S. agrili* has so far failed to establish significant populations in the north and north-eastern U.S. states, where its release efforts were focused, likely due to a mismatch in climatic conditions compared to its native distribution (Herms, 2015).

The current understanding of both the native and North American distributions of *O. agrili*, *S. galinae*, and *T. planipennisi* suggests that these species are adapted to the cooler regions of EAB distribution, though their ranges are still expanding in North America (Duan et al., 2023; MapBioControl, 2023b). Nevertheless, their known distributions indicate potential suitability to the cooler climates of central, western, and parts of northern, Europe, which fall within a similar range of Köppen-Geiger climate types (Kottek et al., 2006). This promotes the feasibility of these species as potential pre-emptive biological control agents against EAB for many European countries. However, the limited

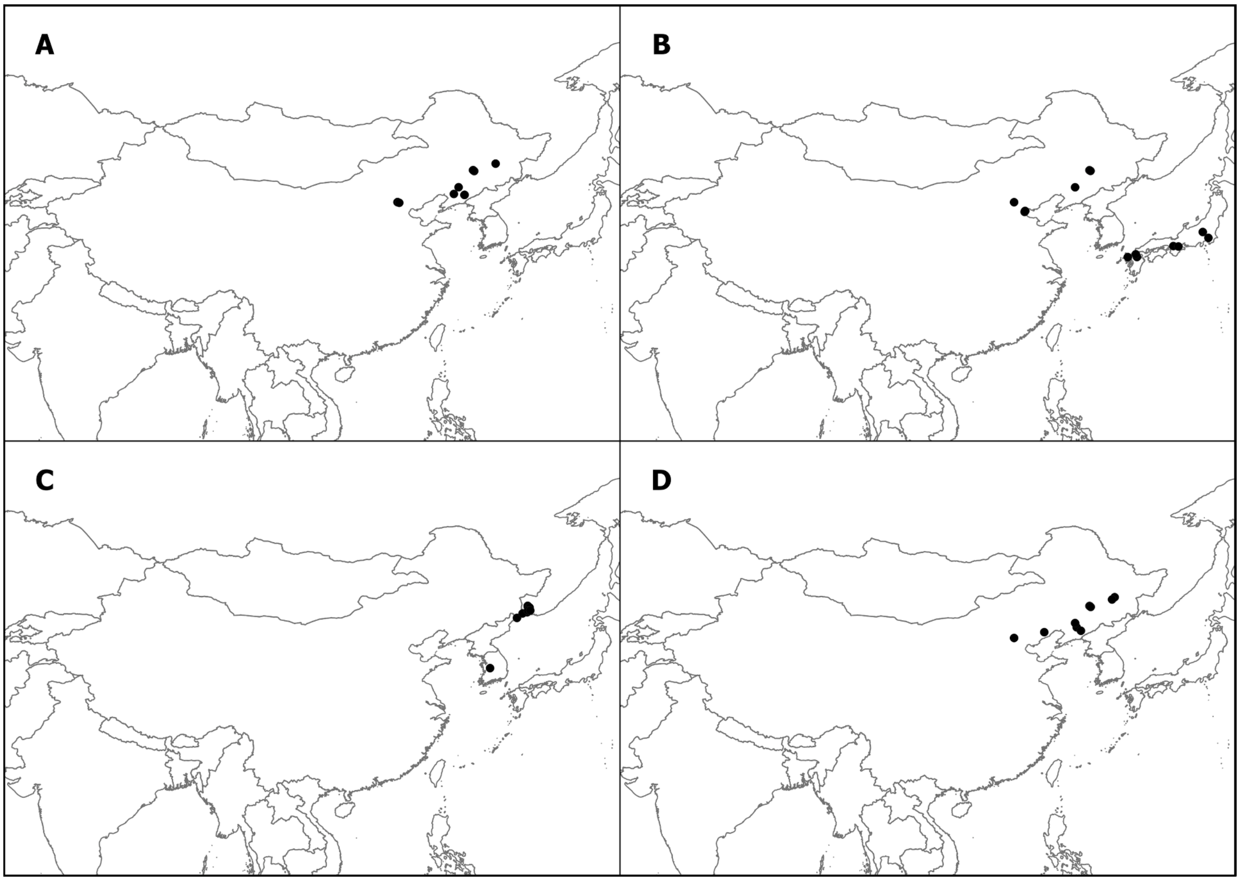

Fig. 1. The known native distributions of (A) *Oobius agrili*, (B) *Spathius agrili*, (C) *Spathius galinae*, and (D) *Tetrastichus planipennisi*. Dots represent occurrence points, which were obtained from the literature in March 2023.

establishment of *S. agrili* in North America suggests that this species could experience similar difficulties if introduced to Europe, which reduces its suitability in a potential pre-emptive biological control programme.

Is information available about the BCA's ability to adapt to different environmental conditions?

Various studies on temperature-related development and tolerances reveal that the aforementioned successes or failures of the EAB parasitoids to establish in North America are largely explainable by their ability to adapt to new, albeit similar, climatic conditions. On the basis of the direct effects of temperature on the development and mortality of *O. agrili*, *S. galinae*, and *T. planipennisi*, these species could theoretically establish throughout the range of EAB in North America, and survive a wide range of climatic conditions (Duan et al., 2014b; Duan et al., 2018b; Hoban et al., 2016; Watt et al., 2016). Furthermore, the supercooling points for these parasitoids range between approximately −25 °C and −30 °C (Chandler et al., 2020; Hanson et al., 2013), similar to the supercooling point range for EAB (Crosthwaite et al., 2011; Sobek-Swant et al., 2012). The supercooling points for *S. galinae* and *T. planipennisi* have additionally been shown to decrease in response to cold acclimation, indicating that cold-tolerance is adaptable to differing climatic conditions (Chandler et al., 2020; Hanson et al., 2013). Nevertheless, Duan et al. (2020) demonstrated that an anomalous extreme cold weather event in the north-eastern U.S. resulted in higher mortality for overwintering parasitoids than for overwintering EAB, suggesting that the cold tolerance of parasitoids may be lower than expected in field conditions. Similar cold tolerance levels have been observed for *S. agrili*, and its apparent difficulty to adapt to the North American environment is instead due to climatic effects on phenology, whereby the timing of adult emergence is asynchronous with the availability of parasitoid-susceptible EAB larvae (Hanson et al., 2013; Jones et al., 2020). This highlights the importance of considering how climatic conditions may interfere with host-parasitoid temporal dynamics when assessing the climatic adaptability of parasitoids. Indeed, the timing of *O. agrili*, *S. galinae*, and *T. planipennisi* emergence in the northern and north-eastern U.S. temporally align with peak availability of the target EAB life stage (Jones et al., 2020; Petrice et al., 2021a; Quinn et al., 2022). However, there are conflicting arguments as to whether this synchrony may be maintained in southern U.S. climates, with some evidence suggesting that both parasitoid and EAB phenology may respond similarly to warmer conditions (Jones et al., 2020; Petrice et al., 2021a).

