Conceptual and technical innovations to better manage invasions of alien pests and pathogens in forests

Edited by

Hervé Jactel, Christophe Orazio, Christelle Robinet,
Jacob C. Douma, Alberto Santini, Andrea Battisti,
Manuela Branco, Lukas Seehausen, Marc Kenis

PENSOFT

NeoBiota 84 (Special Issue)

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Cover photo (clockwise from top left): fruiting bodies of *Austropuccinia psidii* on *Myrtus communis* (symptoms of myrtle rust), Photo by Alberto Santini; rearing systems of *Ips typographus*, Photo by Andrea Battisti; adult of *Anoplophora chinensis*, Photo from wikimedia.org

First published 2023
ISBN 978-619-248-112-4 (e-book)

Pensoft Publishers
12 Prof. Georgi Zlatarski Street, 1700 Sofia, Bulgaria
Fax: +359-2-870-42-82
info@pensoft.net
www.pensoft.net

Printed in Bulgaria, May 2023

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NeoBiota 84: 1–7 (2023)
doi: 10.3897/neobiota.84.104682
https://neobiota.pensoft.net

EDITORIAL

A peer-reviewed open-access journal
NeoBiota
Advancing research on alien species and biological invasions

Management options for non-native forest pests along their invasion pathways

Hervé Jactel[1], Andrea Battisti[2], Manuela Branco[3], Jacob C. Douma[4], Marc Kenis[5], Christophe Orazio[6], Christelle Robinet[7], Alberto Santini[8], Anna Sapundzhieva[9], M. Lukas Seehausen[5], Pavel Stoev[9,10]

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Academic editor: Ingolf Kühn | Received 7 April 2023 | Accepted 24 April 2023 | Published 18 May 2023

Citation: Jactel H, Battisti A, Branco M, Douma JC, Kenis M, Orazio C, Robinet C, Santini A, Sapundzhieva A, Seehausen ML, Stoev P (2023) Management options for non-native forest pests along their invasion pathways. In: Jactel H, Orazio C, Robinet C, Douma JC, Santini A, Battisti A, Branco M, Seehausen L, Kenis M (Eds) Conceptual and technical innovations to better manage invasions of alien pests and pathogens in forests. NeoBiota 84: 1–7. https://doi.org/10.3897/neobiota.84.104682

Not a single year goes by without European forests being invaded by new alien species of insect herbivore or pathogenic fungi. This ever-increasing flow of alien pests (Santini et al. 2013; Roques et al. 2020) is clearly linked to the increase in international trade, in particular to containers containing wood packaging material such as wooden crates, dunnage, pallets, or potted plants where wood- or plant-living insects and pathogens can hide (Meurisse et al. 2019). In 2021, about 60 million containers arrived in European ports (Eurostat 2022), more than 150,000 every day. The largest container ships are now 400 m long and carry more than 20,000 containers on each trip. It is obvious that the inspection effort, although reinforced, cannot deal with such an impressive amount of commodities and it is very unlikely that the flow of international trade will decrease in the years to come owing to our globalized economy. Global warming will certainly not help because new and shorter sea routes are opening up with the melting of the Arctic ice. An increasing proportion of freight in Europe comes from South America, Africa and especially Asia (Eurostat 2022), all hot regions, and the increase in temperatures in Europe may facilitate their establishment. Damage caused by these exotic pests and diseases affects the survival and growth of trees, altering the functioning of forests and thus jeopardizing the multiple ecosystem services they provide. In addition, eradication and management costs of non-native forest pests are considerable. A recent study has shown that the economic cost of biological invasions is of the same magnitude as that of natural disasters (like storms or wildfires). They have been multiplied by 8 between 1980–2000 and 2000–2020, amounting to more than 1,000 billion dollars (Turbelin et al. 2023). Because forests provide irreplaceable goods and materials for people and the European economy, because maintaining healthy forests is essential for their contribution to climate change mitigation through sequestration and storage of atmospheric carbon, it is urgent to develop more effective protective measures against the ever-increasing threat of invasive forest pests.

The invasion process has four main stages, starting with the arrival of individuals (propagules) in a new territory, sometimes followed by a phase of establishment of a population, and its development in an epidemic phase, and finally its geographical expansion (Paap et al. 2022). The principle of preventing the risks associated with these invasive species is to hinder the transition from one phase to the next as quickly and effectively as possible. In this context, the role of researchers is to develop, test and promote the most relevant methods and tools at each stage of the invasion framework, i.e., for the early detection of these invasive alien organisms, for the identification of the species and for the monitoring of their damage and spread, but also for new eradication and control solutions. These are the principles that the European project HOMED (https://homed-project.eu) has applied since 2018 in partnership with 23 research organizations in Europe but also in Australia, China, New Zealand, South Africa and the United States, as they represent the regions of origin of many invasive species in the European forests. This project also benefited from a close collaboration between forest entomologists and pathologists, which was very useful because insects and pathogens often share the same host trees and invasion pathways, or frequently have biotic interactions. The two disciplines are complementary in terms of scales of analysis and investigation methods (Jactel et al. 2020). This project has led to major advances in the management of invasive pests in forests, as illustrated by the 16 articles collected in this special issue of NeoBiota. These publications address the four main stages of the invasion process and its management.

The first phase is the one preceding the arrival of the alien species in the new territory during which the pre-border biosecurity approach is put in place. This is during this preliminary phase when it is necessary to reinforce the preparedness of managers to the risk of invasion and try to identify the alien species likely to invade the territory exposed to the risk. The adoption by a large range of end-users (from customs to nurseries and forest enterprises) of technological innovations for the management of invasive species is highly dependent on the awareness of these organisms by the multiple actors involved in quarantine inspection, management of forest and forest health, plant nurseries, urban parks, garden centres, etc. The adequacy of these new tools also requires attention to the real needs of these stakeholders. This is revealed by the survey conducted among many forest health stakeholders in 15 European countries (Green et al. 2023). When invasive alien pests are known, the susceptibility of potentially exposed woody species can be assessed using sentinel plantations ex patria, i.e., in areas where these pests are already present. This approach is illustrated by Paap et al. (2023) for the Myrtle rust and by Casarin et al. (2023) for the bacterium *Xylella fastidiosa*. From a risk assessment perspective, it is essential to establish the diversity of invasive species known to be harmful to trees as well as their traits or pathways favouring their invasive potential, such as cosmopolitan bark beetles (Grégoire et al. 2023), Buprestidae (Ruzzier et al. 2023), or even all the exotic pests associated with a particular tree species such as the radiata pine (Brockerhoff et al. 2023).

The second phase corresponds to the arrival of invasive species in the new territory, for example exotic pests in European forests. Here, it is necessary to detect them as early and efficiently as possible in order to quickly trigger eradication measures. Trapping methods with generic attractants have been shown to be very relevant for the detection of exotic bark beetles in high-risk areas such as ports and airports (Roques et al. 2023), or with the help of vigilant citizens such as school children (Colombari and Battisti 2023). Rotating spore traps have given encouraging results to detect fungal pathogens such as the one causing ash dieback (Dvořák et al. 2023). Many invasive pathogens go unnoticed in their establishment phase or cause non-specific symptoms. It is therefore very important to have reliable and rapid methods to identify them. For example, molecular methods such as LAMP or real-time PCR allow the simultaneous detection of different organisms, such as *Ophiostoma novo-ulmi* and *Geosmithia* spp. in elm plants or bark beetle vectors (Pepori et al. 2023). The presence of invasive species can also be detected through advanced imaging techniques. For example, the winter nests of the pine processionary moth can now be more accurately detected by drone images analysed by artificial intelligence algorithms (Garcia et al. 2023).

The third phase involves the establishment of invasive species in the new territory, a process that is intended to be interrupted by eradication measures. However, eradication is often complicated and not always accepted by the citizens. A systematic review of the literature has identified the main causes of failure and success of eradication attempts of woody plant pests in Europe, allowing also recommendations for successful implementation (Branco et al. 2023).

The fourth phase starts if eradication measures were not successful and therefore it is necessary to move to long-term management of established populations by limiting their expansion and damage. In particular, studies must be conducted on the climatic conditions and the quantity and vulnerability of host trees that favour the spread of diseases, as shown with the maple sooty disease (Muller et al. 2023) or the small spruce bark beetle (Cocos et al. 2023). Spread models can also be developed to test the role of various potential factors on spread (e.g. human-mediated dispersal, urban trees), and thus better predict the rate and direction of spread of invasive species such as the citrus psyllid (Nunes et al. 2023). Finally, control actions must be considered for the containment of populations of exotic species that have become established in European forests. An interesting solution is that of conservation biological control, which is based on the principle of reinforcing native and generalist natural enemies capable of controlling the populations of new exotic prey. More generally, forest diversification can induce associational resistance by intermingling host and non-host species, leading to less damage from exotic species, as shown in the case of the Douglas-fir midge and Swiss rust (Stemmelen et al. 2023).

The publications collected in this special issue demonstrate that current conceptual, methodological, and technological advances allow a great progress in the anticipation, monitoring and management of invasive pest species in forests. However, it should be noted that each of them, taken alone, is not sufficient to significantly reduce the risk of pest invasion. It is their combination, in a coherent whole, which will effectively reduce the impact of the invasive species on European forests. We therefore call on the community of researchers and practitioners to work together to develop a real strategy for monitoring and managing non-native forest pests by deploying at each stage of the invasion and in the areas at risk, the tools and methods that we contributed to improve or develop. As non-native species can arrive in different parts of Europe, can be highly mobile, borders are not impermeable, and European forests are themselves often transboundary, it is obvious that these strategies should be applied continent-wide. Mobilisation of communities beyond the forest sector and international scientific cooperation should therefore be pursued. It is also necessary to continue to harmonize national biosecurity policies and ideally to establish a European task force capable of reacting rapidly to the arrival or spread of new non-native forest pests, by not only assessing the associated risk and but also proposing actions for detection, surveillance, and control.

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NeoBiota 84: 9–40 (2023)
doi: 10.3897/neobiota.84.95761
https://neobiota.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
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Advancing research on alien species and biological invasions

Awareness, detection and management of new and emerging tree pests and pathogens in Europe: stakeholders' perspectives

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Academic editor: M. Branco | Received 30 September 2022 | Accepted 6 March 2023 | Published 18 May 2023

Citation: Green S, Dehnen-Schmutz K, Drakulic J, Eschen R, Orazio C, Douma JC, Lundén K, Colombari F, Jactel H (2023) Awareness, detection and management of new and emerging tree pests and pathogens in Europe: stakeholders' perspectives. In: Jactel H, Orazio C, Robinet C, Douma JC, Santini A, Battisti A, Branco M, Seehausen L, Kenis M (Eds) Conceptual and technical innovations to better manage invasions of alien pests and pathogens in forests. NeoBiota 84: 9–40. https://doi.org/10.3897/neobiota.84.95761

Abstract

Emerging and invasive tree pests and pathogens in Europe are increasing in number and range, having impacts on biodiversity, forest services, ecosystems and human well-being. Stakeholders involved in tree and forest management contribute to the detection and management of new and emerging tree pests and pathogens (PnPs). We surveyed different groups of stakeholders in European countries. The stakeholders were mainly researchers, tree health surveyors and forest managers, as well as forest owners, nurseries, policy-makers, advisors, forestry authorities, NGOs and civil society. We investigated which tools they used to detect and manage PnPs, surveyed their current PnP awareness and knowledge and collated the new and emerging PnP species of concern to them. The 237 respondents were based in 15 European countries, with the majority from the United Kingdom, France and the Czech Republic. There was a strong participation of respondents with a work focus on research and surveying, whereas timber traders and plant importers were less represented. Respondents were surveyed on 18 new, emerging PnPs in Europe and listed an additional 37 pest species and 21 pathogen species as potential future threats. We found that species on EPPO's list of 'priority pests' were better known than those not listed. Stakeholders working in urban environments were more aware of PnPs compared to those working in rural areas. Stakeholders' awareness of PnPs was not related to the number of new, emerging PnP species present in a country.

Stakeholders want access to more detection and management tools, including long-term citizen-science monitoring, maps showing spread and range of new PnPs, pest identification smartphone apps, handheld detection devices, drone monitoring and eDNA metabarcoding. To help facilitate better forest health across Europe, they called for mixed forest development, reduced nursery stock movement, biosecurity and data sharing amongst organisations. These results indicate that stakeholder knowledge of a few key PnP may be good, but given that the large diversity of threats is so large and future risks unknown, we conclude that multiple and varied methods for generic detection, mitigation and management methods, many in development, are needed in the hands of stakeholders surveying and managing trees and woodlands in Europe.

Keywords
Forest management, invasive alien species, new methods and tools, participatory research, risk management, stakeholder survey, tree health biosecurity

Introduction

Trees and forests provide a multitude of environmental and socio-economic benefits (Trumbore et al. 2015; Baral et al. 2016); however, they face serious threats from new and emerging forest pests and pathogens (PnPs) (Wingfield et al. 2010). Global trade and international travel have resulted in the increasing introduction of invasive non-native tree pests and diseases (Roques 2010; Brockerhoff and Liebhold 2017; Meurisse et al. 2019) and their establishment and impacts are being exacerbated by climate change (Pautasso et al. 2015; Linnakoski et al. 2019) and degradation of forest land. Climate change also puts abiotic stress on trees making them more vulnerable to attack by PnPs (Tubby and Webber 2010; Bentz et al. 2010; Kubiak et al. 2017; Kim et al. 2021). Recent examples of alien PnPs in Europe include: *Xylella fastidiosa* subspecies *pauca* attacking olives and other plants (Desprez-Loustau et al. 2021) where *X. fastidiosa* was identified in Italy in 2013 (Saponari et al. 2019); *Phytophthora pluvialis* attacking Western hemlock, first recorded in UK in 2021 (Pérez-Sierra et al. 2022); and *Agrilus planipennis*, the emerald ash borer, found in European Russia since 2003 and in Ukraine since 2017 (Orlova-Bienkowskaja et al. 2020). An increase in forest damage caused by a native PnP, *Ips typographus*, the European spruce bark beetle, has recently been observed in many European forests, causing increased damage to forests. such as those in the Central German uplands (Zimmermann and Hoffmann 2020).

These alien and emerging PnPs are impacting trees in both natural and planted forests and rural and urban settings, by reducing the ecosystem services they provide. The impacts affect a wide range of stakeholders, thus emphasising the need to involve these groups also in their prevention and management. One of the first steps in this process is the understanding of stakeholder perceptions and suggestions for management solutions (Novoa et al. 2018). For tree and forest health, stakeholder perceptions have been investigated by Marzano et al. (2016); however, stakeholders' views and suggestions for management, in particular at the level of practical tools for management, have not been investigated so far.

Management of PnPs involves multiple stakeholders working in different areas who are involved at the different invasion stages of prevention, eradication, containment and control (Fig. 1) (Ambrose-Oji et al. 2019; Marzano et al. 2020). Furthermore, stakeholder awareness of PnPs and their management options and tools may differ between PnPs depending on PnP abundance or distribution. It is well established that the cost-effectiveness of a management method is inversely related to the abundance/spread of a PnP (Turner et al. 2004). When targeting the first stages of the invasion process, management steps include preventing the introduction of new PnPs, detecting

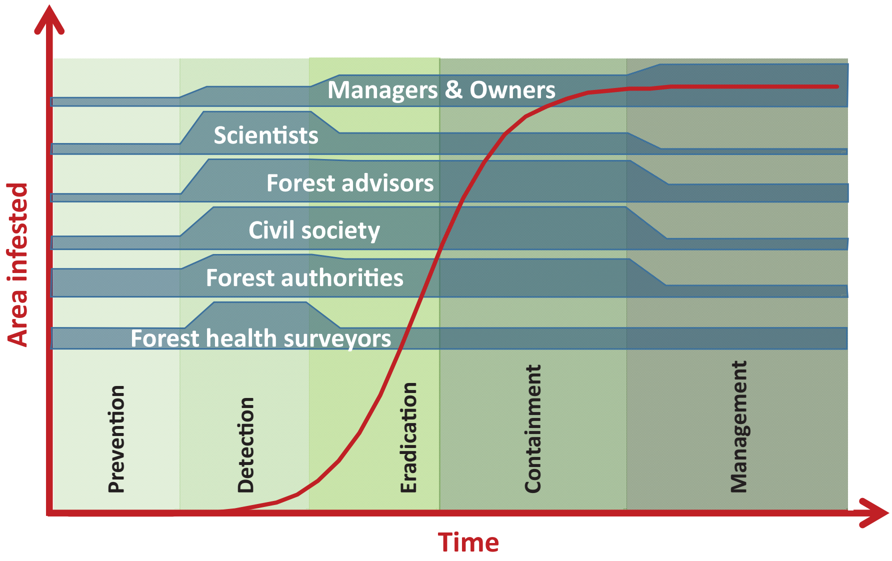

Figure 1. Engagement of different stakeholder groups in the management responses to the different stages of the invasion process of a generalised pest population infestation. Fig. 1 is a conceptual diagram intended to summarise how the various stakeholders interviewed in our survey position their involvement along the various stages of the invasion curve. The thickness of the bars is proportional to their relative involvement in these five steps. The six stakeholder groups were obtained by hierarchically clustering their responses to the questionnaire, assigning a generic name to the group, based on their reported profession. "Managers and Owners" The six stakeholder groups were obtained by grouping the respondents according to their declared professions. "Forest managers" are responsible for the management or maintenance of forests. "Scientists" do research in entomology and forest pathology. "Forest advisors" advise owners on the management of their forest. "Civil society" includes forest users or members of NGOs. The "Forest authorities" are in charge of the implementation of legal measures on forest management. "Forest health surveyors" refers to engineers and technicians in charge of monitoring and controlling forest pests and pathogens. The superposition of the bars corresponding to these six groups does not follow any particular hierarchy. It was chosen to maximise the clarity of the figure.