Information regarding the adaptability of *O. agrili*, *S. galinae*, and *T. planipennisi* to climatic conditions that are similar to that of their native ranges provides convincing evidence that these species would successfully establish throughout most of central, western, and northern Europe (Kottek et al., 2006). Furthermore, these European regions fall within cold-hardiness zones between five and eight, indicating that extreme cold waves causing mortality that may affect their establishment, are highly unlikely (Magarey et al., 2008). This further supports the feasibility of these three parasitoids to be considered for potential pre-emptive biological control against EAB from the European perspective. Conversely, the apparent difficulty of *S. agrili* to phenologically adapt to the climatic conditions of the north-eastern U.S. suggests that much of Europe may also be less suitable, and that it may be less feasible for pre-emptive biological control. However, the conflicting results of studies on cold tolerance and latitudinal variability in host-parasitoid dynamics suggests that these processes are not fully understood for the EAB CBC agents, and that the use of correlative and/or ecophysiological species distribution models could help to refine

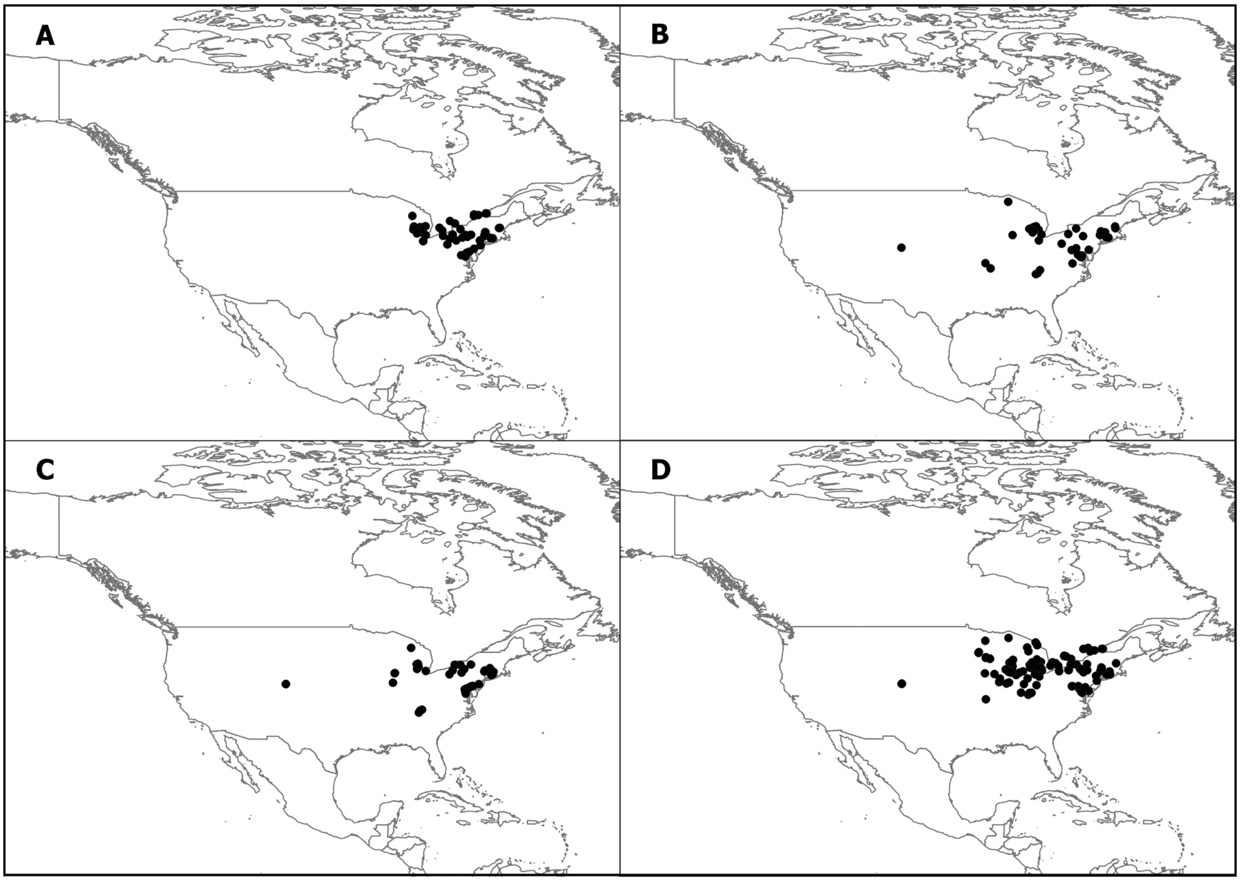

Fig. 2. The known distributions of (A) Oobius agrili, (B) Spathius agrili, (C) Spathius galinae, and (D) Tetrastichus planipennisi in North America, where they have been introduced for classical biological control of EAB. Dots represent sites where the parasitoids have been recovered following release and were obtained from MapBioControl (2023b) in March 2023.

predictions of potential distribution (Fischbein et al., 2019; Hoddle et al., 2015; Hoelmer and Kirk, 2005).

Are there any bioclimatic modelling studies available that could help to confirm climatic suitability/similarity between the BCA's donor and receiving environment?

Limited bioclimatic distribution modelling studies are available for the EAB parasitoids considered in this feasibility assessment. Petrice et al. (2021) present a rate summation approach that considers how the effect of temperature on accumulated development rates of *O. agrili* may influence its potential to establish throughout North America. For *S. galinae*, Wittman et al. (2021) utilise data on supercooling points and overwintering mortality to predict where winter temperatures will allow its survival throughout North America. Although useful for addressing challenges related to the North American CBC programme against EAB, and making inferences regarding the climatic adaptability of these parasitoids, the scope of these studies is limited to phenology and winter survival, and is geographically restricted to North America. To our knowledge, there are no bioclimatic modelling studies available for *S. agrili* or *T. planipennisi*. However, a scarcity of bioclimatic modelling studies is not a deciding factor in the outcome of this assessment considering that reasonable evidence exists from other sources that suggest climatic suitability throughout Europe, as explained under the previous considerations in this section.

Are there any bioclimatic modelling studies for the target pest that could help to confirm potential distribution overlap with the BCA in the new environment?

To our knowledge, there currently exist four bioclimatic modelling studies for EAB that contain predictions for Europe, three of which were developed with MaxEnt, a type of model that compares occurrence points against an environmental background (Phillips et al., 2006). The MaxEnt models presented by Flø et al. (2015) and Meshkova et al. (2023) predict that most of Europe is unsuitable for EAB. However, given the wide distribution of susceptible ash trees in Europe, and the physiological adaptability of the pest to varying climatic conditions throughout both its native and invasive ranges, it is widely accepted that much of Europe would be suitable for its establishment (Baranchikov et al., 2008; Orlova-Bienkowskaja and Bieńkowski, 2016; Orlova-Bienkowskaja and Bieńkowski, 2022b; Valenta et al., 2015). Additionally, Dang et al. (2021) obtained a potential distribution that aligned much more closely to the known EAB distribution by fitting a MaxEnt model using host plant distribution, as opposed to the distribution of the insect. Also, a recent study based on an ensemble modelling approach predicts that most of Europe is suitable for EAB under current and future climate conditions (Rossi et al., 2024). It is possible that ecophysiological niche modelling (such as CLIMEX), which uses a different approach by including parameters related to how climatic conditions affect the developmental biology of a species (Kriticos et al., 2015), may provide more realistic predictions.

If no bioclimatic modelling studies are available, is it possible to develop a climatic suitability model for the selected BCA and the target pest?

Considering the reported native and introduced distribution data, the development of species distribution modelling approaches, such as MaxEnt, should be feasible for the EAB parasitoids considered in this assessment. Furthermore, the developmental and stress responses of these EAB parasitoids in relation to temperature are well-studied, and these ecophysiological data could be used to inform the parameterisation of CLIMEX models. Such modelling approaches would help to refine predictions surrounding the potential for establishment and success of EAB parasitoids in European climatic conditions (Fischbein et al., 2019; Hoddle et al., 2015; Hoelmer and Kirk, 2005), and would be valuable before importation of these parasitoids for further pre-emptive risk assessment.

2.2.3. Biology and ecology of the biological control agent

Is information available about the natural enemy's host specificity?

All four EAB parasitoid species considered in this assessment were subject to host-specificity testing in containment prior to their release in North America. Depending on the parasitoid species, between 14 and 18 non-target insect species from six different families, and either two or three orders, were subject to no-choice tests. Of these, the number of *Agrilus* species ranged from five to nine. From these, *O. agrili*, *S. agrili*, and *S. galinae*, attacked three, five, and one non-target *Agrilus* spp., respectively (Bauer and Liu, 2007; Duan et al., 2015b; Liu and Bauer, 2007; Yang et al., 2008). The only parasitoid that did not accept any non-target species was *T. planipennisi*, and although only five *Agrilus* spp. were tested, it did not accept two species that were attacked by *S. agrili* (Liu and Bauer, 2007; Yang et al., 2008). Of the three parasitoids that exerted non-target attacks, all demonstrated a strong preference towards EAB, and to our knowledge, there are no reports of non-target impacts occurring in the field. Nevertheless, these species are not host-specific and possess the physiological potential to attack some non-target *Agrilus* spp., and we are not aware of non-target impacts in the field having been directly investigated. Furthermore, the relatively low number of non-target *Agrilus* spp. exposed to *T. planipennisi* does not provide conclusive evidence that it is host-specific.

Because Europe has a highly diverse *Agrilus* fauna, comprising approximately 89 species (Lobl and Lobl, 2016), it is not possible to infer the likelihood of non-target impacts from the aforementioned host-specificity studies. This does not exclude them as pre-emptive biological control candidates in Europe, but rather necessitates a more extensive and informed selection of non-target *Agrilus* spp. in order to conduct a robust risk assessment. For instance, the non-target North American *Agrilus* spp. attacked by *O. agrili* during risk assessments were those that exhibited large egg sizes, similar to that of EAB (Bauer and Liu, 2007), and this trait should be prioritised when selecting appropriate European *Agrilus* spp. for non-target risk assessments. Furthermore, there are six European *Agrilus* spp. known to feed on ash, and these should also be prioritised for non-target risk assessments as this shared host plant with EAB may increase the likelihood of encounters with the parasitoids in the field (Jendek and Polakova, 2014; Todd et al., 2015). However, observations in North America suggest that *Agrilus* larval parasitoids, such as *Spathius* spp., are more specific to host size and location within the tree than to the host tree itself (Bauer et al., 2014; Taylor et al., 2012), and this should also be considered when selecting non-target species for risk assessments.

Is information available about the BCA searching and dispersal abilities?

The extent of knowledge on the nature of host-searching differs amongst the EAB parasitoids considered in this assessment. The location of hosts by *S. agrili* involves firstly behavioural attraction to volatiles discharged by ash leaves, followed by the detection of vibrational cues emitted by actively feeding EAB larvae (Wang et al., 2010). Vibrational cues are also known to be utilised by *T. planipennisi* to locate EAB larvae under the bark (Chen et al., 2016; Ulyshen et al., 2011). Although host-location ecology has not been studied for *O. agrili* or *S. galinae*, it is likely that the latter exhibits a similar behavioural-response to *S. agrili* (Ragozzino et al., 2021). However, considering the substantial knowledge on the field efficacy of each of these EAB parasitoids in the context of the North American CBC programme (assessed in detail in section 2.2.4.), an understanding of host-searching efficiency is not of particular importance to this feasibility assessment.

The dispersal abilities of *O. agrili*, *S. galinae*, and *T. planipennisi* are well-studied, with the latter two being strong dispersers. For example in Maryland, established populations of *S. galinae* have been observed up to 45 km away from sites where they were released four years prior, with an estimated dispersal velocity of 10 km/year, indicating rapid establishment and spread (Aker et al., 2022). Similarly at various sites throughout the north-eastern U.S., *T. planipennisi* has been detected up to 20 km from sites where they were released four years earlier (Jones et al., 2019; Quinn et al., 2022; Rutledge et al., 2021), with flight mill experiments demonstrating a similar flight capacity to that of EAB (Fahrner et al., 2015). The dispersal ability of *O. agrili* appears to be much weaker. Indeed, after five years from initial release, studies have observed low saturation of the parasitoid approximately 1 km away from release sites. This may be due to its locomotion being characterised predominantly by walking and occasionally jumping short distances (Abell et al., 2014; Duan et al., 2011a). Release and recovery studies in New York and Maryland observed that *S. agrili* has not successfully dispersed (Aker et al., 2022; Jennings et al., 2016; Jones et al., 2019), but this is likely attributable to its limited establishment rather than a reflection of its inherent dispersal abilities (Jones et al., 2020). The strong dispersal abilities of *S. galinae* and *T. planipennisi*, indicative of their capacity to spread from release sites and locate EAB infestations, provides further support for their consideration for pre-emptive biological control in Europe. However, the weaker dispersal abilities of *O. agrili* should not be viewed as diminishing its candidature for pre-emptive biological control, but rather as knowledge to inform a more intensive release strategy to attain success (Duan et al., 2011a).

Is information available about the BCA parasitism and predation rate?

All four parasitoids considered in this assessment demonstrated high parasitism rates against EAB in their native ranges during exploratory natural enemy surveys, and this was a primary selection criterion for their introduction into North America. This includes observations of parasitism rates of up to 61.50 %, 90 %, 50 %, and 65 % for *O. agrili*, *S. agrili*, *S. galinae*, and *T. planipennisi*, respectively (Belokobylskij et al., 2012; Liu et al., 2007; Yang et al., 2005; Yang et al., 2006). In the north-eastern U.S., the parasitism rates of the three parasitoids that successfully established continue to increase. In Michigan, percentage parasitism of EAB by *O. agrili* increased from 1 % a year after initial releases to 12 % – 30 % after four years (Duan et al., 2015a), and in three north-eastern states, percentage parasitism by *S. galinae* increased from 13.1 % – 49.2 % one year after release to 35 % – 78 % after four years (Duan et al., 2019a; Duan et al., 2021b; Duan et al., 2022b). For *T. planipennisi* in Michigan, percentage parasitism increased rapidly a year after release to 21.20 %, with a further increase to 28.90 % two years later (Duan et al., 2013a; Duan et al., 2015a). Even though parasitism rates for these species are likely to continue to increase (owing to their rapid establishment and spread), field data for *S. galinae* and *T. planipennisi* demonstrate that the current rates are contributing to significant mortality of EAB populations (Duan et al., 2015a; Duan et al., 2022b). Conversely, there are no helpful reports of parasitism rates for *S. agrili* in North America due to its limited establishment. The steady increase in parasitism rates amongst *O. agrili*, *S. galinae*, and *T. planipennisi* in an existing CBC programme (in some cases approaching those observed in their native ranges), provides a positive outlook for their feasibility should they be considered for pre-emptive biological control against EAB in Europe. The especially rapid increase to high parasitism rates observed from *S. galinae* in North America suggests that this species should be viewed with particular interest for a potential future CBC programme.