PnPs early and rapidly responding to those that are introduced and limiting PnP spread (Liebhold et al. 2016). When PnPs have become widespread and abundant, management steps include mitigating their impacts, containing them, protecting assets or salvaging damaged or dead trees (Holmes et al. 2009). As the size of an infested area increases over time, the eradication possibilities become less feasible. Stakeholders' ability to prevent PnP spread as early as possible depends on their awareness of new PnPs or PnPs that have not yet arrived, their role with respect to emerging PnPs, the sources of information available to them, the tools and resources they can use and their motivation of action (Marzano et al. 2015). Likewise, Marzano et al. (2015) also found that the approach to managing established PnPs depends on the focus of a stakeholders' role and the information, tools and resources available to them, regardless of the scale of the outbreak. The stakeholders involved in managing established PnPs are likely different from those involved in early detection and monitoring, so it is important to know what tools and methods stakeholders use currently for PnP detection, identification and management, as well as what stakeholders would like to use to improve management of PnPs in the future.

Gaining understanding of stakeholder awareness of PnPs and their engagement with management tools for PnPs can help to identify groups that may benefit from targeted information about PnPs or highlight areas in need of investment for the development and access to new detection and management tools. The few Europe-wide studies that have been conducted on this also indicate that there is a need to increase the opportunities for knowledge sharing by more experienced tree health practitioners (Marzano et al. 2015; White et al. 2018). Given this, we sought to discover the current knowledge and awareness held by stakeholders in order to provide information about how to target and pitch such knowledge sharing opportunities.

We studied stakeholders' awareness and knowledge of the presence or absence of 18 new and emerging PnPs (Table 1) in their country and of various management practices, using an online questionnaire survey shared with forest health stakeholders from 15 European countries. For our study, forest health stakeholders included all stakeholders engaged with tree work or tree monitoring whether for employment or voluntarily. We formed groups of stakeholders, based on their types of work using cluster analysis and we compared the level of awareness, knowledge and tool use between different groups. Knowledge and awareness are often considered together, for example, Marzano et al. (2016) assessed different levels of knowledge and awareness (without making a distinction) on a scale of four levels ranging from low, where people had never heard about a PnP, to high, where they said they knew a lot about a PnP. However, these responses were not verified. In our study, we use awareness following the definition used by Sudarmadi et al. (2001) as "the attention, concern and sensitivity of the respondent to environmental problems" and for knowledge as "a body of facts and principles concerning the environment that have been accumulated by mankind through study". We, therefore, distinguish awareness, where a stakeholder comments on the status of a PnP independently of whether this is correct or not, from knowledge, where they are correct in their comment about the status of a PnP in their country. Bet-

Table 1. Species and common names of 18 PnPs listed in the survey of forest health stakeholders to answer if they were aware of their presence and abundance in their country and which methods are used to detect and manage it.

The 18 PnPs in the survey	
Common name	**Latin name**
Asian longhorn beetle	*Anoplophora glabripennis*
Box tree moth	*Cydalima perspectalis*
Asian chestnut gall wasp	*Dryocosmus kuriphilus*
Oak processionary moth	*Thaumetopoea processionea*
Douglas-fir needle midge	*Contarinia pseudotsugae*
Emerald ash borer	*Agrilus planipennis*
Eucalyptus snout beetle	*Gonipterus platensis*
Black twig borer	*Xylosandrus compactus*
Oak lace bug	*Corythucha arcuata*
Shot-hole borer	*Euwallacea fornicatus*
Pine wood nematode	*Bursaphelenchus xylophilus*
Pine pitch canker	*Fusarium circinatum*
Pine red band needle blight	*Dothistroma septosporum*
Pine brown spot needle blight	*Lecanosticta acicola*
Root rot fungi	*Heterobasidion irregulare*
Phytophthora ramorum blight	*Phytophthora ramorum*
Ash dieback	*Hymenoscyphus fraxineus*
Xylella wilt	*Xylella fastidiosa*

ter information about these two levels of understanding of stakeholders is important to guide communication about PnPs. Furthermore, we analyse how stakeholder awareness and knowledge are dependent on a range of factors, including presence/absence of PnPs in their country, the urban/rural setting of their work, regulatory status and taxonomic group of the PnPs. We then asked which other PnPs outside our survey list of 18 species were of concern to them. We modelled the responses to determine if the tools used by stakeholders for detection and management depended on their type of work, the PnP species and how long they had experienced the PnP and gathered suggestions for other tools they want access to or to see developed.

Three PnPs were chosen as case studies (*Phytophthora ramorum*, Asian longhorn beetle *Anoplophora glabripennis* and Oak Processionary Moth *Thaumetopoea processionea*) to gain further insight into how effective detection and management methods were perceived to be and whether the methods used varied according to the PnP species and the urban/rural setting of stakeholders' work. These data will help policy-makers, researchers and communicators to appreciate the current understanding and wishes of tree health stakeholders working in different countries, roles and scales, to be able to create tools and resources that are more effective to protect forests from PnPs.

Materials and methods

The study was conducted using an online survey distributed within Europe from October 2019 to March 2020. The survey was designed in English (see Suppl. material 1) and then translated into eight further languages (French, Czech, Italian, Bulgarian, German, Portuguese, Dutch and Swedish). For each language version, there was a national contact person who translated the survey and who was responsible for its distribution to stakeholder groups in the country and, later, the translation of results into English. The questionnaire was designed using the 'Online surveys' platform (https://onlinesurveys.ac.uk/) and first tested via a pilot version in English.

We used a snowball approach in order to reach a wide range of target groups involved in the tree health sector and working across the invasion stages. Initially, a volunteer project partner in each country sent the survey and an explanation of its aims, to relevant academic and professional contacts in their networks. They also e-mailed a list of suggested contacts in a variety of relevant work sectors, generated by other project partners, which included local and national interest groups and forestry newsletter editors. Those contacted were encouraged to share the survey link further in their relevant networks. In addition, we shared the survey link of the questionnaire in relevant languages on social media such as Facebook, Twitter and LinkedIn, encouraging readers to share it with their social media networks.

Questionnaire design

The questionnaire (see Suppl. material 1) addressed issues relating to new, emerging forest pests and diseases and was organised into three sections. The first section asked about the socio-environmental characteristics of respondents. The second section asked about their awareness and knowledge of 18 new and emerging forest PnPs in Europe (Table 1). The third section asked about the tools and methods that they used and would like to use, for detecting, identifying and managing new, emerging tree PnPs. The survey questions included some with required answers. The respondents selected which categories they found most relevant to them using their own judgement and experiences of their environment. Most of the questions were closed-ended, of which some were binary, some had a mixture of multiple possible answers and some had free text answers.

The socio-environmental characteristics asked about in section one comprised the main country and sector(s) of the stakeholder's work role, where their work relates with regard to the invasion stages, geographic scale of their work and urban/rural focus of their work. In section two, respondents were asked to comment on the presence in their country of a list of 18 PnPs and to name any other PnPs they were concerned about. In order to explore the knowledge and perceptions of stakeholders in more depth, this section enabled respondents to give further details of three PnPs (Asian longhorn beetle (ALB), Oak processionary moth (OPM) and *Phytophthora ramorum* (PRA)) regarding how long each PnP had been in their country, the main method used to manage the PnP and how effective they found their chosen management method. The third section asked respondents to select which tools and methods they use from a list of 17 for detecting and identifying PnPs and eight for managing PnPs, then asked open-ended questions for the tools and methods they would like to use in the future.

Informed consent was obtained from all participants. Personal data and responses were stored separately and processed in accordance with the UK General Data Protection Regulation 2016 (UK GDPR 2016) and the Data Protection Act 2018. The survey was approved through Coventry University's ethical approval review process (CU ethics number – Project P90536). A limitation of the study was the time that could reasonably be asked of stakeholders to complete the survey and that stakeholders may suffer from survey fatigue (Fan and Yan 2010). We shortened the survey to focus on further questions for three PnPs rather than the 18 listed and formed the case studies of three PnPs on Asian longhorn beetle, Oak processionary moth and *Phytophthora ramorum*. The choice of 18 PnPs reflected a mix of pests and pathogens at various invasion stages within the European continent. Once a stakeholder had answered the questions on one of the 18 listed PnPs (Table 1), they could not add it to be counted to their list of 'PnPs of concern' in the free text. This means that, within this study, it was not possible to compare the level of stakeholder concern between various PnPs.

Data analysis

The results from all surveys were translated to English where applicable and combined into one dataset. For each country and for each of the 18 PnPs listed, we determined their status at the time when the survey was conducted using the EPPO database (EPPO 2019) and checked if a PnP was listed in the European Commission's list of priority pests (Commission Delegated Regulation (EU) 2019/1702 2019). All statistical analyses were conducted in R version 4.2.2 (R core team 2022).

Clustering and groups

The survey question on stakeholders' work role was multiple choice and, from the 17 roles listed, respondents could choose all categories that applied to them. From the responses provided, we applied a clustering method to detect six separate groups of respondents in terms of their sectors of work. The input variables were binary. We used hierarchical clustering of a distance matrix calculated using a Euclidean distance measure (Hastie et al. 2001). The six groups formed (Table 2; Suppl. material 2: fig. S1) are used in our subsequent analyses to help understand stakeholder experiences and awareness of forest PnPs, as well as stakeholders' tool use for PnP identification, detection and management.

Stakeholder awareness of the 18 PnPs listed in the survey

We analysed stakeholders' awareness and knowledge from their responses to the question in the survey asking them about their experience of the 18 listed PnPs (see survey question 7 in Suppl. material 1). Any response, independent of whether the response was correct with regard to a PnP's presence or absence in a country, was regarded as being aware of a PnP, whereas any other responses ('not applicable', 'I don't know this PnP') or if respondents chose not to answer were interpreted as being unaware.

Table 2. Composition of six stakeholder groups.

Stakeholder group number and name	Stakeholder Group composition
Group 1 – Managers and Owners	This group is formed of 45 stakeholders mainly working in forest and tree management, some of whom may also be woodland or forest owners and, to a lesser extent, some stakeholders may also work in landscape architecture, NGOs, consultancy, education or arboreta. An example of a member in this cohort is a forest owner with a plant nursery, working in forest and tree management.
Group 2 – Scientists	This group comprises 28 people who selected their work as scientific researchers only. An example is a scientist researching tree pests and pathogens.
Group 3 – Forest Advisors	This is the largest group, 66 respondents, who are generalist practitioners and advisers. Many may work in scientific research, as well as forest and tree health surveys. Some combine these roles with forest and tree management, education or consultancy, plant health law, plant nursery or a related role. An example member is a respondent who works in scientific research and at an arboretum.
Group 4 – Civil Society	This is a group of 44 respondents with a mixed variety of roles, many relating to civil society, with a garden or amenity horticulture and plant nursery focus. Volunteers and interested citizens who are also researchers or working for NGOs may be included. An example is a respondent who works in gardens and amenity horticulture and scientific research, is a volunteer or interested citizen and a woodland or forest owner.
Group 5 – Forest authorities	Members of this group of 36, may be working in plant health law enforcement and forest authority organisations and at once be involved with forest and tree health surveys and policy-making. Examples of a member of this group is a respondent who works in plant health law enforcement and another respondent, involved in policy-making, forest authority work, forest and tree health surveys. as well as plant health law enforcement.
Group 6 – Forest Health Surveyors	This group comprises 16 stakeholders who are forest and tree health surveyors, one of whom is also a woodland or forest owner. An example is a respondent doing forest and tree health surveys.

Generalised linear mixed effect models (GLMMs) with binomial error distribution were then used with awareness (yes/no) as the dependent binary variable in our first model (model 1a). The independent variables were all categorical variables: respondents' stakeholder group allocation (six groups as described in Table 2); working scale (local/regional/national/European/global); PnPs, invasion status of PnPs (present, absent, eradicated); and the number of 18 PnPs present at country level. All explanatory variables were included as fixed effects, whilst country and respondent ID were included as random effects (see Suppl. material 2: table S2 for all variables).

In a second model (model 1b), we replaced the individual PnPs with two variables, "EU priority pest" (yes/no) and "insect" (Insect pest or not). This was done to avoid fitting an overly complex model while still being able explore further variables. Our list of 18 PnPs included 10 insects with the remaining species being fungal and bacterial pathogens and one nematode (Table 1). We also included the variable "urban" (yes/no). This variable was not included in model 1a because of missing values; however, model 1 including this variable is shown in the Suppl. material 2: table S3).

Stakeholder knowledge about the invasion status of the 18 PnPs in the survey

We then analysed respondents' knowledge of presence and absence of the 18 PnPs in their country by comparing their answers to the status (presence or absence) of the PnPs in the EPPO Global Database at the time when the survey was conducted (EPPO 2019). We excluded all responses of whether a PnP was 'unknown' to respondents or that no answer was given for, as well as responses where the pest had been eradicated in a country (227 observations) because respondents' choice of answer could not be consistently evaluated as correct or incorrect. We then modelled the correct score (yes/

no) at the level of each response for each PnP as a dependent variable using the same modelling framework as above (model 2a, b).

For the last GLMM analysis (model 3), we aggregated the data at the respondents' level to examine what determines a respondents' accuracy about the status of a pest. This was measured as the proportion of PnPs they reported correctly as present/absent for their country out of the total number they scored. Observations of eradicated PnPs were again excluded from this analysis. We also included the number of PnPs scored by each respondent (our measure of awareness) and the respondents' answers with regard to their main focus of work as a series of seven binary variables (detection, education, control, restoration, research, recording, adaptation).

All these models were analysed in R using the package glmmTMB for fitting GLMMs (Brooks et al. 2017).

In the final analysis, we considered the three status categories of the PnPs for each country: present, absent (never present) or eradicated (absent, but was previously present). We then considered three answers from the stakeholders: (present, absent, eradicated) and scored their answers as correct or incorrect according to a confusion matrix (Suppl. material 2: table S1). We awarded a score of one if their answer matched the PnP invasion status or zero if it was different, in addition to awarding a score of one if a respondent said a PnP was eradicated when the PnP status was actually absent. We calculated the overall percentage of correct scores for the list of 18 PnPs per respondent, then pooled the responses from each country to create a country-wide percentage score. Finally, we used countries as "replicates" to calculate the mean and standard error of these percentages of correct score to produce the results.

New and emerging PnPs of concern to stakeholders

Respondents' free text responses regarding further new, emerging tree pests and diseases of concern were gathered, translated and cleaned to remove ambiguous entries or broad groups of organisms. The data were then grouped by frequency, organism type and country of the respondent. The 18 PnPs listed in the survey in Table 1 were excluded. A combined list of all EPPO priority pests (from the EPPO A1 List of pests recommended for regulation as quarantine pests, EPPO A2 List of pests locally present in the EPPO region and EPPO Alert list of pests possibly presenting a risk to EPPO member countries) was edited to exclude non-tree pests. Then, species listed by respondents that were currently, or had ever been, EPPO priority pests (EPPO 2019) and EU Priority pests (EFSA 2019) were noted. From the combined EPPO list, a percentage was calculated to show the proportion of the list which was represented in the free text responses.

Case studies on Asian longhorn beetle, Oak processionary moth and *Phytophthora ramorum*

Further information was gathered about which primary management method was used by respondents against three case study PnPs, (Asian longhorn beetle, Oak processionary moth and *Phytophthora ramorum*) and how effective the method was perceived to be. To reflect the invasion stage of each PnP in a country, data were obtained from the EPPO distribution maps in April 2022 (EPPO 2022a).

Ninety responses were received for Asian longhorn beetle, 119 for *Phytophthora ramorum* and 104 for Oak processionary moth. The null hypothesis that there was no difference in the use of each management method for each PnP was tested using a Chi squared test for twelve degrees of freedom in R (R core team 2022). The observed frequency of use of each method was compared to the expectation that use of each tool would be equally represented if the null hypothesis were true. Efficacy perception ratings were transcribed into scores where five points corresponded to the most effective rating and one point for the least effective. Mean scores were calculated for the perceived efficacy of each method used against each PnP and for perceived efficacy of management for each PnP according to the urban/rural setting of respondents' work. A top-down approach of a maximal linear model was taken to analyse efficacy scores. The PnP, management method, urban/rural setting of respondents' work and all interaction terms were included initially as explanatory variables. The least significant terms were removed one at a time in a stepwise fashion until all variables with *p* values less than 0.05 could be identified.

Use of tools for detection and management of PnPs

A PERMANOVA (Anderson 2017) was used to study differences in detection and management tools and methods used across stakeholders. The binomial distance was used to calculate the distance amongst respondents in terms of the methods they used against PnPs. Next, we tested whether the distances between groups was significantly larger than within groups. In case the overall test revealed a significant effect of stakeholder groups, a post hoc test with Bonferroni correction was used to show which stakeholder groups were significantly different from each other.

Stakeholders' desired tools and methods

Respondents' answers to open ended questions relating to tools and methods used and those desired to help with PnP detection and management, along with their suggestions for future tool development, yielded a large number of diverse responses. These were extracted, analysed and presented in the Results as tables of the most frequent themes, together with a description of the themes, as drawn from stakeholder comments.

Results

Participant profile

The survey was completed by 237 respondents from 15 European countries. The majority of respondents were from the United Kingdom (69 responses), France (46) and the Czech Republic (28) (Suppl. material 2: fig. S2a for other countries). Respondents could select multiple foci of their work regarding tree PnPs (prevention of entry at border, early detection/rapid response, recording, control and management, adaptation, restoration, education, research). Early detection and rapid response was selected by the greatest number of respondents as their focus of work regarding PnPs, (n = 117; Suppl. material 2: fig. S2b), followed closely by those with a control or management focus (n = 113). Research and recording, for example, surveillance, were well-represented with over a quarter of respondents answering for each (n ≥ 87). Adaptation, or the change of cultural techniques and practices (n = 45), then restoration were the least represented answers (n = 25) and just ten respondents selected "other" as their working focus.

We formed groups of stakeholders for the analysis from the clustering methods, relating to the respondents' sectors of work (Table 2). Group 1 is formed of 'Managers and Owners', respondents who are mainly working in forest and tree management. Group 2, 'Scientists', is formed of scientific researchers only. Group 3, 'Forest advisors', is the largest group of 66 respondents and contains generalist practitioners and advisors. Group 4, 'Civil Society' includes volunteers, NGO workers and those with a mix of backgrounds relating to civil society. Group 5, 'Forest Authorities', include respondents working in plant health law enforcement, forest authority organisations, tree surveying and policy-making. Group 6, 'Forest Health Surveyors' is the smallest group of 16 respondents, who exclusively work in forest and tree health surveying. Table 2 also describes an example respondent from each group. Stakeholder groups differed in their awareness of PnPs and this is described in the results section on stakeholder awareness.

Research scientists were the best represented group by work role profile (n = 91; Suppl. material 2: fig. S2c; Question 3 in the survey in Suppl. material 1), followed by forest and tree health surveyors and those working in forest and tree management (n ≥ 70 each), whereas timber traders and plant importers were less represented (n < 10 each). The remaining groups covering forest authorities and policy-makers, educational and horticultural practitioners contained between 11 and 35 respondents each. Respondents were working at spatial scales from less than a hectare to worldwide. The majority of respondents were working at national scale (n = 79, 33%; Suppl. material 2: fig. S2d) and regional/sub-national scales (n = 60, 25%). Far fewer were working at European (n = 24, 10%) and worldwide (n = 19, 8%) scale. Seventeen (7%) respondents were working at 10 km^2 to regional area scales. Amongst the local scales, most respondents worked at scales between one and 99 hectares (ha) (n = 22, 9%), followed by 100–999 ha (n= 7, 3%) with few working at less than one ha (n = 6, 3%).

The majority of respondents worked with trees in mostly rural (n = 124, 52%) or completely rural (n = 54, 25%) environments. Considerably fewer worked in mostly urban (n = 34, 14%) or completely urban (n = 3, 1%) areas.

Stakeholder awareness of the 18 PnPs in the survey

Of the overall 4266 scores received for the 18 PnPs, 58% indicated that respondents were aware of the respective PnP (i.e. they said they were aware of a PnP, independent of whether they scored presence/absence correctly). The remaining 42% of scores related to responses where either no score was received or the respondents did not know the PnP. On average, respondents were aware of 10.5 (SE 0.32) of the 18 listed PnPs, ranging from four respondents not answering to any of the PnP scoring questions of the survey to 19 respondents scoring all of them.

Results from model 1a (Suppl. material 2: table S4) demonstrate that respondents' awareness was dependent on whether a PnP was present in their country or not ($F = 103.87$, $df = 2$, $p < 0.001$) with respondents more likely to be aware of PnPs that were present in their country. Awareness of PnPs differed significantly between the stakeholder groups ($F = 28.5$, $df = 5$, $p < 0.001$). Awareness of the individual PnPs varied significantly ($F = 466.24$, $df = 17$, $p < 0.001$), but the total number of PnPs present in a country did not have an impact on the probability that respondents were aware of individual species ($F = 2.35$, $df = 4$, $p = 0.672$).

When individual PnPs in model 1b (Suppl. material 2: table S4) were replaced with variables stating if a species were an insect or not, its status as EU priority pest and the urban or more rural working scale variable (reducing the number of observations), we found that all these variables were significant to explain increased awareness by respondents (Insect: $F = 47.18$, $df = 1$, $p < 0.001$; EU priority: $F = 221.72$, $df = 1$; Urban/rural working scale: $p < 0.001$, $F = 6.66$, $df = 1$, $p < 0.01$; Suppl. material 2: table S4). As in the full model, the status of the PnP species was significant ($F = 449.65$, $df = 2$, $p < 0.001$), as was the stakeholder group ($F = 29.44$, $df = 5$, $p < 0.001$). In particular, respondents in the 'Civil Society' group were less likely to be aware of a PnP, but respondents in the 'Forest Authority' group were more likely to be aware. Neither the number of PnPs present in a country ($F = 1.75$, $df = 1$, $p = 0.185$) nor the working scale ($F = 2.13$, $df = 4$, $p < 0.712$) had an impact on the probability of being aware of a PnP.

Stakeholder knowledge about the invasion status of the 18 PnPs in the survey

Respondents scored on average 8.2 (SE 0.29) of the 18 PnPs correctly with regard to their presence or absence in their country, with a range from two respondents (of 234) not getting any correct scores to four respondents being correct about the status of all of the PnPs in their country. The correctness of respondents' knowledge (model 3) was highly dependent on the PnP itself ($F = 97.19$, $df = 17$, $p < 0.001$; Suppl. material 2: table S3), but whether a PnP was an insect or an EU priority species did not correspond to differences in correctness. There were significant differences in correctness according to the scale stakeholders were working on ($F = 23.31$, $df = 4$, $p < 0.001$), with stakeholders working at national scale significantly more likely to know the status of a PnP correctly. Amongst the PnPs, the status of ash dieback (*Hymenoscyphus fraxineus*) was scored with the highest accuracy, whereas root rot fungi (*Heterobasidion irregulare*) were most likely to be scored incorrectly.

When we aggregated the data to look at the proportion of PnPs for which individual respondents reported the correct invasion status (model 3, Suppl. material 2:

table S5), we found that the scale people worked at still strongly corresponded with their ability to correctly report the invasion status of the PnPs (F = 29.06, df = 4, p < 0.001). Once again, those working at national scales had the highest likelihood to be correct. Those reporting their main work focus to be on detection (F = 4.52, df = 1, p = 0.036), education (F = 4.77, df = 1, p = 0.029) or research (F = 4.18, Df = 1, p = 0.04) were most likely to be correct; however, correctness across all the PnPs invasion statuses did not differ significantly between the stakeholder groups. The level of awareness (i.e. number of PnPs scored by individual respondents) was not a significant factor explaining the proportion of PnPs scored with the correct invasion status.

Looking at correctness across all respondents for individual PnPs, stakeholders were overwhelmingly correct (~ 80%) about the presence or absence of PnPs, but few knew about past eradications (< 20%). Stakeholders seem to know more about absence than presence (Fig. 2), as correct negative responses were consistently more common than correct positive responses.

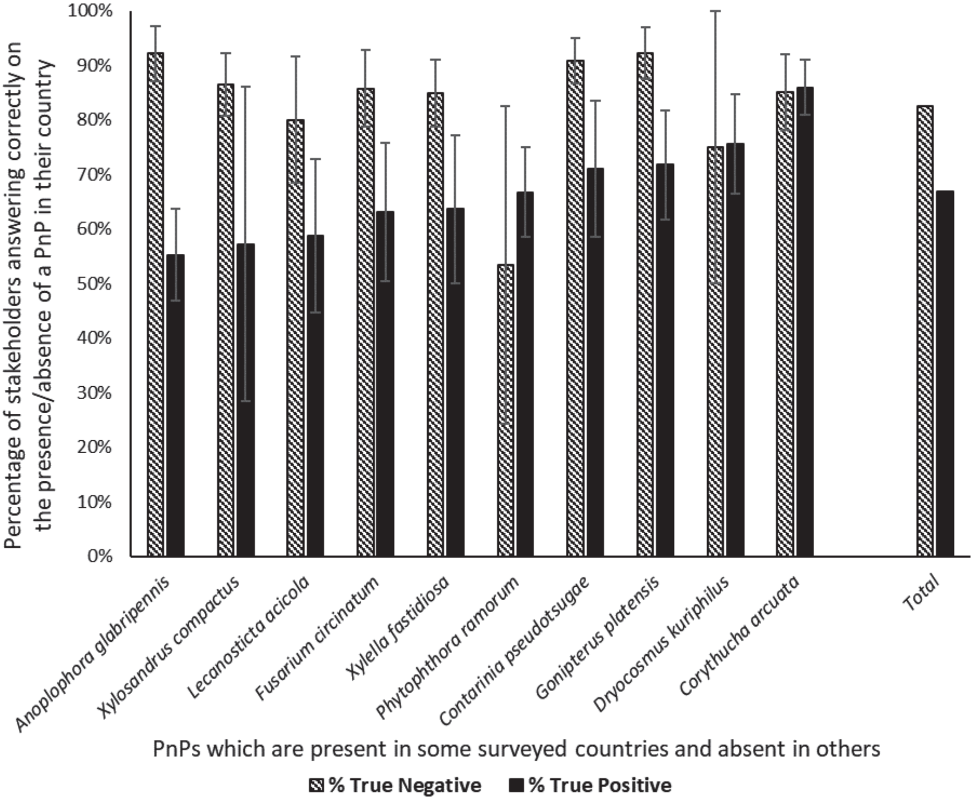

Figure 2. Stakeholder knowledge about the invasion status of PnPs, showing the percentage of true positive and negative results of PnP awareness for named PnPs which are both present in some countries and absent in others.

New and emerging tree PnPs of concern to stakeholders

Further to the 18 PnPs listed in the survey structure, (Table 1) respondents listed 37 additional invertebrate (Table 3) and 21 pathogen species (Table 4) of concern to them. Nine of the invertebrate and four pathogen species are EPPO-listed species, while just five invertebrates and no pathogens are EU priority pests. The species listed by our respondents represent 6% of the species on the combined lists of EPPO priority pests and pathogens of trees. Most pests reported were beetles (Coleoptera), comprising longhorn (Cerambycidae), jewel (Buprestidae), bark

Table 3. Invertebrate pests of trees and the frequency and countries of stakeholders that listed them as organisms of concern in addition to the list of 18 PnPs referred to in the survey.

Latin name	Common name	Frequency	Countries
Anoplophora chinensis‡	Citrus longhorn beetle	9	CR, F, SWI
Agrilus anxius†‡	Bronze birch borer	5	CR, UK
Ips typographus	Larger eight-toothed European spruce bark beetle	5	B, N, UK
Vespa velutina	Asian hornet	4	F, P, UK
Xylotrechus chinensis†	Tiger longhorn beetle	4	F, GE, GR
Cameraria ohridella†	Chestnut leaf miner	3	B, UK
Dendrolimus sibiricus‡	Siberian silk moth	3	F
Popillia japonica‡	Japanese beetle	3	I, SWI
Thaumetopoea pityocampa	Pine processionary moth	3	P, UK
Xylosandrus crassiusculus†	Granulate ambrosia beetle	3	F, GE
Aromia bungii†‡	Red-necked longhorn beetle	2	F, I
Dendroctonus micans	Spruce bark beetle	2	F, UK
Ips sexdentatus	Six-toothed bark beetle	2	CR, F
Phloeomyzus passerinii	Poplar woolly aphid	1	F
Eriosoma lanigerum	Woolly aphid	1	F
Dryocoetes himalayensis	Himalayan bark beetle	1	CR
Euwallacea whitfordiodendrus	Polyphagous shot-hole borer	1	UK
Gnathotrichus materiarius	American utilizable wood bark beetle	1	CR
Pityophthorus juglandis†	Walnut twig beetle	1	CR
Ips amitinus	Small spruce bark beetle	1	SWE
Ips cembrae	Larch bark beetle	1	SWE
Xylosandrus germanus	Black timber bark beetle	1	CR
Melolontha hippocastani	European forest cockchafer	1	F
Melolontha melolontha	Cockchafer	1	F
Trachymela sloanei	Small eucalyptus tortoise beetle	1	P
Phoracantha semipunctata	Australian Eucalyptus longhorn beetle	1	P
Psacothea hilaris†	Yellow spotted longhorn beetle	1	I
Tetropium gabrieli	Larch longhorn beetle	1	SWE
Thaumastocoris peregrinus†	Bronze bug	1	P
Oxycarenus lavaterae	Lime seed bug	1	CR
Corythucha ciliata	Plane lace bug	1	UK
Halyomorpha halys	Brown marmorated stink bug	1	I
Leptoglossus occidentalis	Western conifer seed bug	1	F
Glycaspis brimblecombei†	Red gum lerp psyllid	1	P
Trioza erytrae	African citrus psyllid	1	P
Hylobius abietis	Large pine weevil	1	F
Rhynchophorus ferrugineus†	Red palm weevil	1	F
Total organisms = 37			

Key: † = EPPO priority pest (past or present); ‡ = EU priority pest; B = Belgium; CR = Czech Republic; F = France; GE = Germany; GR = Greece; I = Italy; N = the Netherlands; P = Portugal; SWE = Sweden; SWI = Switzerland; UK = United Kingdom.

Table 4. Tree pathogens and the frequency and countries of stakeholders who listed them as organisms of concern in addition to the list of 18 PnPs referred to in the survey.