Is information available about the BCA reproductive potential?

The basic reproductive biology of the four parasitoid species is another key consideration that was studied prior to their introduction into North America. Data from such studies that are relevant to reproductive potential are summarised in Table 1. Despite variation between the parasitoid species, these data generally suggest a quick generational turnover, long window of oviposition potential relative to the target EAB life stage, and high fecundity, which are characteristics of a high reproductive rate that should support rapid population growth. The much larger brood size of *T. planipennisi* may translate into lower parasitism rates, but this must be considered in the wider context of a

Table 1
Life history traits related to the reproductive potential of the four emerald ash borer parasitoids considered in this assessment. Data are presented as averages from experiments conducted at 25 °C. A range of averages are provided where data was obtained from more than one study.

	Oobius agrili	*Spathius agrili*	*Spathius galinae*	*Tetrastichus planipennisi*	References
Life cycle (days)	20–25	33	36.80	27	Bauer & Liu (2007); Duan et al. (2011b); Gould et al. (2011); Watt et al. (2016)
Adult longevity (days)	14.00	29.10–60.80	45.50–49.00	42.00	Bauer and Liu (2007); Duan et al. (2014b); Duan et al. (2014c); Gould et al. (2011); Watt et al. (2016); Yang et al. (2010)
Fecundity (eggs/female)	24–70.6	23.30	31.00–42.60	57.00–81.60	Bauer and Liu (2007); Duan et al. (2011b); Duan et al. (2014b); Duan et al. (2014c); Duan et al. (2018b); Hoban et al. (2016); Larson and Duan (2016); Watt et al. (2016); Yang et al. (2010)
Brood size (progeny/host)	1	5.40–9.20	7.35	67.70	Gould et al. (2011); Hoban et al. (2016); Ulyshen et al. (2010); Wang et al. (2015); Yang et al. (2010)
Pre-ovipostion period (days)	–	9.90–19.50	5.33	0.00	Duan et al. (2011b); Gould et al. (2011); Watt et al. (2016); Yang et al. (2010)

CBC programme that may consider the importation of multiple parasitoid species with low interspecific competition (assessed in detail in section 2.4.2.).

Nevertheless, because a CBC programme already exists involving these four parasitoids, from which substantial data is available on their performance under natural conditions (Duan et al., 2015a; Duan et al., 2022b), information on their reproductive potential under laboratory conditions is of little value when considering the potential for pre-emptive biological control against EAB in Europe. For example, the high reproductive output of *S. agrili* bears negligible importance in light of its limited adaptability to the climatic conditions of North America (Hanson et al., 2013; Jones et al., 2020).

Is information available about the BCA phenological synchrony with the target host?

Host-parasitoid synchrony for all four EAB parasitoids considered in this assessment is another key attribute that was investigated prior to their introduction into North America. In their native ranges, each of the parasitoids were found to be phenologically synchronous with the availability of parasitoid-susceptible EAB life stages. Furthermore, *O. agrili* was shown to have two generations, *S. galinae* up to three generations, and *S. agrili* and *T. planipennisi* up to four generations per year, allowing the parasitoids to respond to peaks in EAB populations throughout the season (Belokobylskij et al., 2012; Liu et al., 2007; Yang et al., 2006; Yang et al., 2010). Data from North America reveals that in the north-eastern U.S., *O. agrili*, *S. galinae*, and *T. planipennisi* all show close phenological synchrony with EAB, with each generation of emerging adults being temporally aligned with the peak of parasitoid-susceptible EAB life stages (Jones et al., 2020; Petrice et al., 2021a; Quinn et al., 2022). However, it has been demonstrated that photoperiod and climatic conditions can alter the timing of diapause, and therefore seasonal phenology, in *O. agrili*, though it is unknown whether this would result in a similar response, or cause a mismatch, with the phenology of EAB in more southerly U.S. latitudes (Petrice et al., 2019; Wetherington et al., 2017). Furthermore, field data from Virginia, below the 40th parallel and a warmer climate than the north-eastern U.S., shows little overlap in the timing of peak *S. galinae* emergence and the presence of parasitoid-susceptible EAB larvae (Ragozzino et al., 2020). Nevertheless, the close host-parasitoid synchrony observed in the north-eastern U.S. suggests that this is likely to be observed also throughout the similar climatic conditions found in central, western, and northern Europe (Kottek et al., 2006). This further bolsters the feasibility of these three parasitoids for consideration towards potential future pre-emptive biological control programmes against EAB in Europe. Conversely, the limited establishment of *S. agrili* in the north-eastern U.S. due to a phenological mismatch with EAB populations (detailed in section 2.2.2.) provides further indication that priority should be given for the assessment of the other three species and this parasitoid should only be reconsidered for pre-emptive biological control in these European climates as new information becomes available.

Is information available about the BCA's ability to survive at low host/prey densities?

There is limited direct study on the ability of the EAB parasitoids considered in this assessment to maintain their populations at low host densities. However, Bauer et al. (2015) reports that *O. agrili* is established at several sites in Michigan where EAB densities are low. Field observations in the native range of *S. agrili* also demonstrated that there was no relationship between host density and the number of parasitized hosts (Wang et al., 2007). It has been posited that *S. galinae* must have persisted on the presumed low densities of EAB that occurred in the Russian Far East prior to the 21st century, when EAB populations exploded in association with widespread planting of more susceptible American ash species (Orlova-Bienkowskaja and Volkovitsh, 2018). Furthermore, Duan et al. (2012) reported consistent observation of *S. galinae* broods and a higher parasitism rate at low host densities in the Russian Far East. This pattern has also been observed in both the native and introduced ranges of *T. planipennisi* (Duan et al., 2015a; Liu et al., 2007). These findings suggest that all of the EAB parasitoids considered in this assessment possess the ability to locate and parasitize hosts, which in turn enables the persistence of their populations, at low EAB densities. However, despite evidence supporting the current efficacy of these parasitoids in the North American CBC programme (Duan et al., 2015a; Duan et al., 2022b), more field studies assessing the population dynamics of EAB parasitoids at low host densities is recommended to inform the long term outcome when EAB populations are smaller. This may also enable the assessment of whether alternative hosts may be targeted at low EAB densities.

Is information available about potential natural enemies of the BCA that may be present in the area of introduction?

We are not aware of any reports of hyperparasitoids or other natural enemies of any of the EAB parasitoids considered in this assessment, or of any of their congeners in Europe, that may diminish their efficacy against EAB or pose risks to closely related European species. Given the well-reported CBC programme in North America, it is unlikely that any natural enemies of these parasitoids occur there. However, the lack of reporting could be commensurate to the lack of studies investigating potential natural enemies, and this would be an important consideration as part of possible future risk assessments for these parasitoids (van Lenteren, 1997).

Is information available about closely related species in the area of introduction that may be at risk to hybridise with the BCA?