Latin name	Common name	Category	Freq.	Country listing
Bretziella fagacearum (syn. *Ceratocystis fagacearum*)	Oak wilt	Fungus	14	F, N, SWI
Cryphonectria parasitica	Chestnut blight	Fungus	7	CR, N, SWI, UK
Ceratocystis platani	Plane wilt	Fungus	5	F, N, SWI, UK
Sphaeropsis sapinea (syn. *Diplodia pinea*)	Tip blight & canker	Fungus	4	F, SWE
Phytophthora cambivora	Root rots/Ink disease of European sweet chestnut	Oomycete	3	F, UK
Phytophthora cinnamomi	Root rots/Ink disease of European sweet chestnut	Oomycete	3	F
Cronartium flaccidum	Blister rusts of Scots Pine	Fungus	2	F, SWE
Sphaerulina musiva	Poplar leaf spot	Fungus	2	F
Pseudomonus syringae pv. *aesculi*†	Horse chestnut bleeding canker	Bacterium	1	B
Erwinia amylovora	Fireblight	Bacterium	1	P
Geosmithia morbida†	Thousand cankers disease	Fungus	1	CR
Melampsora larici-populina†	Poplar rust	Fungus	1	F
Sirococcus tsugae†	Sirococcus blight	Fungus	1	UK
Chrysomyxa abietis	Needle rust of fir	Fungus	1	SWE
Chrysomyxa weirii	Spruce needle rust	Fungus	1	SWE
Cronartium ribicola	White pine blister rust	Fungus	1	F
Cryptostroma corticale	Sooty bark disease of Maple	Fungus	1	N
Lecanosticta acicola	Pine needle blight	Fungus	1	A
Ophiostoma novo-ulmi	Dutch elm disease	Fungus	1	B
Splanchnonema platani	Massaria disease	Fungus	1	UK
Thekopsora areolata	Cherry/spruce rust	Fungus	1	SWE
Total organisms = 21				

Key: † = EPPO priority pest (past or present); A = Austria; B = Belgium; CR = Czech Republic; F = France; GE = Germany; GR = Greece; I = Italy; N = Netherlands; P = Portugal; SWE = Sweden; SWI = Switzerland; UK = United Kingdom.

(Scolytinae), leaf (Chrysomelidae) and chafer (Scarabaeidae) beetles. The largest number of species from these groups were bark beetles (11 species), then longhorn beetles (six species). The three pests reported most frequently were beetles, *Anoplophora chinensis* (Citrus longhorn beetle), *Agrilus anxius* (Bronze birch borer) and *Ips typographus* (Larger eight-toothed European spruce bark beetle; Table 3). Citrus longhorn beetle, the pest named by the most respondents, affects broadleaved trees and conifers. Furthermore, of the thirteen pests reported more than once, six affect broadleaves, five affect conifers, one affects both and one does not directly affect trees (*Vespa velutina*).

The pathogens most frequently listed of concern to respondents (Table 4) were *Bretziella fagacearum* (Oak wilt), *Cryphonectria parasitica* (Sweet chestnut blight) and *Ceratocystis platani* (Plane wilt). Most species listed were fungi (17/21) and there were just two species each of bacteria and oomycetes.

Eleven respondents listed groups of invertebrates of concern. The most frequently mentioned group was non-European bark beetles and *Ips* species (n = 6). There were two mentions of *Hylobe* species and one entry each for *Xylosandrus* species, *Contarina* species and tropical xylophagous species (data not shown). Thirteen respondents described groups of pathogens or diseases of concern. Of these, five related to *Phytophthora* species, two each for *Ceratocystis* species and *Armillaria* species, plus one mention each for needle diseases of fir and pine, fungal root rot and *Fusarium* dieback.

Case studies on Asian longhorn beetle, Oak processionary moth and *Phytophthora ramorum*

The primary management method used for each of the three case study PnPs (Asian longhorn beetle (ALB), Oak processionary moth (OPM) and *Phytophthora ramorum* (PRA)) varied significantly between organisms (F = 82.99, df = 12, p < 0.001; Fig. 4). The greatest number of respondents (40%) said that eradication was the primary management method used against ALB, followed by surveillance/monitoring (23%) and early detection/rapid response (20%). Control and management was the primary tool used for OPM (35%) and PRA (25%). The other two frequently chosen methods for OPM were surveillance/monitoring (23%) and education (21%). The other three methods selected more frequently for PRA were eradication (21%), surveillance (18%) and early detection (17%).

The perceived efficacy of the primary management method used most frequently was high for ALB (eradication: mean score = 4.3 ± 0.18 SE, Suppl. material 2: table S6), but lower for OPM and PRA (control and management: 3.4 ± 0.23, OPM; 3.6 ± 0.25, PRA). For PRA, early detection and rapid response received the highest efficacy rating (3.85 ± 0.24), whereas for OPM eradication was perceived as the most effective (4 ± 0.32). The lowest efficacy score for all PnPs was found when the respondents selected "no management" (2.5 ± 0.29, ALB; 2.86 ± 0.46, OPM; 2.5 ± 0.87, PRA).

Perceived efficacy scores of the primary management method used (Suppl. material 2: fig. S4) against ALB were consistently higher across all urban/rural working remits compared to those used for OPM and PRA. Perceived efficacy of methods used against OPM and PRA were similar in all urban/rural settings. The PnP was highly significant in the linear model (Suppl. material 2: table S7) to account for variation in efficacy score of the primary management method used ($p < 0.001$). The method used was also strongly significant in determining the efficacy perception ($p = 0.002$), whereas the urban/rural setting of respondents' work was only significant at the 10% level ($p = 0.057$). There is a slight trend for efficacy to be perceived less positively the more rural the respondent's work remit. Where urban/rural was not deemed applicable to their work, respondents gave the lowest efficacy scores for the primary management method for OPM and PRA (mean score 3 ± 0.49 SE, OPM; 3.11 ± 0.48, PRA).

Use of tools for detection and management of PnPs

Survey respondents answered whether they used 17 tools and methods for detecting and identifying PnPs or eight tools for managing PnPs. Most respondents indicated that they use monitoring of infected areas, books, websites, experts or tree health advisory services, plant health policies and advice and research publications for detecting and identifying PnPs (Fig. 3a).

We found there were significant differences in methods used for detecting and identifying PnPs across stakeholder groups (F = 5.29, df = 5, p < 0.001; Suppl. material 2: fig. S3a). The 'Managers and Owners' group use different tools compared to

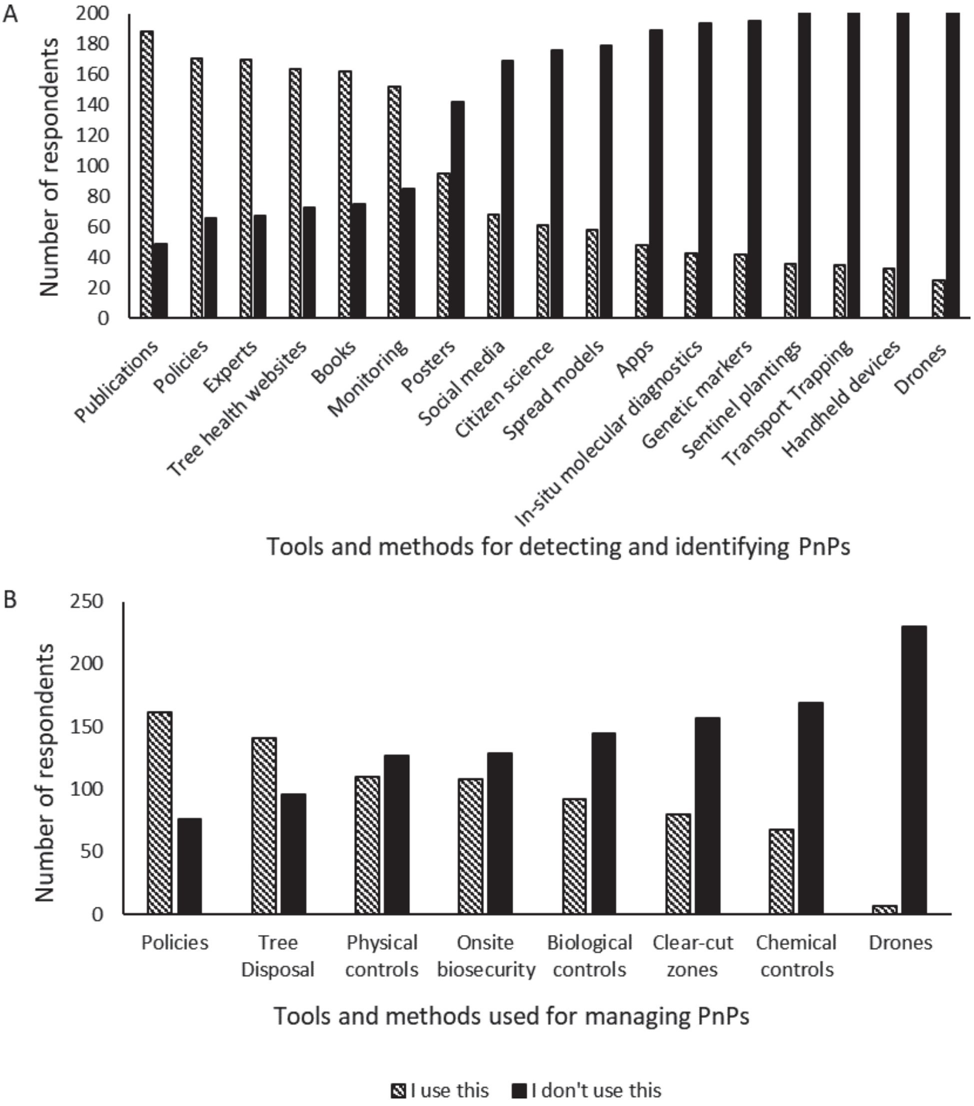

Figure 3. Number of respondents who said they used each of (**A**) 17 tools and methods used for detecting and identifying and (**B**) eight tools and methods for managing new and emerging forest PnPs (required answer for all respondents).

'Forest Advisors', 'Forest Health Authorities' and 'Forest Health Services'. Likewise, 'Civil Society' use different tools to 'Forest Advisors', 'Forest Health Authorities' and 'Forest Health Surveyors'. The 'Forest Advisors' used more of the detection and identification tools in total.

Some tools and methods for detection and identification had very low use by certain groups, with no responses from 'Civil Society' for the use of drones, which was the least used method across all groups. Other than for 'Forest Advisors', the use of

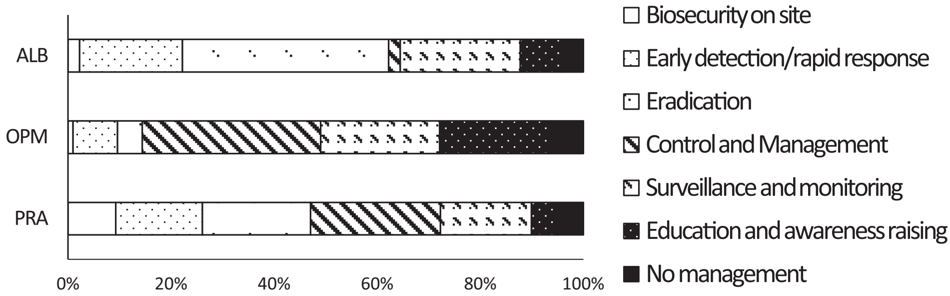

Figure 4. The primary management tool used by stakeholders against three case study PnPs (ALB = Asian longhorn beetle; OPM = Oak processionary moth; PRA = *Phytophthora ramorum*). Tools displayed left to right are listed in order top to bottom in the key from biosecurity on the left to no management on the right.

genetic markers, transport trapping, *in situ* molecular diagnostics, hand-held devices, spread prediction models, sentinel plantings and identification and recording apps were also low. Citizen-science reporting was not widely used by any groups, except 'Civil Society' and 'Forest Advisors' where around one in three and one in four used this method, respectively.

For management of PnPs (Suppl. material 2: fig. S3b), most respondents used plant health policies (68%) and disposal of infected trees or tree parts (59%). In contrast, most respondents did not use biosecurity, biological control, clear-cut zones, chemical or physical controls or drones. Drones with sensors and sprayers were the least used method for managing PnPs.

Stakeholders' desired tools and methods

There were 403 stakeholder comments and suggestions for future development and access to tools and methods for PnP detection and management beyond those listed within the survey, which fell into six themes: surveillance and trapping; education, information and data sharing; tools and techniques; citizen science and 'eyes on the ground'; inspections and import restrictions; experiments and research. The numbers of comments in each theme are shown in Table 5.

Surveillance and trapping

The comments within this theme centred on the use of pheromone, multilure and spore traps, as well as drones, sniffer dogs, aerial surveillance and LiDAR. Respondents desired trapping and surveillance to be more widely used, including for domestic and public gardeners to use pheromone traps. However, there were concerns about the (unspecified) limitations of drones, to whom the financial costs of surveillance and trapping would fall and when in a plant's life trapping and surveillance should be performed.

Table 5. Themes of stakeholder comments for their desired future detection and management tools.

Theme of stakeholder comments	**For detection and identification**		**For management**		**Total number of comments**
	No. of stakeholder suggestions for developments of isted tools and methods for PnP detection and identification	No. of stakeholder comments on other tools and methods used, and wanted for IEFP detection	No. of stakeholder suggestions for developments of listed tools and methods for PnP management	No. of stakeholder comments on other tools and methods used, and wanted for PnP management*	
Surveillance and trapping	"Trapping devices = 4, Monitoring = 11, Drones=10	19	NA	13	57
Education, information and data sharing	Plant Health policies and advice = 18, Books = 3,Research pubilcations = 10,Tree Health advisor services and Experts = 7	24	Plant health policies and advice = 8	29	99
Tools and techniques	Genetic Markers = 2,Prediction models = 5, in-situ molecular diagnosis = 3, handheld devices = 3	35	On-site biosecurity practices = 11, Physical control methods = 6, Chemical control methods = 7,Biological control methods = 7,Clear-cut zones = 7, Disposal = 2, Drones with multisensors, processors and sprayers = 4	47	139
Citizen science and "eyes on the ground"	Social media = 12, Citizen Science = 13, Apps =10	9	NA	4	48
Restricted imports and inspections	Posters = 10	5	NA	20	35
Experiments and research	Sentinel plantings = 8	3	NA	14	25
Total	129	95	52	127	403
Numbers shown are the no. of respondent comments					

*this includes comments on tools, bodies or regulations stakeholders would like to see developed.

Education, information and data sharing

Stakeholder suggestions encompassed ideas on accessibility, social media, information sharing and an educational network with training opportunities. The range of professionals that stakeholders rely on come from many sectors: governmental officers, charities, industry, academia and volunteer networks. Collaborations and knowledge sharing were called for amongst plant health bodies, professionals, industry and interested citizens. Respondents wanted access to maps showing range and recent sightings of PnPs. They recommended using social media for horizon scanning and sharing cases of interceptions. One suggestion called for long-term establishment of existing citizen-science tree health programmes with sufficient expert support.

Respondents envisaged that Pest Risk Analysis following horizon scanning and liaising with networks of scientists and experts inside and outside the country could be further developed. Stakeholders found search engine landing pages which synthesise the most up-to-date and relevant content for forest health the most useful.

Tools and techniques

While some stakeholders saw a need for vastly improved biosecurity, particularly at borders, others found biosecurity recommendations impractical and unrealistic and saw a need for revision of required practices in proportion to the risk, invasion stage and mobility of the organism. There was a wish to develop secure methods for onsite biosecurity and movement, cleaning and management of suspected and affected material and to work together with local neighbours for better biosecurity outcomes.

An increased hesitancy in using chemical control methods was expressed by stakeholders. Prohibitive legislation and an appreciation of environmental harm were given as reasons for this. Stakeholders also noted that approval of new chemicals is slow. Desired methods include chemical insecticide netting on woodpiles, spray, injection, fumigation and electric current. It was also noted that chemical tools vary in their 'greenness' and there was a call for a list of disinfectants and accompanying information on their efficacy against different pests and pathogens.

Other tools suggested by respondents include better and quicker diagnostic tools, such as *in situ* tests, particularly 'cheaper devices for more widespread use' for rapid confirmation of *Phytophthora* spp. and *Xylella fastidiosa*. Furthermore, they wanted field tests and molecular test kits that were easy to use, ways to diagnose from eDNA in air or water samples including non-destructive meta-barcoding approaches, LAMP, qPCR, electronic noses, the ability to send samples for identification in laboratories, drone monitoring of spectral signatures and insect identification from picture galleries. These suggestions were made mainly by tree health surveyors, who may also be working in other sectors concurrently.

Other stakeholder suggestions relate to biodiversity and better underlying plant and ecosystem health to limit the impact of PnP outbreaks.

Citizen science and 'eyes on the ground'

Training, funding, automatic warning systems and better integration of citizen science into official monitoring programmes were suggested to improve the current offer. Interested citizens and professionals reported their use of social media for the detection and identification of PnPs. Further suggestions include to develop a daily PnP learning update to be shared via Twitter. Eight percent of respondents named Facebook and 6% of respondents named Twitter as a social media method they use for detection and identification of PnPs. Stakeholders wanted future developments of apps including an app with keys for identifying PnPs, illustrating symptoms of specific diseases or pests, plus pictures of other types of tree damage that could be confused with damage caused by the pathogen or pest. They had concerns regarding privacy, data sharing, access and record validation within such apps.

Inspections and import restrictions

There was a common desire towards locally-sourced and grown trees instead of importing them, for imports to have greater restrictions with checks implemented by more inspection personnel at borders and inland and inspection checks to be performed for high-risk plants from retail to final planting. Several respondents wanted more content to be displayed on posters and for these to be placed at all departure and arrival areas in transport hubs. Consistency of branding was deemed important and it was suggested that posters could show maps that highlight the range and spread of recent PnP sightings locally to raise public awareness of current issues.

Experiments and research

Other suggested research topics were to improve isolation of pathogens in pure culture from infected plants and find new fungicides. Stakeholders suggested that both formal International Plant Health Sentinel Network sites and informal sentinel trees and plantings could be used to support further research, such as identifying tolerance levels of trees to widespread PnPs. They called to extend citizen-science tree health projects to monitor local trees as sentinels. Plus, stakeholders perceive that it is important to develop high throughput screening for effective selection of resistant breeding stocks alongside traditional breeding.

Discussion

We found that the stakeholders of European forests that we surveyed are relatively aware and knowledgeable about the 18 PnPs we selected for this study, although our group does not represent all stakeholder types or locations equally, with a particular need to look in more detail at those working in the timber trade and from countries other than UK, France and Czech Republic. We also found that there were gaps in awareness and knowledge held by stakeholders, but there is a demand for better support and access to tools for PnP detection and management.

Stakeholder awareness of tree pests and diseases in Europe has been previously studied by Marzano et al. (2016) in a survey of 392 tree and forest professionals in nine countries. When asking these stakeholders about their level of awareness for five PnPs, Marzano et al. found, on average, about 20% of respondents were not aware of these PnPs. In our survey, we found an average of 42% of respondents were not aware of the PnPs we listed. However, our sampling approach differed, as our longer list of PnPs included species that are in an early invasion stage. For the three species included in both surveys (Emerald ash borer, Asian longhorn beetle, Ash dieback), we found similar levels of 'non-awareness' to the study of Marzano et al. (2016). For Emerald ash borer, in our sample, 34.6% of respondents were not aware of the species, compared to 36.3% in Marzano et al. (2016), whereas awareness for Asian longhorn beetle was lower (25.7% vs. 20.4%) despite the fact that several additional outbreaks of Asian longhorn beetle have been recorded in Europe in the meantime (Branco et al. 2022), which could have resulted in an increase in awareness of this species. Only for Ash dieback did we find awareness had improved, from 21.1% of non-awareness in Marzano et al. (2016) to 16% in our sample. This could be attributed to the increasing spread and impacts of the disease, particularly in Britain (Enderle et al. 2019; Hill et al. 2019), where a large number of our participants were based and consistent media coverage during this timeframe. Our respondents' awareness also differed significantly between the 18 PnPs included in our survey, potentially because certain PnPs may pose a larger threat to forests in their country, symptoms are easier to spot or the PnP is easier to identify compared to others (Boa and Nations 2003). Changing taxonomy species, particularly fungi, could be another barrier to accurate stakeholder knowledge (Steenkamp et al. 2018), contributing to the finding that, of the 18 PnPs in the survey, *Heterobasidion irregulare* was most likely to be scored incorrectly by stakeholders.

We found stakeholders seem to be better informed about pests that were absent in their countries than those present. This could suggest that their knowledge is lagging behind the actual invasions and there is a need for better information on newly-established PnPs or it may indicate that stakeholders are well prepared to initiate specific prevention measures against PnPs not yet present. It is important to acknowledge the risk of the yet unknown PnPs and their potential pathways and the need for pathway focused prevention measures (Evans 2010; Webber 2010) and Jactel et al. (2020) also recommend the development of generic tools or methods for pest and pathogen management and capacity building for all stakeholders involved in forest health. Stakeholders knew EU priority pests better than non-priority pests, suggesting that EU and EPPO priority pest lists and plant health authorities' dissemination work (EFSA 2019, EPPO 2022a) has been effective. People working in urban environments were aware of more PnPs compared to people working in rural areas. This could be because of higher diversity of tree species occurring in urban areas, as more imported stock is planted in cities or first arrives in a country via trading ports close to urban areas (Branco et al. 2019). By assigning individual respondents into groups according to their working roles, we were able to identify socio-environmental factors that are linked with varying levels of awareness and knowledge about PnPs. Stakeholders in Civil Society were less likely to know a PnP than those working in Forest Authorities, but all groups can benefit from improved forest health communication, collaboration and knowledge exchange.

The PnPs of concern listed by respondents included more pests than pathogens – consistent with the EU priority list which has very few pathogens and combined EPPO lists, in which, of 260 species that can affect trees, 164 (63%) are insects and mites. The low representation of EPPO priority pests and pathogens affecting trees (6%) by our respondents indicates that, if we are to effectively involve stakeholders in prevention activities, then communication and awareness raising for priority tree PnP species needs to be increased. Frequently-listed species were widespread across the continent (e.g. both *Cryphonectria parasitica* and *Agrilus anxius* were listed from the Czech Republic to UK and *Xylotrechus chinensis* was listed from Greece to France). The high number of bark beetles mentioned could perhaps mirror the visual damage level that has been caused by these groups or simply the great diversity of tree health problems they cause. (Christiansen and Bakke 1988; Ploetz et al. 2013). Interestingly, there was an absence of nematodes and viruses listed by respondents: stakeholders may lack awareness of these groups and diagnostic symptoms can closely resemble those of other pathogens and abiotic stresses (Boeri and Chung 2012; Hassan et al. 2013). Although the diagnostic symptoms for many nematode and virus infections may be hard to separate from other pathogens and abiotic stresses, literature shows that at least one new syndrome, Beech Leaf Disease (BLD), recently found to be associated with the nematode *Litylenchus crenatae mccannii* (Carta et al. 2020), has symptoms that are recognisable and suitable for tree PnP citizen-science surveillance (Woodland Trust 2022).

A limitation in the interpretation of the data is that, since half of the respondents are from the UK and France, the results reflect the situation in these countries more than for other parts of Europe; future work is needed to gather more data for comparison between all European countries. In addition, responses largely reflect stakeholders involved in research and tree health surveying. We did not gather information on the forest types (forest management practices, forest legislation etc.) in the different countries investigated which would be an interesting topic for further study.

Regarding the three case study PnPs (Asian longhorn beetle, Oak processionary moth and *Phytophthora ramorum*), the primary method reported for their management varied significantly depending on the organism and this could reflect their different stages of the invasion process (Blackburn et al. 2011) and/or the policy direction from plant health authorities. As is consistent with EU policy (Commission Delegated Regulation (EU) 2019), eradication of Asian longhorn beetle was listed as the primary method of management by most respondents, which accurately reflects that the species has been eradicated in most of Europe, with only restricted populations remaining in France and Italy as of 2022 (EPPO 2022b). This approach was perceived as highly successful and significantly more so than the approaches used for managing Oak processionary moth and *Phytophthora ramorum*. Oak processionary moth is native to many countries in continental Europe (Austria, Belgium, France, Germany, Italy and the Netherlands (Moran et al. 2015)), but the emerging nature of the problem is reflected in that respondents who said no management was performed against Oak processionary moth gave the lowest efficacy scores.

There was a weak trend in the efficacy scores for the primary management method used against PnPs where stakeholders in urban environments thought the methods were more effective than for stakeholders working in mostly or completely rural environments. However, stakeholders with a national perspective (where urban/rural labelling of their working remit did not apply) were even more sceptical of the primary management methods' efficacy. We suggest that this may be because their perceptions of what is expected, or possible, varies. In urban areas, interventions may be more noticeable and stakeholders in urban areas may be more likely to detect new pests and diseases (Branco et al. 2019). Furthermore, fragmented private ownership of trees and greater oversight by plant health authorities in urban areas could lead to faster intervention and lower costs of interventions incurred by individuals. This is consistent with Branco et al. (2019) who found that eradication in open-field environments is more difficult to achieve than in urban areas and highlights that PnP management in rural areas, which is a target for improvement. It is also consistent with (Paap et al. 2017) who found that urban trees, for example, in botanical gardens and arboreta, may be useful for detecting PnPs in the initial stages of establishment, where early detection in urban environments offers the only realistic prospect of eradication.

Stakeholders reported a range of suggestions for tools to be developed and made available for use in the field for detection and management of PnPs in the future. This shows that, for developers of new tools, there is much to learn from working closely with stakeholders to provide information for their designs and that there is a widely held desire to update the forest health management practices that stakeholders currently rely on.

There is substantial scope to improve usability, visibility and uptake of forest health citizen science, smartphone applications, social media and public information posters. The results indicate that conventional methods of sharing information, i.e. discussion amongst peers and networks, publications, posters, pictures, websites and correspondence, are the preferred means for identifying PnPs currently, which is consistent with previous reports (White et al. 2018). Good examples of peer-to-peer communication exist in Europe, such as UK networks of stakeholders that have been provided with an arena for social learning about tree health (O'Brien et al. 2021). Posters and social media were used successfully to raise public awareness for the eradication of longhorn beetles *Anoplophora chinensis* and *A. glabripennis* in the Lombardy Region of Italy after first detection in 2010 (Ciampitti and Cavagna 2014; Marchioro and Faccoli 2021). Initiatives to educate children about forest health demonstrate the different levels to which effective forest health communications can be pitched. The 'Izzy the Inspector' character and cartoon in the UK (APHA 2020) which is available in two European languages and school citizen-science projects, such as 'backyard beetles' in Italy (Colombari et al. 2022) are diverse examples of ways to engage children. Their efficacy hinges on creating memorable learning experiences to convey understanding of the threats to forest health from PnPs.

Recruiting citizen scientists to monitor tree health (Slawson and Moffat 2020) helps raise the profile of forest health and fulfils stakeholders' wishes to stop delegating surveillance to professionals. Forest health data provided by citizens can be used by local authorities and scientific community, but requires significant effort for moderation and verification (Baker et al. 2021; Balázs et al. 2021). If reports can be incorporated into the Global Biodiversity Information Facility (GBIF 2022) or similar repository, rather than local databases, data sharing is more accessible (Saarenmaa 2005).

Suggested improvements to smartphone applications include featuring spread models, keys for identifying PnPs, illustrations of PnP symptoms, comparative images of easily-confused symptoms and phytosanitary guidance and pest profile information that is available already online (EFSA 2020, IEFC 2022). Ideally, stakeholders could use a single app that is applicable to all of Europe. Europe-wide tools currently under development, such as silvalert.net, could be developed to meet these expectations (Orazio 2019).

The development of molecular tools that are more accessible for civil society may help better PnP identification and we suggest that the lower level of tool use by 'Civil Society' compared to 'Forest Authorities' is partly due to having less access to methods, such as molecular diagnostics, that are traditionally delivered by professionals. The capability for citizens to engage in molecular methods is being demonstrated in the case of fungal biodiversity recording in the UK and USA, by using PCR 'bento boxes', where amateur mycologists barcode specimens and contribute to publications. Their data provide policy-makers with evidence to grant sites with protected status and, thus, preserve their biodiversity, in addition to increasing the output that could be achieved by the professional sector alone (Douglas 2020; Bierend 2021). The bottleneck to productivity caused by the limited availability and high costs of laboratory testing for samples from damaged trees could be alleviated by engaging groups usually excluded from molecular technology, be they surveyors, forest managers, traders or supportive citizens.

Our stakeholder group called for updating forestry practices to better protect forest health. They suggested improving forest biodiversity and planting species mixtures as a means to improve forest resilience in relation to PnPs, an approach which is supported by recent research findings (Randall and Smith (2019) and Jactel et al. (2017)). Local provenance planting stock was favoured as a way to reduce the risks of international trade; however, business problems (e.g. variability in commercial demand) associated with local tree nurseries (Alonso Chavez et al. 2019) and a lack of scientific literature on the topic of local sourcing prevents this from being viable at present.

Stakeholders also saw the value in biosecurity practices (preventing PnP spread by controlling movement of plants and practising hygiene and quarantine). They specified a need for better-developed on-site biosecurity procedures and to expand the labour force of inspectors and administrators who could ensure tree health is checked before, during and after trade, including at final planting and into maturity. However, given that less than half of the study's respondents were using biosecurity tools, it follows that finding ways to overcome the barriers to uptake, such as inconsistency between countries and lack of evidence for the efficacy of practices (Eschen et al. (2015) is worth investigating in future studies (Marzano et al. 2018, 2021). Important concepts around complex epidemiological and political issues were raised during to the COVID-19 pandemic and widespread discussion of key messages from public information campaigns has helped raise the profile and explain the principles of plant health (MacLeod and Spence 2020). This parallel with COVID-19 may continue to serve as a useful framework to influence biosecurity uptake. Poignant concepts that apply in each scenario include that the need for biosecurity extends beyond borders, that risk management is key to mitigating harm (MacLeod and Lloyd 2020) and that biosecurity is a shared responsibility for all (White et al. 2019; Nahrung et al. 2023).

Conclusions

Our results showed that stakeholder knowledge of a few key PnP is good, but the broad diversity of threats may be too large to expect stakeholders to be able to be aware of them all (only 6% of the EPPO list came up in free text). This could be solved by a better Europe-wide communication strategy with alerts and the ability to see which PnPs are causing problems in neighbouring countries. This means that international cooperation is necessary and desired, inside and outside of the EU, highlighting valuing the importance of EPPO and EFSA. There is still much to be done to reach safe standards for trading and biosecurity practices and improving localised nursery stock production is essential to lower the demand on high-risk trade practices.

Stakeholders are using and developing multiple tools and methods for PnP identification and management and show desire for access to new tools to help with PnP early detection and rapid response, as well as improved data sharing across Europe. Engaging new audiences across both urban and rural environments and equipping more people to monitor and detect PnPs can help increase surveillance levels and promote better forest health.

Acknowledgements

This manuscript is a result of collaborative work by the HOMED (HOlistic Management of Emerging forest pests and Diseases) Project which received funding from the European Union's Horizon 2020 Research and Innovation Programme under grant agreement No 7771271.

The authors wish to thank the stakeholders who responded to the surveys, those who distributed the surveys to stakeholders working with trees and all respondents and colleagues who provided their comments and suggestions. We are particularly grateful to the translators who made the survey accessible in nine languages and for the input of our colleagues in HOMED for helping share the survey with their networks. In particular, we thank Manuela Branco, Armand Clopeau, Fryni Drizou, Miloň Dvořák, André Garcia, Luisa Ghelardini, Susana Rocha, Anna Sapundzhieva, Lukas Seehausen, Pavel Stoev and Sarah Yoga.

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Supplementary material 1

HOMED stakeholder survey

Authors: Samantha Green, Katharina Dehnen-Schmutz, Jassy Drakulic, René Eschen, Christophe Orazio, Jacob C. Douma, Karl Lundén, Fernanda Colombari, Hervé Jactel

Data type: PDF file

Link: https://doi.org/10.3897/neobiota.84.95761.suppl1

Supplementary material 2

Supplementary tables and figures

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Data type: tables and figures

Explanation note: fig. S1: Role types and group sizes represented within each of the six stakeholders' groups produced by cluster analysis; fig. S2: Respondent profiles regarding country, sector, focus and geographic range of work relating to PnPs; fig. S3: Tools and methods used by the 6 stakeholder groups for (A) detection and identification of PnPs and (B) for management of PnPs; fig. S4: Efficacy rating for methods to manage three PnP case studies: ALB = Asian longhorn beetle (*Anoplophora glabripennis*), OPM = Oak processionary moth (*Thaumetopoea processionea*), PRA = *Phytophthora ramorum*. table S1: Confusion matrix; table S2: All GLMM variables; table S3: Model including urban variable in first model shown in table S4; table S4: Factors explaining stakeholders' awareness of PNPs (model 1 a, b), i.e. if they scored a species or not and how correct (Knowledge) their scores were with regard to presence or absence of PNPs in their country (model 2a, b). Anova results of GLMMs. Both models are presented in two versions, with the second version replacing the PNP variable with the binary variables "insect" and "EU-priority" pest; table S5: Factors explaining individual respondents' proportion of PnPs scored correctly as present or absent in their country (model 3). Anova results of the GLMM model; table S6: Efficacy of three PnP methods (no urban/rural grouping); table S7: Linear model for efficacy rating of primary management methods used against 3 PnP case studies.