Species in Europe that are closely related to the four EAB parasitoids considered in this assessment include five *Oobius* spp. (Noyes, 2019), 15 *Spathius* spp. (Hedqvist, 1976), and 93 *Tetrastichus* spp. (Hansson and Schmidt, 2020). In Europe, 64 parasitoid species were identified to attack 24 *Agrilus* spp., although some of the records may be erroneous host-parasitoid associations or misidentifications (Kenis et al., 2024). Of these, one *Oobius* spp. (Noyes, 2019), seven to eight *Spathius* spp. (Kenis et al., 2024; Kenis and Hilszczanski, 2004), and four *Tetrastichus* spp. (Hansson and Schmidt, 2020), are known to attack *Agrilus* spp., possibly increasing the likelihood of encountering their congeneric EAB parasitoid/s, should they be introduced, due to shared host niche (Havill et al., 2012). Further assessment of hybridisation risk is difficult due to the generally poorly understood phylogenetic relationships within these genera. However, of the seven European *Spathius* spp. that attack *Agrilus* spp., five belong to the *Spathius exarator* L. (Hymenoptera: Braconidae) species group, to which both *S. agrili* and *S. galinae* belong (Belokobylskij, 2003; Belokobylskij et al., 2012). Furthermore, a molecular phylogeny for the *Spathius* genus reveals that *S. agrili* and *S. galinae* are closely related to these European species (Zaldívar-Riverón et al., 2018), which may pose risks of hybridisation. *Spathius polonicus* Niezabitowski (Hymenoptera: Braconidae), the only European parasitoid currently known to attack EAB in European Russia (Orlova-Bienkowskaja and Belokobylskij, 2014; Orlova-Bienkowskaja, 2015), would almost certainly interact with any *Spathius* spp. introduced into Europe to control EAB, and therefore should be prioritised when considering the risk of hybridisation. However, this parasitoid is not considered to belong to the *S. exarator* species group (Belokobylskij et al., 2012). The relatively large *Tetrastichus* fauna in Europe also confounds the uncertainty surrounding hybridisation risk for *T. planipennisi*. Although closely related European insects exist for each of the parasitoids considered in this assessment, hybridisation is rare within CBC programmes (Havill et al., 2012), and we are not aware of any examples of hybridisation between species of these genera. Any risk of hybridisation should also be considered within the wider context of risk–benefit analysis. For example in this case, even if hybridisation with native species is biologically possible, the extent of interbreeding could be limited for CBC agents that have a low chance of encountering congenerics due to targeting different host species. This could in turn limit the potential environmental impacts of hybridisation (Havill et al., 2012). Nevertheless, uncertainty surrounding the risk of hybridisation alone does not discount the potential feasibility of these EAB parasitoids for pre-emptive biological control.

2.2.4. Known effectiveness of the BCA against the target host abroad from field and/or lab studies

Is information available about the BCA's performance and efficacy against the target host in its native range?

As detailed in section 2.2.3., the high parasitism rates, reproductive potential, and physiological synchrony with EAB in the native ranges of the four EAB parasitoids considered in this assessment, provide evidence for their likely efficacy. However, specific studies on how these parasitoids contribute to field mortality of EAB, and to ash stand survival and/or recovery, are required for further inferences of efficacy in their native ranges.

Is information available about the BCA's performance, efficiency and efficacy against the target host in its adventive range if self-introduced?

This consideration is not applicable because none of the EAB parasitoids considered in this assessment are known to have self-introduced outside of their native ranges or where they have been deliberately introduced for CBC.

If any biocontrol programmes have been started with the selected pest abroad, is there information about their success or failure?

The information regarding parasitism rates, reproductive potential, and physiological synchrony with EAB in North America, as detailed in section 2.2.3., provides some evidence regarding the efficacy of the EAB parasitoids as part of a CBC programme. However, the contribution to field mortality of EAB, and to ash stand survival and/or recovery, are yet to be quantified for *O. agrili* because recovery of EAB eggs inside the crevices of ash bark is labour intensive, making standardisation for sampling the impact of this egg parasitoid on EAB population dynamics challenging (Abell et al., 2014; Petrice et al., 2021b). Additionally, *S. agrili* can be presumed to lack efficacy due to poor establishment in North America. Conversely, recent analysis of field data from ash dominated hardwood forests after the release of *S. galinae* and *T. planipennisi* in several north-eastern U.S. states observed a 76 % reduction in EAB larval densities during an outbreak phase between 2015 and 2020, which has contributed to ash recovery and regeneration at these sites (Duan et al., 2022b). Similarly, *T. planipennisi* provided a substantial contribution to an observed 90 % decline in EAB larval densities at field sites in Michigan (Duan et al., 2015a). It was also found to have reduced the net growth rate of EAB populations at these field sites by over 50 %, with healthy saplings and younger trees surviving despite high pest densities (Duan et al., 2017; Margulies et al., 2017). Interestingly, growing evidence suggests that differences in morphology, ecology, and life-history traits promote niche-partitioning and co-existence of *S. galinae* and *T. planipennisi*. Compared to *S. galinae*, the significantly shorter ovipositor of *T. planipennisi* limits its ability to penetrate the thicker bark of mature ash trees (Abell et al., 2012). Parasitism rates of *T. planipennisi* in sapling sized ash trees are therefore higher than that of *S. galinae*, whereas the reverse is observed in mature ash trees (Duan et al., 2017; Duan et al., 2019a; Margulies et al., 2017). Furthermore, *S. galinae* parasitise more larvae, but deposit smaller brood sizes per host compared to *T. planipennisi*, providing a trade-off in reproductive output that mediates the niche-partitioning and co-existence between these species by reducing the chances of multi-parasitism (Ulyshen et al., 2010; Wang et al., 2015).

The ability of *S. galinae* and *T. planipennisi* to co-exist not only indicates that their combined application may provide a greater impact on EAB populations than if either were released alone, but crucially also suggests that each promotes the recovery and protection of ash stands at different ages. This further bolsters the feasibility of these two parasitoids with regards to their likely efficacy in future CBC programmes, and the potential for their combined release should be considered for pre-emptive biological control in Europe. However, to justify prioritisation on this basis, it would be necessary to investigate whether niche-partitioning based on tree age would also occur in European ash. Despite the paucity of data on the extent to which *O. agrili* contributes to EAB mortality and ash recovery, it should not be excluded from consideration for pre-emptive biological control in Europe due to the substantial evidence for other measures of efficacy (detailed in section 2.2.3.), and that it may complement the larval parasitoids by targeting a different life stage of EAB.

2.2.5. Availability of closely related BCA species that could be used as an alternative or in synergy

Is there any information on closely related resident BCAs in the receiving environment that could be used as either alternative BCAs or in synergy with our candidate BCA?

Amongst the known European fauna that are congeneric to the EAB parasitoids considered in this assessment, and that target at least one *Agrilus* spp., are one *Oobius* spp. (Noyes, 2019), seven *Spathius* spp. (Kenis and Hilszczanski, 2004), and four *Tetrastichus* spp. (Hansson and Schmidt, 2020). Of these European species, only one, *S. polonicus*, is known to target EAB in European Russia, with up to 50 % parasitism observed (Orlova-Bienkowskaja, 2015; Orlova-Bienkowskaja and Belokobylskij, 2014). However, little is known about the nature and extent of *S. polonicus* parasitism of EAB, or whether it dispersed eastwards from Europe upon the arrival of EAB or represents a previously undetected biotype in the region. We recommend that such attributes be investigated for *S. polonicus* to resolve whether it could provide an alternative to CBC in certain countries. Until this is undertaken, it should not be considered as a viable alternative to, or a complementary component of, the well-studied CBC agents considered in this assessment. It would also be useful to investigate whether *T. planipennisi* could experience the same niche-partitioning pattern with *S. polonicus* as it does with *S. galinae*, in which case *S. polonicus* could be considered as providing complementary parasitism (Duan et al., 2017; Margulies et al., 2017).