Link: https://doi.org/10.3897/neobiota.84.95761.suppl2

NeoBiota 84: 41–46 (2023)
doi: 10.3897/neobiota.84.95823
https://neobiota.pensoft.net

SHORT COMMUNICATION

A peer-reviewed open-access journal
NeoBiota
Advancing research on alien species and biological invasions

Myrtus communis in Europe threatened by the pandemic and South African strains of the myrtle rust pathogen *Austropuccinia psidii* (Sphaerophragmiaceae, Pucciniales)

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Academic editor: Andrea Battisti | Received 30 September 2022 | Accepted 15 November 2022 | Published 18 May 2023

Citation: Paap T, Santini A, Rodas CA, Granados GM, Pecori F, Wingfield MJ (2023) *Myrtus communis* in Europe threatened by the pandemic and South African strains of the myrtle rust pathogen *Austropuccinia psidii* (Sphaerophragmiaceae, Pucciniales). In: Jactel H, Orazio C, Robinet C, Douma JC, Santini A, Battisti A, Branco M, Seehausen L, Kenis M (Eds) Conceptual and technical innovations to better manage invasions of alien pests and pathogens in forests. NeoBiota 84: 41–46. https://doi.org/10.3897/neobiota.84.95823

Abstract

Austropuccinia psidii, the causal agent of myrtle rust, has emerged as a significant threat to Myrtaceae in planted and natural woody ecosystems. The first detection of *A. puccinia* in South Africa was from severely infected ornamental *Myrtus communis.* This raised concern that *M. communis*, the sole Myrtaceae species native to Europe and an important component of vegetation in Mediterranean regions, could be threatened by the rust. In light of the potential threat to this unique species, seed was collected from 12 Italian provenances of *M. communis*, including mainland and island (Sardinia and Sicily) populations. We assessed the susceptibility of these provenances to both the pandemic and South African strains of *A. psidii*. In Colombia, where the pandemic strain of *A. psidii* is native, seedlings rapidly became infected by natural inoculum. In South Africa, a preliminary screening of seedlings by artificial inoculation with a single-uredinium isolate produced high levels of disease. Finally, plants of each of the 12 provenances were planted and monitored in Florence, Italy. To date, these showed no signs of disease, but will continue to be monitored. This study highlights the significant threat that both the pandemic and South African strains of *A. puccinia* pose to *M. communis* in Europe.

Keywords
biosecurity, emerging diseases, invasive forest pathogens, microbial invasions, sentinel plants

Austropuccinia psidii (G. Winter) Beenken (basionym *Puccinia psidii*, Sphaerophragmiaceae, Pucciniales) has emerged as an important invasive plant pathogen, threatening Myrtaceae in planted and natural woody ecosystems globally (Coutinho et al. 1998; Carnegie et al. 2016; Roux et al. 2016; Beenken 2017). Originating in South America (Coutinho et al. 1998; Glen et al. 2007), the pathogen is the causal agent of a rust disease with common names including guava rust, eucalyptus rust, ʻōhiʼa rust and myrtle rust. *Austropuccinia psidii* has a wide host range of over 480 species of Myrtaceae (Soewarto et al. 2019) and currently represents the major biosecurity threat for the Myrtaceae family.

In 1998, *A. psidii* was added to the EPPO Alert List, based on the threat the pathogen posed to *Eucalyptus*, which are considered important trees in Mediterranean countries. In 2003, however, the listing was deleted, following the conclusion from PRA (02-9886 & 9891) that the risk was low due to the climatic requirements of the rust (EPPO 2003). In the two decades that have passed since this decision, *A. psidii* has invaded diverse geographic regions including Africa, Asia-Pacific and Oceania (Carnegie et al. 2010; Giblin 2013; Roux et al. 2013, McTaggart et al. 2016; du Plessis et al. 2019), with evidence that the pathogen is able to spread rapidly and results in devastating impacts (Carnegie and Pegg 2018).

A globally-important new record of *A. puccinia* was when it was first detected in South Africa on ornamental *Myrtus communis* Linn. (Roux et al. 2013). *Myrtus communis* is native to southern Europe, North Africa and West Asia. It is the sole Myrtaceae species native to Europe and an important component of vegetation in Mediterranean regions. In addition to its occurrence in natural ecosystems, it is also widely cultivated for its ornamental value and aromatic properties. In particular, its berries, leaves, seeds and essential oils are natural sources of several nutrients and bioactive compounds and, in the Italian region of Sardinia, berries are used in the production of an important local liqueur, ʻ*Mirto di Sardegna*ʼ (Sumbul et al. 2011; Giampieri et al. 2020). Observations from South Africa (Roux et al. 2013, 2016) indicated that *A. psidii* infection resulted in severe disease development, highlighting the threat posed to this unique plant. This prompted the question as to the relative threat of *A. psidii* to *M. communis* in Europe.

Seeds were collected from 12 Italian provenances of *M. communis*, including mainland and island (Sardinia and Sicily) populations (Table 1). Seeds were cleaned and prepared at the Institute for Sustainable Plant Protection - National Research Council (IPSP-CNR) facilities in Florence, Italy, before being sent to Colombia and South Africa, to be screened for susceptibility to the pandemic and South African strains of *A. psidii*, respectively. In Colombia, seeds were sown in June 2019 at the phytopathology laboratory of Smurfit Kappa (Restrepo, Valle del Cauca: 3.855278, -76.5075). Germination rates varied from 23–100%, with the number of seedlings per provenance varying from 170–920. Plants were maintained outside; however, these rapidly became infected by natural inoculum present on planted *Syzygium jambos* (L.)

Alston. A known susceptible host of *A. psidii*, the identification of the pandemic strain of *A. psidii* was previously confirmed on this stand of *S. jambos* by Granados et al. (2017). Yellow masses of urediniospores were frequently observed on shoots and young leaves and stems of the *M. communis* plants, with telia also occasionally developing. By March 2022 (33 months after germination), extensive mortality had occurred, due to repeated natural infection (Fig. 1). Mortality varied by provenance, ranging from 85.3–100% mortality (mean = 95.4%).

In South Africa, seeds were sown in February 2021 in a phytotron maintained under controlled conditions (ca. 25 °C, 75%–85% relative humidity, 12 hr natural daylight/12 hr night) at the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria (Pretoria, Gauteng: -25.755537, 28.235440). Artificial inoculation of a selection of seedlings (seven replicate plants from nine provenances) was conducted in September 2021 (7 months after germination). The inoculation was carried out using a single pustule isolate of the South African strain, originally collected from *Eugenia natalitia* Sond. (syn. *Eugenia capensis* subsp. *natalitia*) and mass produced on seedlings of *S. jambos*, as described by Roux et al. (2016). The plants were assessed for disease symptoms after 14 days. Symptoms started to develop within a few days following inoculation. After 14 days, all individuals (n = 63) showed typical symptoms of infection by *A. psidii* (Fig. 1). Pustules with uredinia developed on young leaves and stems of nearly all plants, with telia also developing on many individuals.

Plants of each of the 12 provenances were planted at the experimental facility of the IPSP-CNR in Florence, Italy (43.771944, 11.177500). The planting was established in line with the "Sentinel Planting" approach, to optimise early detection of alien pests and pathogens (for detail, see Migliorini et al. 2022). Monitoring of these plants is ongoing; to date they remain disease-free.

The results arising from the natural infection in Colombia and a preliminary screening using artificial inoculation in South Africa, indicate that a wide range of provenances of *M. communis* are highly susceptible to both the pandemic and South African strains of *A. psidii*. The 2003 decision by EPPO to delist this pathogen was based on the finding that the risk of pathogen establishment in Europe was low due to

Table 1. Italian provenances of *Myrtus communis* screened for susceptibility to *Austropuccinia psidii*.

Region	Municipality and Province	Location
Sardinia	Sinnai (Cagliari)	Pineta di Sinnai
Sardinia	Villa Cidro (Mediocampidano)	Cannamenda
Sicily	Buccheri (Siracusa)	CDA: Santa Maria
Sicily	Militello Rosmarino (Messina)	Luco
Sicily	Eraclea Minoa (Agrigento)	CDA: Borgo Bonsignore
Sicily	Caronia (Messina)	CDA: Pagliarotta
Sicily	Noto (Siracusa)	RNO: Vendicari
Sicily	Ragusa	CDA: Cava Dei Modicani
Sicily	Randazzo (Catania)	CDA: La Guardia
Sicily	Buseto Palizzolo (Trapani)	CDA: Scorace
Tuscany	Pisa	San Rossore
Tuscany	Cecina (Livorno)	Cecina

climatic requirements. However, in the two decades that have followed, *A. psidii* has invaded many new regions. The spread of the pathogen into warm to cool temperate climates in Australia and New Zealand has provided opportunities to study the biology and epidemiology of *A. psidii* under conditions different to those found in the tropical and subtropical areas from which the pathogen originates. For example, Beresford et al. (2020) demonstrated that the uredinial stage of *A. psidii* is capable of overwintering (in a latent phase) in cool temperate climates. A CLIMEX model developed by Kriticos et al. (2013) showed *A. psidii's* preference for moist climates with moderate temperatures throughout the wet tropics and sub-tropics; however, the model also identified some cool regions with a mild Mediterranean climate as climatically suitable areas. It is likely that revising the model, including distribution data from more recently invaded areas, will demonstrate an increase in the range of areas suitable for the pathogen to establish.

The original EPPO listing was based on the threat *A. psidii* posed to *Eucalyptus* in Mediterranean areas of Europe. While the pathogen continues to threaten this economically important species (Hakamada et al. 2022), there are growing concerns regarding the threat that *A. psidii* poses to native ecosystems, particularly in regions with high levels of Myrtaceae diversity and endemism. A number of Australian species are now threatened with biological extinction, as a direct result of continued infection

Figure 1. top – natural infection of ornamental *Myrtus communis* in Cape Town (Western Cape) (*Austropuccinia psidii* South African strain) (left); natural infection of Italian provenances of *M. communis* in Colombia (*A. psidii* pandemic strain) (centre and right); bottom – disease development and prolific production of urediniospores14 days after artificial inoculation (*A. psidii* South African strain).

(Pegg et al. 2017; Makinson et al. 2020). We believe the findings communicated here should prompt a re-evaluation of the risk *A. psidii* presents to Europe, taking into consideration the demonstrated threat that both the pandemic and South African strains of *A. puccinia* pose to *M. communis*.

Acknowledgements

This study was funded by the European Commission Horizon 2020 Research and Innovation Programme 'HOlistic Management of Emerging Forest Pests and Diseases' (HOMED) (grant No 771271). The authors would like to acknowledge Dr. Giancarlo Perrotta, Regione Siciliana, Dipartimento Regionale Sviluppo Rurale e Territoriale - Ufficio provinciale Azienda foreste demaniali di Siracusa and Dr. Antonio Vinci, Forestas, Agenzia forestale regionale per lo sviluppo del territorio e dell'ambiente della Sardegna, for providing the *Myrtus communis* seeds from Sicily and Sardinia, respectively.

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NeoBiota 84: 47–80 (2023)
doi: 10.3897/neobiota.84.90024
https://neobiota.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
NeoBiota
Advancing research on alien species and biological invasions

Measuring the threat from a distance: insight into the complexity and perspectives for implementing sentinel plantation to test the host range of *Xylella fastidiosa*

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Academic editor: Alberto Santini | Received 6 July 2022 | Accepted 8 November 2022 | Published 18 May 2023

Citation: Casarin N, Hasbroucq S, López-Mercadal J, Miranda MÁ, Bragard C, Grégoire J-C (2023) Measuring the threat from a distance: insight into the complexity and perspectives for implementing sentinel plantation to test the host range of *Xylella fastidiosa*. In: Jactel H, Orazio C, Robinet C, Douma JC, Santini A, Battisti A, Branco M, Seehausen L, Kenis M (Eds) Conceptual and technical innovations to better manage invasions of alien pests and pathogens in forests. NeoBiota 84: 47–80. https://doi.org/10.3897/neobiota.84.90024

Abstract

The sentinel plantation concept consists of assessing the impact of exotic factors, such as pests and pathogens, on plants of interest by planting them out of their native range. This tool is a way to enhance knowledge for pest risk analysis (PRA) by guiding decisions on how quarantine organisms should be regulated and where to focus prevention and surveillance efforts for an early detection. In this study, the sentinel method was used in the case of research on *Xylella fastidiosa*, a plant pathogenic bacterium that has recently been found established in southern Europe, but whose potential impact and possible host range are still poorly documented in northern areas where the bacterium is not known to occur. To improve knowledge on the susceptibility of potential hosts of *X. fastidiosa* in northern Europe, a sentinel plantation of *Prunus domestica* cv. Opal, *Quercus petraea* and *Salix alba* was established in the *X. fastidiosa*-infected area of Majorca. In order to assess the circulation of the bacterium in the sentinel plot and around it, surveys of the local flora and insect vectors were carried out, as well as the planting of a network of rosemary "spy plants". Symptomatic monitoring and molecular analyses were performed on the sentinel plants for four years. During these years, *X. fastidiosa* was never detected in our sentinel plants most likely because of the low infectivity pressure recorded in the surroundings. This study underlines the complexity of conducting sentinel plantation assays combined with *X. fastidiosa* research, highlighting the need for long-term investigation and questioning the efficiency of the sentinel tool. However, this study is placed in perspective with other valuable sentinel plantations. It also highlights the complementarity of the tool and proposes elements to improve or re-orientate the implementation of future sentinel projects.

Keywords
biological invasions, ex-patria planting, Majorca, northern Europe, pest risk analysis, *Prunus domestica*, *Quercus petraea*, *Salix alba*

Introduction

The world sustainability is threatened by outbreaks of invasive pests and pathogens increasingly spreading around the globe (Simberloff et al. 2013; Diagne et al. 2021). These organisms largely travel to new areas through global trade, with living plants or with wood packaging material, which are considered as the main pathways of plant-related organism introductions (Kenis et al. 2007; Liebhold et al. 2012; Santini et al. 2013; Meurisse et al. 2019). These agents often expand by outcompeting native species because they are transported far from their natural enemies ("enemy release hypothesis"; Keane and Crawley 2002; Colautti et al. 2004), allowing them to allocate resources to growth and fecundity instead of defence, enhancing their fitness ("evolution of increased competitive ability" hypothesis; Blossey and Notzold 1995; Manfredini et al. 2013). They may trigger epidemics, sometimes on new hosts whilst they were less harmful to their native hosts, as they have not co-evolved with the new local plants that lack of specific defence mechanisms (Pimentel et al. 2001; Aukema et al. 2011). Apart from trade and globalisation, climate change and intensive land-use are also factors enhancing outbreaks by decreasing the resilience of the agricultural production systems and of forests (Walther et al. 2009; Bosso et al. 2016).

Preventing the introduction and the establishment of pests and pathogens in new areas is the most efficient tool for mitigating the consequences of a disease in terms of cost, biodiversity conservation and human impact (Barham et al. 2016). This includes the implementation of a pest risk analysis (PRA), which is an assessment giving biological, scientific and economic information on a particular organism (Aukema et al. 2011; Tomoshevich et al. 2013; EFSA PLH Panel 2018) to understand its potential impact and how it should be regulated (Parker et al. 1999; EC 2000; Liebhold et al. 2012). If considered harmful, the first measure taken to avoid its introduction might be its inclusion in a quarantine list implying either thorough inspections of imported plants before or after the importation, plant production in pest-free areas or sites of production or complete prohibition of trade or production of its native host plants (EC 2000).