For the remaining European fauna congeneric to the EAB parasitoids considered in this assessment, little else can be elucidated regarding their potential to target EAB. Amongst the North American parasitoid fauna belonging to these genera, several *Spathius* spp. have been observed attacking EAB larvae, albeit at very low rates, which is not the case for species belonging to the remaining genera (Duan et al., 2014a; Lyons, 2015; Taylor et al., 2012; Triapitsyn et al., 2015). It is therefore possible that the European parasitoid fauna has the potential to target EAB, but since it is not their primary host, parasitism would likely be very low. However, the potential for native parasitoids to switch to EAB, and the extent of their contribution to overall parasitism, should be investigated upon the establishment of the pest throughout Europe. For example, native *Atanycolus* spp. occasionally exert high parasitism rates against EAB in North America (Abell et al., 2012).

If closely related BCAs are identified in the receiving environment, is information available about their potential to compete with our candidate BCA and potentially reduce its effectiveness?

If any of the European parasitoid fauna were to target EAB, it could potentially result in competition with the EAB parasitoids considered in this assessment (Saunders et al., 2022). As described above, with the exception of *S. polonicus*, there is no evidence to conclusively suggest that the resident European parasitoid fauna would target EAB. In this regard, consideration of potential competition should be given to *S. polonicus* given that it is the only parasitoid in Europe known to attack EAB (Orlova-Bienkowskaja, 2015; Orlova-Bienkowskaja and Belokobylskij, 2014). However, given the aforementioned paucity of knowledge regarding the nature and extent of *S. polonicus* parasitism of EAB in European Russia, or how this may eventuate in other European regions, it is not possible to accurately infer the risk of competition with EAB parasitoids considered in this assessment. It would again be pertinent to consider the potential for *T. planipennisi* to avoid competition with *S. polonicus* via niche partitioning (Duan et al., 2017; Margulies et al., 2017). For the remaining European parasitoid fauna, the extent of any parasitism of EAB is likely to be low, and in turn, unlikely to exert a degree of competition that would interfere with the EAB CBC agents under pre-emptive consideration, as has been observed in North America (Duan et al., 2015a; Lyons, 2015; Taylor et al., 2012). Although the contemporary literature provides useful information to consider regarding the potential for competition between the European parasitoid fauna and the parasitoids considered in this assessment, there is insufficient evidence to support the elimination of any candidate parasitoid on this basis.

2.3. Challenges to be encountered during pre-emptive biological control risk assessment

2.3.1. Non-target species to test

Is there sufficient information about the fauna that is closely related to the target pest to make an informed selection of non-targets to include in pre-emptive risk assessment tests?

The *Agrilus* fauna of Europe, comprising 89 species, and their distribution at the country scale is well-catalogued (Lobl and Lobl, 2016). Host plant records for all known *Agrilus* spp. are also catalogued (Jendek and Polakova, 2014), and a molecular phylogeny of the *Agrilus* genus that includes almost half of European species and EAB, is also available (Kelnarova et al., 2018). The selection of non-target species can therefore be sufficiently informed on the basis of occurrence in certain countries, phylogenetic relationship to EAB, and ecological niche overlap with EAB, and this applies equally to the four EAB parasitoids considered in this assessment. However, given that the only *Agrilus* spp. attacked by *O. agrili* during pre-release risk assessments in North America exhibited large egg sizes similar to that of EAB (Bauer et al., 2008; Bauer and Liu, 2007), this would be crucial to consider for non-target species selection for this parasitoid. Anecdotal observation in North America suggests that *Agrilus* larval parasitoids also tend to be more specific to host size than to host tree (Bauer et al., 2014; Taylor et al., 2012), which may be worth considering for the selection of non-target species for the EAB larval parasitoids. Overall, there exists ample information on various attributes of both potential non-target organisms, and host-specificity of the EAB biological control candidates, to make a well-informed selection of non-target organisms for pre-emptive risk assessments from the European perspective (Fig. 3).

Is it possible to collect non-target species from the field or source them from a collaborator's lab to do risk assessment in containment?

The comprehensive cataloguing of host plants for European *Agrilus* spp. (Jendek and Polakova, 2014) will enable the targeting of certain plant species to locate and collect specific non-target species. There are also established methodologies for collecting *Agrilus* spp., in particular debarking for larvae (Martel et al., 2022), and trapping and tree-beating for adults, which could be adapted for the collection of species selected for non-target risk assessments (Martel et al., 2022; Moraglio et al., 2013). However, the rarity of some native European *Agrilus* spp., for example *Agrilus litura* Kiesenwetter (Coleoptera: Buprestidae) (Kwast, 2020) and *Agrilus pseudocyaneus* Kiesenwetter (Coleoptera: Buprestidae) (Gutowski et al., 2019) could make sourcing challenging. This could complicate the attainment of an informed selection of non-target species for risk assessment as some regulatory frameworks, including that of the European and Mediterranean Plant Protection Organisation (OEPP/EPPO, 2018), may require the consideration of rare and/or protected non-target species. Nevertheless, with the knowledge available on the host plants of *Agrilus* spp., and well-established collection methods, it should be feasible to collect a reasonable selection of non-target species from the field for all of the EAB pre-emptive biological control candidates in question.

Is it possible to establish non-target colonies for risk assessment? If not, would host testing with field-collected insects be feasible?

As reported in the published literature, there are few *Agrilus* spp. for which colonies have been established. However, rearing methodologies are well-established for EAB, and require ash sticks and leaves for oviposition and larval development (Duan et al., 2013b; Duan et al., 2021a). These methodologies have also been adapted to establish colonies of *Agrilus biguttatus* Fabricius (Coleoptera: Buprestidae) (Reed et al., 2018) and *Agrilus auroguttatus* Schaeffer (Coleoptera: Buprestidae) (Lopez and Hoddle, 2014), which feed on *Quercus* (oaks). The existing methodologies for establishing colonies of EAB can be applied for rearing European *Agrilus* spp. that inhabit ash, and the same can be applied for the European species that feed on *Quercus*. For European *Agrilus* spp. that inhabit plants other than ash or *Quercus*, it can be assumed that these methodologies could also be adapted to rearing them using their respective host plants. This provides a reasonable indication that colonies can be established for selected non-target species of the four EAB pre-emptive biological control candidates. However, the only European *Agrilus* sp. for which a rearing protocol is established is *A. biguttatus*, and the feasibility of rearing other non-target species cannot be conclusively confirmed until similar methodologies are adapted for them.

Is it possible to test selected non-target species in our containment facility? If not, is it possible to conduct host testing of non-targets in a collaborator's laboratory abroad?

In Europe, several countries possess containment facilities with high enough security clearance to do research on EAB and its natural enemies. For example, the quarantine facilities at the Swiss Federal Institute for Forest, Snow and Landscape Research (WSL) bear the necessary security level in Switzerland (level 3). Research on EAB in this quarantine laboratory has been approved and rearing is ongoing (Doonan et al., 2023). This would be a logical facility to undertake host-specificity testing for the EAB parasitoids considered in this assessment. Research on EAB and imported parasitoids is also on-going in the containment facilities at the Forest Research Holt Laboratory, UK (N. Audsley, personal communication, 2023).

2.3.2. Logistics

Is it possible to get all permits needed from regulatory agencies to import the selected BCA for pre-emptive biocontrol risk assessment?