However, these measures are not fully effective by themselves. Inspections can fail to intercept all the potential pests and pathogens travelling through plant trade (Kenis et al. 2007; Eschen et al. 2015, 2017, 2019). First, these agents can be invisible to the naked eye because of their intrinsic nature or because they are in a latent form or in an endophytic stage on their traded hosts, leading to asymptomatic infections (Stergiopoulos and Gordon 2014; Migliorini et al. 2015). Secondly, despite the prioritisation of inspected organisms through PRA, the massive volume of traded materials makes the systematic control of each plant inoperable, with only batches being thoroughly examined (Britton et al. 2010; Eschen et al. 2015). Finally, PRA relies on prior awareness and knowledge of a pest and this knowledge is not always available; several agents, including non-catalogued taxa, harmless in their native region, are unknown to be invasive and pathogenic prior their introduction in a new land and escape controls (Brasier 2008; Britton et al. 2010; Tomoshevich et al. 2013; Vettraino et al. 2018). The few of them that manage to establish and cause significant damage are then often discovered too late to avoid outbreaks. Such is the case for some of the most damaging organisms of temperate forests that have occurred in recent years, which were unknown as pests prior to their introduction in a new area (Britton et al. 2010). Examples are the epidemics of Dutch elm disease caused by *Ophiostoma ulmi* Buisman and *O. novo-ulmi* Brasier that decimated billions of elm trees in Europe and America in the 20th century (Brasier and Buck 2001) or the massive damage to pines in Asia (Zhao et al. 2008) and Europe (Soliman et al. 2012) caused by the pine wood nematode, *Bursaphelenchus xylophilus* (Steiner & Bührer) Nickle, an organism well tolerated by its native pine hosts in North America (Akbulut and Stamps 2012).

A way to enhance knowledge about potentially damaging organisms to improve biosecurity systems would be to expose plants of interest out of their native range to study their susceptibility to local organisms in specific relevant locations, for example, a frequent plant exporting country (Roques et al. 2015; Barham et al. 2016). These plants would represent sentinels for their species in the foreign land. They provide an early warning for potential threats and additional information for PRA to set prevention measures and to know where the efforts for plant protection should be focused (Barham et al. 2016; Mansfield et al. 2019). An EPPO standard document was published in 2020, “PM 3/91 Standard on Sentinel woody plants” (EPPO 2020), to explain the approach and to provide guidance to carry out sentinel plant studies to identify new pest risks.

Sentinel plant research can be carried out by different ways (Britton et al. 2010). A first way is through botanical gardens and arboreta gathering a collection of specimens from all over the world, which are generally out of their area of origin and exposed to local agents. For such studies, the International Plant Sentinel Network (IPSN), working closely with National Plant Protection Organisations (NPPOs), was created. It connects the botanical gardens and arboreta staff around the world and gives them tools and expertise to monitor and to identify new pests and pathogens (Barham et al. 2016). Tomoshevich et al. (2013), for example, discovered 29 new pest-host associations (of which 18 were noticeably damaging for European trees) by studying European and Eurasian trees in Siberian gardens in Russia. However, in botanical gardens and arboreta, the number of representatives of each plant species is generally limited

(Roques et al. 2015), the trees are often large and hence difficult to examine in detail and they are usually subject to pesticide treatments or other management practices, which ensure plant health in the gardens (Eschen et al. 2019). Furthermore, gardens are often located in urban areas distant from the habitats of potential pests. All these reasons reduce the likelihood for an organism to reach and infect a specific plant species in an arboretum (Britton et al. 2010). A second way to conduct sentinel plant research is directly establishing actual plantations of exotic plants of interest in an environment where we want to study the impact of local pests and pathogens, the so-called "sentinel plantations" (Roques et al. 2015) or "ex-patria plantings" (Eschen et al. 2019). For example, Roques et al. (2015) and Vettraino et al. (2015) established two sentinel plantations of European tree species in China to investigate new pest-host associations potentially threatening to Europe that may emerge as a result of trade.

On the other hand, some well-known pathogens are still restricted to one part of the world and their potential host range in non-infected areas is uncertain and must be investigated. Such is the case of the phytopathogenic bacterium *Xylella fastidiosa* Wells et al., with more than 650 reported host plant species and for which the host range continues to extend as the bacterium enters new areas (EFSA 2022). While the threat of *X. fastidiosa* is definite for the European Mediterranean flora, the potential impact for northern areas is uncertain as most of the flora in these regions has never been exposed to the bacterium and probably contains many unreported hosts. The objective of our study was, therefore, to establish a sentinel plantation with European northern trees in a *X. fastidiosa* infected area in order to study the potential host range for these still-uninfected regions.

The gammaproteobacterium *X. fastidiosa* (Xanthomonadaceae) is strictly limited to the foregut of xylem sap-feeding insect vectors, mainly leafhoppers and spittlebugs (Hemiptera, Cicadomorpha) (Redak et al. 2004; Almeida et al. 2005; Chatterjee et al. 2008) and to the xylem vessels of its host plants. While many listed hosts are asymptomatic, the bacterium causes severe outbreaks on several crops, ornamental plants and shade trees generally provoking leaf-scorching that could lead to plant death (EFSA PLH Panel 2015). First limited to the Americas, the bacterium is currently regulated in Europe as a quarantine organism under the Council Directive 2000/29/EC (EC 2000). Between 2014 and today, the Europhyt database recorded 51 interceptions of *X. fastidiosa* in plants for planting and four interceptions of leafhoppers (EUROPHYT Online database 2022). Despite the border controls and EU prevention measures, a first focus of *X. fastidiosa* in Europe was discovered in 2013 in Apulian olive groves (Italy), for which more than 21 million olive trees were estimated to be affected in 2018 (Saponari et al. 2019b). The bacterium was then identified in mainland France, in Corsica (Denancé et al. 2017), in mainland Spain, in the Balearic Islands (Olmo et al. 2017), in another region of Italy (Tuscany) (Saponari et al. 2019a) and in Portugal (EUROPHYT Online database 2022). Divided into several subspecies (mainly subsp. *fastidiosa*, subsp. *multiplex* and subsp. *pauca*: Schaad et al. 2004) and more finely according to its sequence type (ST) (Scally et al. 2005; Yuan et al. 2010), 11 different STs were identified throughout Europe revealing multiple independent *X. fastidiosa* introduction events (Cunty et al. 2022). Phylogeny studies allowed to date back the different entries of *X. fastidiosa* in the specific European regions, indicating entrance in the 1980s, 1990s and 2000s according to the area, i.e. well before the official identification of the pathogen's establishment on the continent. *Xylella fastidiosa* is, therefore, a perfect example of an organism escaping control due to the complexity of detection given the asymptomatic pool of hosts, the potentially long latent period limiting visual inspection and the number of reported and supposed/unreported hosts, as well as the lack of specific surveillance programmes and the limited availability of specific diagnostic tools in the past. Its movement into Europe has been caused in part by the trade of asymptomatic coffee plants imported from Latin America (EFSA PLH Panel 2015; Denancé et al. 2017).

However, it has been shown that eradication of *X. fastidiosa* may be complex if not impractical once it is well established and has reached a large geographical extent (Strona et al. 2017; EFSA PLH Panel 2019). Therefore, while entries can hardly be prevented, early detection is of prime importance to limit damage of outbreaks. The probability of early detection would increase by improving knowledge on where the bacterium is most likely to establish in order to conduct effective surveillance. In fact, performing detection tests on every plant in random areas is neither efficient nor conceivable as it would exceed any diagnostic capability considering the wide range of potential host plants (EFSA PLH Panel 2022). Targeting the main host plants and establishing a prioritisation list is essential to know where to focus resources and monitoring efforts.

To enhance knowledge on the susceptibility of potential hosts of *X. fastidiosa* in northern Europe, a sentinel plantation of northern plant species *Prunus domestica* cv. *Opal*, *Quercus petraea* and *Salix alba* was established in the *X. fastidiosa*-infected area of Majorca (Balearic Islands, Spain). There, the bacterium is considered widespread and well established. Three different STs belonging to two subspecies (*X. fastidiosa* subsp. *fastidiosa* ST1 and *X. fastidiosa* subsp. *multiplex* ST81 and ST7) have been identified on several hosts including wild olives, cultivated olives, almonds, grapes and figs (Olmo et al. 2021). They are mainly transmitted by the *Philaenus spumarius* Linnaeus (Aphrophoridae) vector and, to a lesser extent, by *Neophilaenus campestris* Fallén (Aphrophoridae) (López-Mercadal et al. 2021). This study experiments with the sentinel plantation tool in the case of *X. fastidiosa* research. The outcome questions the efficiency of the method, at least in this particular case and highlights the complexity of its implementation. However, it provides a methodology and several perspectives for future sentinel projects.

Methods

Preliminary tests and plant movement

The establishment and the monitoring of the sentinel plantation was achieved with the collaboration of the Applied Zoology and Animal Conservation group of the University of the Balearic Islands (UIB). First, the agreement of the local government and the UIB authorities had to be obtained. Then, the plant material was bought at the Calle-Plant Nursery in Wetteren, Belgium. It consisted of dormant material: 30 *Salix alba* 0/1 80/120, 30 *Quercus petraea* 2/0 80/100 and 30 *Prunus domestica* cv. Opal 2 years grafted on Myrobolan or St Julien. Although all the plants were equipped with a phytosanitary certificate, *X. fastidiosa* specific detection tests were performed on several twigs of each plant to make sure the initial material was free of the bacterium. For this purpose, three branch parts of each plant were collected and bark peeled. They were chopped and their DNA was extracted according to the CTAB-based DNA extraction protocol specific for *X. fastidiosa* plant samples ("PM 7/24 (4) *Xylella fastidiosa*", EPPO 2019). The detection was then performed by PCR (Minsavage et al. 1994). After this double check, the ninety plants were wrapped in hessian bags filled with wood chips and were brought by truck from Belgium to the UIB campus in Palma (Majorca, Balearic Islands) in March 2018. The chips were humidified during the 2-day trip to avoid root dryness.

Location and establishment

The location of the plot was chosen with the UIB collaborators mainly based on the ease of connection to an irrigation system, as well as on the observation of *Philaenus spumarius* and *Neophilaenus campestris* nymphs on the ground vegetation and the presence of host plants, such as wild olive and almond trees. For the positioning of the plants in the plot, the JMP software was used to generate nine blocks, each one composed of three plants of each species randomly distributed (Fig. 1). The scheme was divided by blocks to take into account the potential gradients such as the slope, irrigation distribution or sunlight. The trees were planted directly into the ground to promote the growth of the root system and to enable them to survive throughout the season (Fig. 2). The soil was compact and rocky and was dug thanks to machines (Fig. 3). In every hole, about 20 litres of breeding soil were poured. The trees were separated from each other by 1.50 m and the whole plantation covered a total area of 144 m^2. The irrigation system was established in the second year of the plantation. It consisted in three closed loops of pipe with one dripper per plant, allowing a constant pressure in all pipes and the same amount of water per plant (Fig. 1). The climatic data were followed through the season thanks to an HOBO device placed in the middle of the plantation.

Exploring the surroundings

To monitor the circulation of the bacterium in the plot and around it, a 100-m demarcated area was organised around the plantation. In this area: i. a floristic inventory was carried out; ii. insect vectors were sampled; iii. a rosemary "spy plant" network was established (Fig. 4).

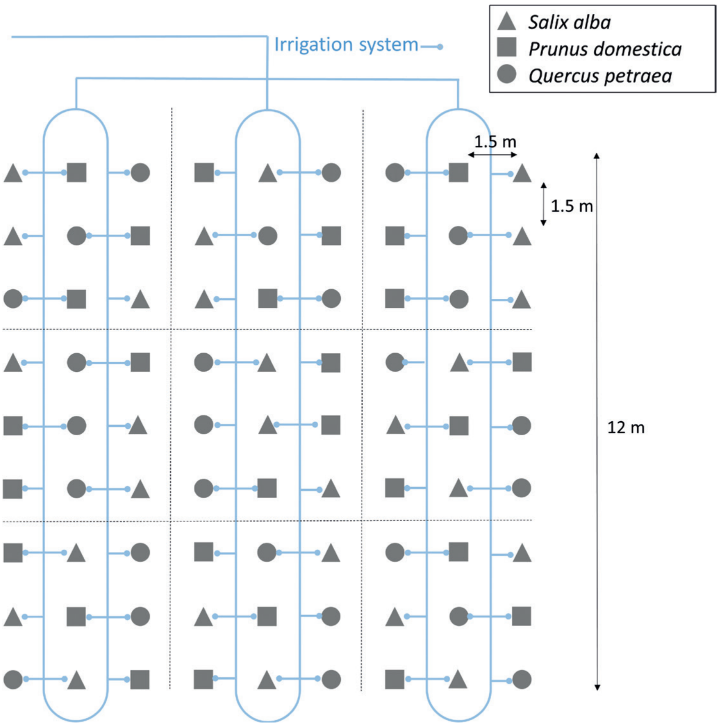

Figure 1. Scheme of the sentinel plantation of *Salix alba*, *Prunus domestica* cv. Opal and *Quercus petraea*. The dotted lines delimit nine blocks in which there are three plants of each species distributed randomly (JMP). The solid blue line is for the representation of the irrigation system consisting in three closed loops of pipe with one dripper per plant.

Figure 2. The sentinel plantation in Palma (Majorca, Balearic Islands) in May 2018.

Figure 3. Overview of the planting of the sentinel trees. Pictures highlighting the difficulty of establishing the sentinel plantation in the compact and rocky soil of the area.

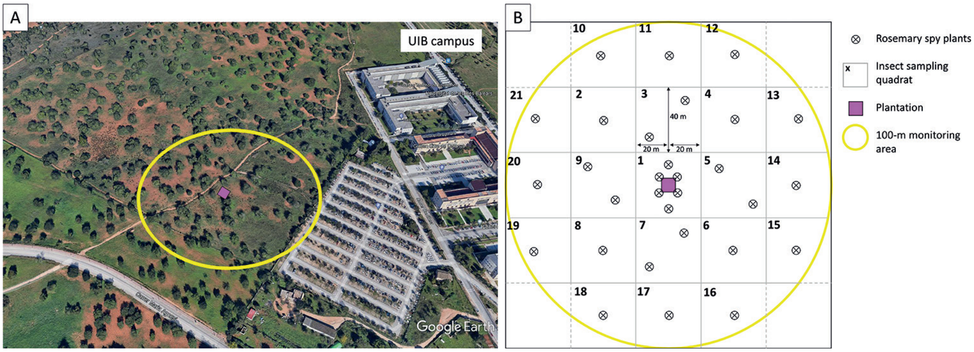

Figure 4. Surroundings of the sentinel plantation **A** Google Earth view (Google Earth Pro, satellite image of 6 May 2021) of the UIB campus with the location of the sentinel plantation (purple square) and the 100-m demarcated area around the plantation (yellow circle) **B** scheme of the plantation and the demarcated area. In the demarcated area, a floristic inventory was carried out, insect vectors were sampled in the determined quadrat and a rosemary "spy plant" network was established by planting evenly seedlings around the plantation.

Floristic inventory

To locate and assess the proportion of *X. fastidiosa* host plants in the area and to follow the eventual appearance of symptoms, a floristic inventory was carried out. It consisted in identifying and mapping the tree layer of the demarcated area. An identification of the main herbaceous species was also performed with the help of local collaborators and of two determination keys for the local flora (Gil and Llorens 1999; Herbari Virtual del Mediterrani Occidental 2018).

Rosemary network

A total of 44 *Rosmarinus officinalis* were planted around the campus: 32 plants evenly positioned in the demarcated area (Fig. 4B) and 12 plants in other places of the campus. The idea was to choose a robust plant adapted to local environmental conditions and which is quite susceptible to several subspecies of *X. fastidiosa*. Planting and regularly sampling these susceptible plants for bacterial detection provide a spy network allowing us to control the circulation of the bacteria in the vicinity of the plantation. The plants were bought in a local nursery in March 2018. They were first checked for *X. fastidiosa* presence with molecular tests before planting them, consisting of a CTAB-based DNA extraction followed by PCR of Minsavage et al. (1994). For sampling, about 15 leaves were collected on each plant, starting with symptomatic ones and were processed right away in the local laboratory. The mid-rib and the petiole were sectioned and the total DNA was extracted with the CTAB-based extraction procedure (EPPO 2019). The DNA samples were then sent to Belgium and were processed at UCLouvain by PCR of Minsavage et al. (1994) in the first three years and by real-time PCR of Harper et al. (2010) in the fourth year-final testing. In this final year, about five twigs per plant were collected as well and were processed in the same way.