Importing BCAs into containment facilities for risk assessments is generally regulated and possible in several European countries (EPPO, 1999; Mason et al., 2017). For example, the Swiss governments

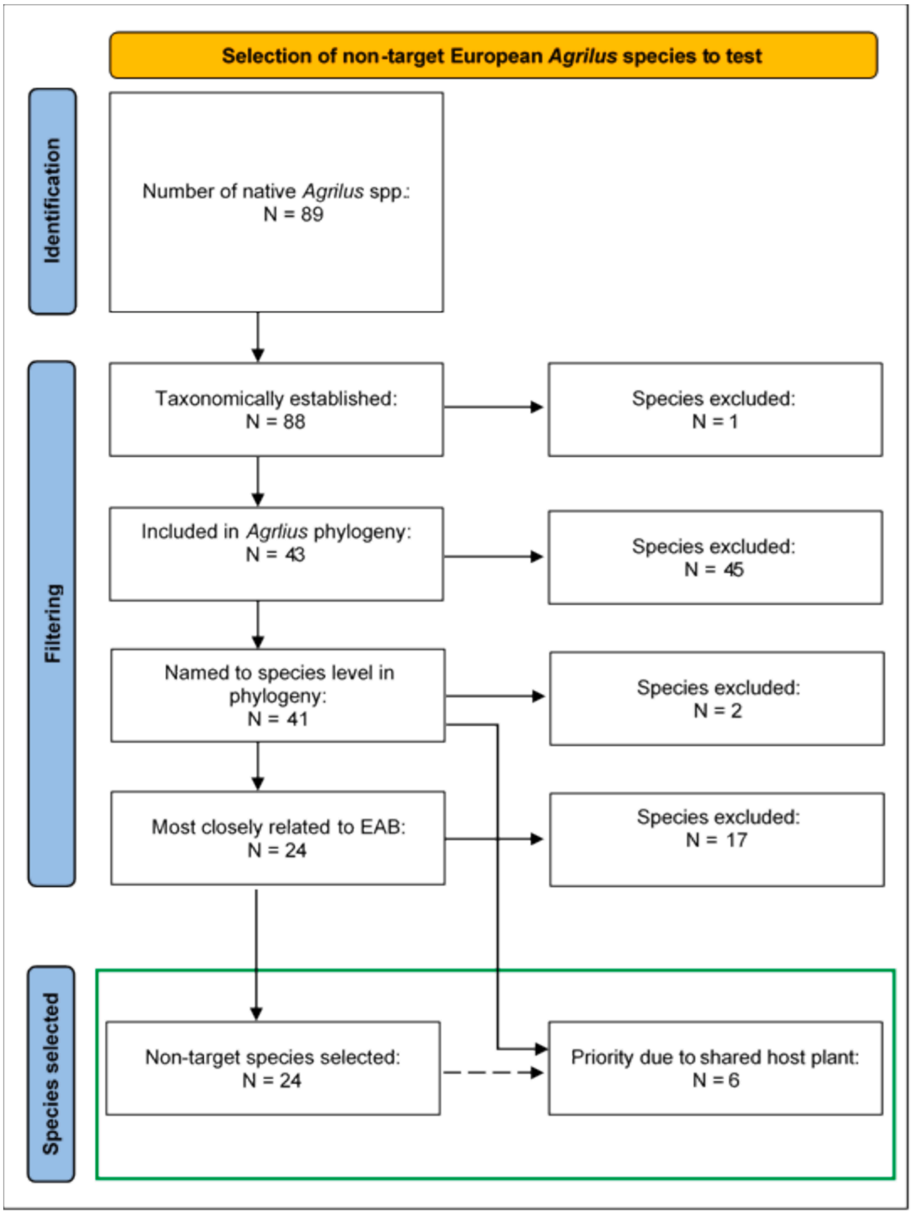

Fig. 3. A flowchart example of selecting non-target species for biosafety risk assessment of EAB parasitoids, beginning with all known native European Agrilus species, and filtering the final selection based upon taxonomy (Lobl and Lobl, 2016), phylogenetic proximity to EAB (Kelnarova et al., 2018), and shared host tree niche with EAB (Jendek and Polakova, 2014). The dashed arrow illustrates that there is some overlap of species between the two final selection criteria.

Ordinance on Handling Organisms in Contained Systems (FOEN, 2012) provides the relevant legislature for importing alien organisms into containment. It is stipulated that risk assessments must be undertaken to class risk, and that organisms deemed low risk simply require notification to authorities, whereas those deemed moderate to high risk require official authorisation from authorities. Generally, host range and risk to non-target and/or native species must be considered for risk assessments and decisions surrounding authorisation. It would therefore be feasible to apply these regulatory frameworks to the potential pre-emptive importation of the four EAB parasitoids to European countries. The non-target risk assessments conducted on these parasitoids for North America suggest that *O. agrili* may pose greater risk than *S. galinae* and *T. planipennisi*, which could make it less suited in relation to obtaining approvals for importation. However, this should be considered in the wider context of risk–benefit analysis during the application process (Sheppard et al., 2003). For instance, the EPPO decision-support scheme for import and release of biological control agents, and a recently modified version of this scheme, could be utilised by EPPO countries to support such assessments (OEPP/EPPO, 2018; Seehausen et al., 2023). In the UK, CBC agents can be imported into containment by notifying the Animal and Plant Health Agency through the import of products, animals, food and feed system (IPAFFS), to which the importer must be registered (APHA and DEFRA, 2024).

Is a suitable containment facility available for pre-emptive biocontrol risk assessment?

The relevant information for this key consideration is discussed in Section 2.3.1.

Is there a reliable collaborator/supplier identified to provide a constant supply of the candidate BCA for pre-emptive biocontrol risk assessment? If not, is it possible to collect the BCA from a suitable area for importation?

The USDA and Canadian Forest Service have a well-established system in place for the rearing and supply of all of the biological control agents imported and released for the control of EAB (Gould et al., 2021). Collaborations have already been established with both the USA and Canada (e.g. in Switzerland through CABI and WSL) and the reliable supply of the candidate CBC agents should be possible through these connections.

Is there a rearing methodology for the BCA available?

As stated above, rearing methods for all EAB parasitoids released in the USA and Canada, including *O. agrili*, have been developed. This includes mass rearing for field releases (Gould et al., 2021). However, because no artificial diet currently exists for EAB, mass rearing it, and therefore also its parasitoids, requires the harvest of fresh ash logs and leaves or the greenhouse cultivation of fast-growing tropical ash (*Fraxinus uhdei* (Wenz.) Lingelsh) (Lelito et al., 2015). This is both time consuming and labour intensive, but does not preclude feasibility for potential pre-emptive biological control risk assessment in European countries (Gould et al., 2021).

Has a shipping option to expedite rapid transport of the BCA been identified?

Transportation of a non-native organism requires adherence to national and international regulations, of which the most relevant in this case is the European Commission's TRACES framework for the certification of imported animals and/or animal products (European Commission, 2023). This requires an official shipping agent that agrees to transport biological control agents, and both the sender and receiver need to be registered with TRACES. For example, in Switzerland this shipping option has been used successfully to import parasitoids of *Popillia japonica* Newman (Coleoptera: Scarabaeidae) from Canada into CABI's containment facility (CABI, 2024), which proves that this is an operational solution.

If any of the above are not feasible, can pre-emptive risk assessment be conducted in a collaborator's laboratory abroad?

This consideration is not applicable to this case when considering Europe because supply, shipping, and importation should be feasible, as outlined above. However, for some countries in Europe, it may be difficult to obtain permits or suitable containment facilities may not be available. Thus, collaborations between different European countries will be key to do the pre-emptive risk assessment for Europe.

Is it possible to get approval to bring the target pest into containment to take part in the BCA's pre-emptive biocontrol risk assessment work?

Although the risk may be rated as too high in some European countries, it is generally possible to bring the target pest into containment facilities, given that several European countries (1) have containment facilities with high enough security levels to conduct experiments with non-native species, (2) regulate and allow the import of certain non-native species, and (3) the shipping of non-native species is regulated through the TRACES system and has been proven possible. This is evidenced by the importation of EAB into containment in Switzerland (Doonan et al., 2023).

3. Outlook of pre-emptive biological control for EAB from the European perspective

When applying the above guidelines to assess the feasibility of pre-emptive biological control of EAB in Europe using four candidate parasitoids, we were able to draw upon the plenitude and quality of information resulting from research on the successful and ongoing CBC programme against EAB in North America (e.g. Duan et al., 2022a; Duan et al., 2023). When applied to the European context, this enabled thorough scrutiny of the majority of the key considerations for each of the parasitoids considered in this assessment. Overall, the pre-emptive biological control candidates, with the exception of *S. agrili*, performed comparably well with substantial supporting evidence and met the key criteria for feasibility. Considering the significant economic and environmental threat that EAB poses to Europe and the mounting evidence from the North American experience that CBC is currently the only viable and sustainable control approach (Duan et al., 2023; Musolin et al., 2021; Petter et al., 2020), the outcomes of this feasibility assessment reveal pre-emptive biological control as a promising component of readiness against the apparently imminent spread of EAB further into Europe. We therefore recommend that European countries implement pre-emptive risk assessments for *O. agrili*, *S. galinae*, and *T. planipennisi*. However, scientists and regulators must adapt the generalised interpretations in this assessment to the particularities of the target country (Mason et al., 2017).

In the case of pre-emptive biological control for EAB, national adaptations would be of particular importance to the key considerations surrounding the feasibility of non-target risk assessment. Indeed, the 89 *Agrilus* spp. that are known to occur in Europe differ substantially between countries/regions. For instance, Switzerland and the UK are known to accommodate 33 and nine of these *Agrilus* spp., respectively (Duff, 2012; Lobl and Lobl, 2016). These differences could influence the feasibility of ascertaining a reasonable selection of non-target species for risk assessment, their field-collection, and their laboratory rearing. Furthermore, despite its widespread distribution throughout continental Europe, *S. polonicus* has been reported only once from the UK (according to GBIF (2024)), where it is presumed to be non-indigenous (Broad et al., 2016; Orlova-Bienkowskaja, 2015). This would certainly affect interpretations of the key considerations surrounding the presence of closely related resident natural enemies of EAB, potential future risk assessments, and it could potentially be considered as a pre-emptive biological control candidate in this context. Such differences between European countries could not be specifically accounted for in our feasibility assessment as it is not applied to a national scale, and this assessment should thusly be viewed as a guide for countries considering pre-emptive biological control against EAB, as opposed to having a catch-all application.

Regarding the overall relative performance of the EAB parasitoids subject to this feasibility assessment, Table S1 summarizes the strengths and weaknesses of the considered species and highlights the areas where more research is needed for conclusive results. It becomes clear that *S. agrili* has the lowest suitability for pre-emptive biological control in European countries due to its apparent difficulty to adapt to, and establish in, new climatic conditions in North America (Hanson et al., 2013; Jones et al., 2020). Evidence of successful establishment in more southern North American climates, below the 40th parallel (Bauer et al., 2015; Hooie et al., 2015), should become available before this recommendation is reconsidered. Despite the strong performance of *O. agrili*, *S. galinae*, and *T. planipennisi* throughout the feasibility assessment, there exist non-negligible differences within the wider outlook of a potential pre-emptive biological control programme that could influence decisions pertaining to their use in such a programme. The ostensibly broader host-range exhibited by *O. agrili*, and its possible preference towards host size as opposed to host species, during pre-release risk assessments in North America (Bauer and Liu, 2007), suggests that it may present a greater biosafety risk to the speciose *Agrilus* fauna in Europe. However, non-target exposure assays using an informed selection of European *Agrilus* spp. would be required to conclusively define this risk. Also, the lack of data on the impact of *O. agrili* on EAB population dynamics and ash recovery is an important distinction (Abell et al., 2014; Petrice et al., 2021b). Not only does substantial evidence support field efficacy for *S. galinae* and *T. planipennisi*, but also demonstrates that niche-partitioning promotes their co-existence and control of EAB in different age cohorts of ash forests (Duan et al., 2017; Duan et al., 2019a; Wang et al., 2015). However, given the widespread presence of the resident *S. polonicus* throughout Europe, which has been anecdotally observed exerting high parasitism against EAB in European Russia (Orlova-Bienkowskaja, 2015; Orlova-Bienkowskaja and Belokobylskij, 2014), its potential to compete with these two larval parasitoid candidates and interfere with their efficacy should be considered carefully. As a part of pre-emptive biological control efforts against EAB in Europe, research should therefore be conducted into the nature of the new association between *S. polonicus* and EAB, to clarify whether this could have implications for the success of a potential CBC programme. Nevertheless, given the evidence surrounding biosafety and efficacy, we posit that combined releases of *S. galinae* and *T. planipennisi* should be prioritised within the wider context of risk–benefit analysis for potential pre-emptive biological control programmes against EAB in Europe.

For further evaluation of the parasitoids for biological control, we identified that more research is needed in the following areas: 1) differences in biological characteristics of the parasitoids due to biotypes, strains, subspecies, or cryptic species present in Asia; 2) geographic distribution of the parasitoids in Asia; 3) bioclimatic modelling studies to determine the potential distribution of the parasitoids in the area of introduction; 4) the parasitoids ability to survive at low host densities; 5) the risk of hybridization and competition with species that are native in the area of introduction; and 6) methods to establish laboratory colonies of non-target *Agrilus* spp. for testing the host specificity of the considered parasitoids.

4. Conclusions

Our study utilised a recently developed decision-based framework to assess the feasibility of candidate biological control agents for use in pre-emptive biological control against high-risk pests. We found that three of the four candidate EAB parasitoids considered in this assessment – *O. agrili*, *S. galinae*, and *T. planipennisi* – would be feasible for pre-emptive biological control against this pest from a generalised European perspective. These findings can be used by scientists and regulatory bodies in European countries to justify biosafety risk assessments for these parasitoids that could support applications to pre-emptively approve their importation and release, conditional to the establishment of EAB. This case study represents a first step towards the development of biological control preparedness against this high-risk pest that is predicted to spread widely throughout Europe and pose a severe economic and environmental threat in the coming decades. We hope that this work will promote further research towards biosecurity preparedness against EAB in Europe, to mitigate the consequences of a delayed response to its arrival.

Author statement.

KJH, MLS, RM and JC conceptualised the study. MLS, RM and JC acquired funding. KJH collected and compiled data. KJH, RED and NA wrote the original draft manuscript. All authors reviewed, edited and approved the final draft.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocontrol.2024.105641.

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