Sowing ground vegetation

As the planting of the sentinel plants with machines had removed the herbaceous layer in the sentinel plantation, which could prevent insects from reaching the trees, it was decided to re-sow grass in February of the second year to reconstitute this layer. The seed consisted of a universal mix of Asteraceae, Fabaceae and Poaceae.

Insect sampling and testing

Insects were sampled with two objectives. On one hand, they were collected to be tested for *X. fastidiosa* presence by PCR (Minsavage et al. 1994) and quantitative PCR (Harper et al. 2010) to check for the circulation of the bacterium around the plantation. On the other hand, during the first year, the vector population density was assessed every month to determine the variability of the potential transmission during the season. For this study, the 100-m area around the plot was divided in 25 blocks (Fig. 4B). In each block, the same numbers of insect samples were taken. According to the development stage of the insect, the sampling method was adapted. For the nymphal stage, a 50 cm × 50 cm frame (0.25 m^2) was thrown randomly four times in each block. The nymphs present in the surface delimited by the frame were counted.

In total, 84 samples were undertaken throughout the demarcated area and the number of nymphs/m^2 could be estimated. Although *X. fastidiosa* is lost after every moult, the nymphs can also become infected with it (Purcell and Finlay 1979; Redak et al. 2004). Therefore, in addition to the density study, three nymphs of *P. spumarius* and three nymphs of *N. campestris* were collected in each block for bacterial detection to determine if they already have an indication of the circulation of the bacteria in the plot. This quantity was chosen in order not to affect the vector abundance around the plot for the rest of the season.

Regarding insects at the adult stage, the sampling was carried out with sweeping nets. Two samples per block were taken in the ground layer, one sample corresponding to ten sweepings. The sweepings were undertaken homogeneously in each block in order to cover all the area. In total, 42 samples were taken throughout the demarcated area and the number of adult/sweep was measured. Again, only three insects per species (*P. spumarius* and *N. campestris*) were collected per block. Due to the small number of insects found in summer, the tree layer was also sampled. All the wild olive, almond and carob trees in the demarcated area were hit fifteen times with sweeping nets, distributed evenly on the plant in order to cover its entire attainable foliage surface. The number of adults per tree could be assessed.

The insects collected were placed at -20 °C, then stored in ethanol 70% and were sent to Belgium where they were processed. The eyes were removed and the DNA of the head together with the mouthparts was extracted using the CTAB-based protocol (EPPO 2019). The extracted DNA was then processed by PCR of Minsavage et al. (1994), by nested PCR of Cruaud et al. (2018) or by quantitative PCR of Harper et al. (2010).

Sentinel plantation monitoring

Visual inspections were carried out for each sentinel tree. The appearance of *Xylella*-like symptoms was cautiously observed and wilting, shoot dieback, desiccation, defoliation or any change in leave colour were reported. The evolution of the size of the different plants was also monitored, as well as the presence of *Xylella*-vectors or of other pests or organisms. In parallel, molecular analyses were performed on each plant. One sample per plant was collected, consisting of ten leaves per plant and 4–5 small twigs collected from all sides of the plant, but prioritising symptomatic areas if there were any. DNA extractions were carried out with the CTAB-based protocol (EPPO 2019) on leaf mid-ribs, on petioles and on the twigs after bark peeling and cutting them into small pieces. The DNA samples were then sent to Belgium where they were processed by PCR of Minsavage et al. (1994) in the first three years. In the final testing of the fourth year, two samples per plant were collected, one sample consisting of 10 different twigs distributed throughout the plant together with 10 to 20 leaves, always prioritising symptomatic parts. After extraction, they were processed by PCR of Minsavage et al. (1994), as well as by real-time PCR of Harper et al. (2010). No fertiliser was applied and no pruning was carried out in the winter, to allow the plants to develop naturally and not to cut potentially infected sections.

Calendar of the establishment and monitoring of the plantation and demarcated area

The planning of the plantation monitoring during the four years is available in Table 1. The first year, it was decided to monitor the plantation and the demarcated area almost every month of the vector-season to assess the vector density fluctuation and to measure the rate of infection, if any, of the different plant species. In March, nymphs were sampled while, from May to October, insect adults were monitored. Several rosemary plants had desiccated already in May of the first year. Therefore, the dead ones were replaced in May and also in February of the following year. From the second year onwards, the sampling periods were chosen to correspond more or less to the beginning and the end of the highly infectious period of *X. fastidiosa* carried by the insect vectors, respectively, June and October, avoiding the aestivation periods of insects. The third year was impacted by the Covid-19 crisis and only one sampling campaign could be carried out in October 2020.

Results

Insight into surrounding plants

About 170 trees were inventoried: 134 carob trees, 31 wild olive trees, five almond trees and two pine trees. Their distribution can be observed in Fig. 5. The wild olive trees and the almond trees are both host plants of *X. fastidiosa*. Therefore, 36 host plants of the bacterium were identified in the 100 m around the plot (Fig. 5B). Amongst these host plants, 64% showed leaf scorching symptoms similar to those caused by *X. fastidiosa* (Fig. 5C). Concerning the ground vegetation, the identified plants were mainly: *Conium maculatum* (Apiaceae), *Foeniculum vulgare* (Apiaceae), *Cichorium intybus* (Asteraceae), *Dittrichia viscosa* (Asteraceae), *Galactites tomentosa* (Asteraceae), *Euphorbia medicaginea* (Euphorbiaceae) and many Poaceae (*Oryzopsis* sp. and others).

Table 1. Four-year schedule of the establishment and monitoring of the plantation and of the demarcated area.

Task	2018						2019			2020		2021	
	March	May	Jun	Jul	Sept	Oct	Feb	Jun	Oct		Oct	Jun	Oct
Sentinel establishment	▨												
Rosemary network establishment	▨	▨					▨						
Floristic inventory	▨									C O V I D			
Vector density	▨	▨	▨	▨	▨	▨							
Vector sampling	▨	▨	▨	▨	▨	▨		▨	▨		▨	▨	▨
Sentinel plants monitoring and testing	▨	▨	▨	▨	▨	▨		▨	▨		▨	▨	▨
Rosemary monitoring and testing	▨	▨	▨	▨	▨	▨		▨	▨		▨	▨	▨
Sowing herbaceous vegetation							▨						

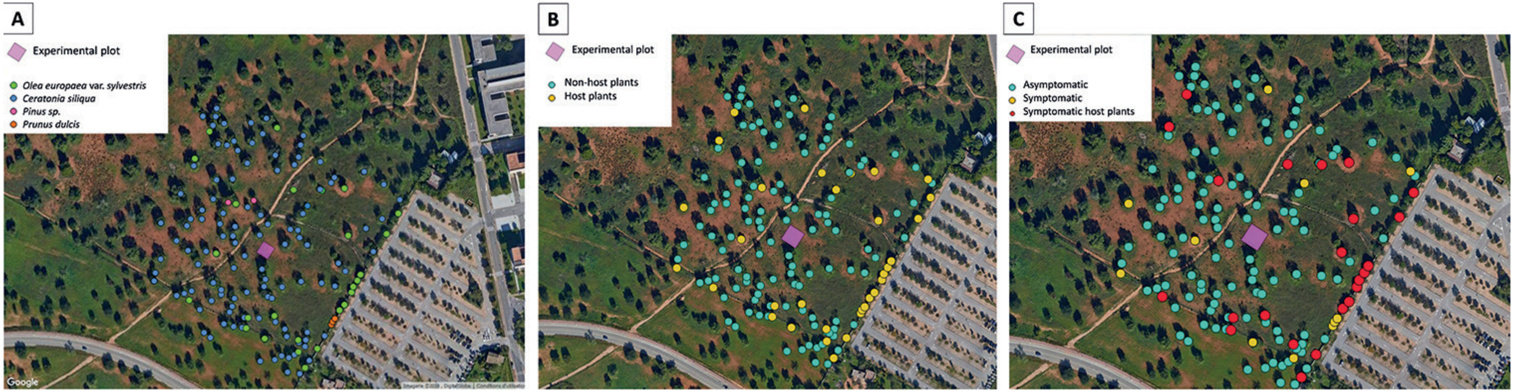

Figure 5. Tree species inventory, host and health status around the sentinel plantation **A** map of the different tree species in the area of 100 m around the plantation. The pink square is the experimental plot (the sentinel plantation). The green dots are for *Olea europaea* var. *sylvestris* (wild olive tree), the blue dots for *Ceratonia siliqua* (carob tree), the pink dots for the *Pinus* sp. (pine tree) and the orange dots for the *Prunus dulcis* (almond tree) **B** map of the host status of the trees located in the area of 100 m around the plantation. The green dots are the non-host plants of *X. fastidiosa* and the yellow dots are the host plants of the bacterium **C** map of the symptomatic trees located in the area of 100 m around the plantation, presenting typical *X. fastidiosa* leaf scorches. The green dots are the asymptomatic plants, the yellow dots the symptomatic plants and the red dots the symptomatic plants that are host plants of the bacterium. The maps were created with the QGIS software with maps from Google Earth, Imagery 2018, DigitalGlobe.

Regarding the rosemary spy plants, molecular tests carried out over four years have not detected any bacteria in the collected samples. The rosemary plants suffered from the heat and many of them died. In May of the first year, the 12 rosemary planted in the campus were already all desiccated. The following year, they were replaced, as well as six rosemary plants located in the demarcated area. However, they did not last one year. Soil tilling performed in the demarcated area by the local gardeners also removed several plants from the ground. Only 12 out of 44 rosemary plants survived the four years of the experiment. In the first year, symptoms similar to those caused by *X. fastidiosa* already started to appear in May and, at the end of the first season, two thirds of the plants presented these symptoms, starting with chlorosis at the tip of the leaves, which extended to all the leaf surface and turned necrotic (Fig. 6).

Insect sampling

Molecular tests carried out over four years have never detected any bacteria in the collected insects of the demarcated area.

During the first season, the amount of sampled insects of both species fluctuated depending on the month. This fluctuation can be observed in Fig. 7. In March, the foam produced by the nymphs could be easily observed and in total, 40 nymphs of *P. spumarius* (1.9 nymphs/m^2, mainly at nymphal stage 3–4) and 89 nymphs of *N. campestris* (4.2 nymphs/m^2, mainly at nymphal stage 2–3) were sampled (Fig. 7A, B).

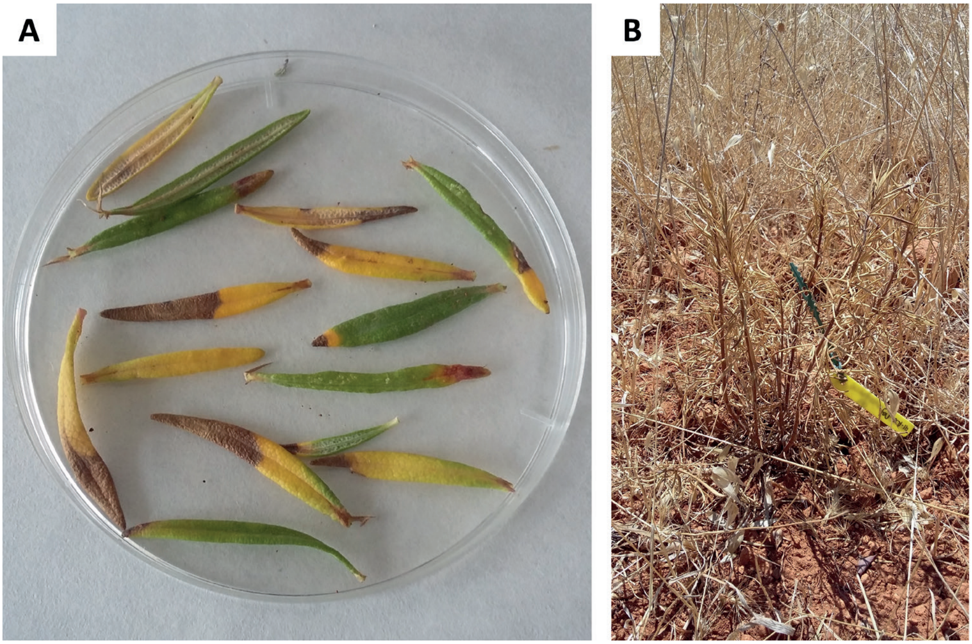

Figure 6. Rosemary health state **A** sampled leaves of rosemary presenting *X. fastidiosa*-typical leaf scorch symptoms (May 2018) **B** dry and dead rosemary on the field (July 2018).

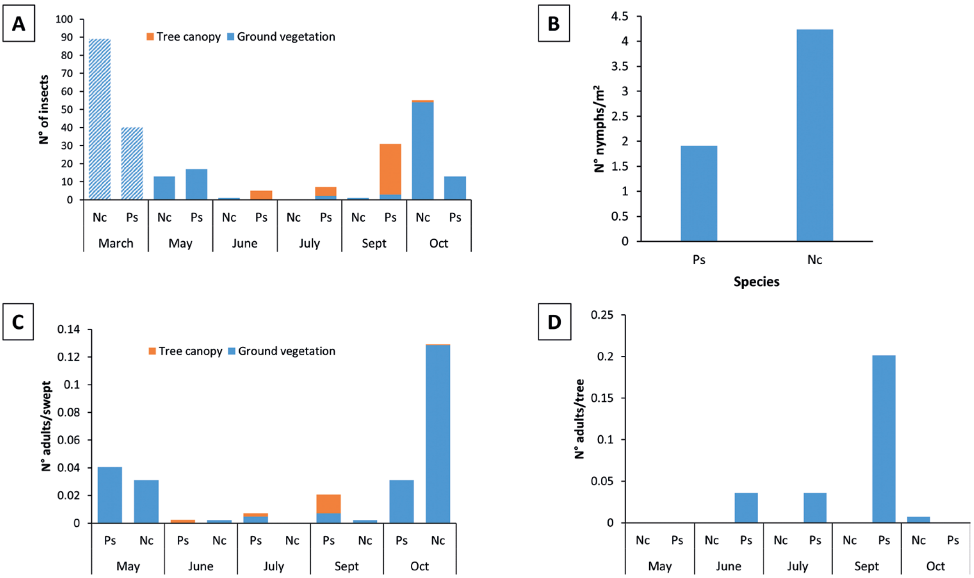

Figure 7. *Philaenus spumarius* (Pc) and *Neophilaenus campestris* (Nc) samples in 2018 in the 100 m area around the sentinel plantation **A** number of insects sampled through the different months. The striped pattern represents the nymphs and the plain pattern represents the adults **B** number of nymphs per m^2 sampled in March **C** number of adults per sweep sampled through the different months **D** number of adults per tree (wild olive, almond or carob tree) sampled during the different months.

The nymphs of *N. campestris* were always found on Poaceae, while those of *P. spumarius* were sampled on Asteraceae (*Carduus* sp.), Euphorbiaceae and other herbaceous plants. At the beginning of May, local collaborators observed nymphs of *P. spumarius* on one *S. alba* plant in the plantation, as well as two adults of *P. spumarius* on *P. domestica*.

At the end of May, the adult stage was already present and the sampling on the ground vegetation revealed less individuals than when nymphs were sampled the previous months. The number of adults per sweep was below one, with 0.04 *P. spumarius*/sweep and 0.03 *N. campestris*/sweep (Fig. 7C). In June, the herbaceous layer had dried and almost no insects were found in the ground vegetation. Very few insects were also sampled in the tree canopy. In September, more *P. spumarius* adults were sampled in the tree canopy (Fig. 7D); however, the number remained low with about 0.2 adults/tree. In October, new fresh herbs had grown and the highest number of *N. campestris* over the season was reached in the ground vegetation (0.13 adults/sweep), while a similar density as the one sampled in May was found for *P. spumarius* (0.03 adults/sweep).

The following years, the number of insects collected around the plantations varied between months and years (Fig. 8A, B) with a maximum in October 2020 of 0.06 *P. spumarius*/sweep and 0.13 *N. campestris*/sweep, sampled in the ground vegetation for both species. In total, four *P. spumarius* in October 2019, one *P. spumarius* in October 2020 and one *N. campestris* in October 2020 were found in the herbaceous layer of the sentinel plantation, showing that few insects were also circulating amongst the trees.

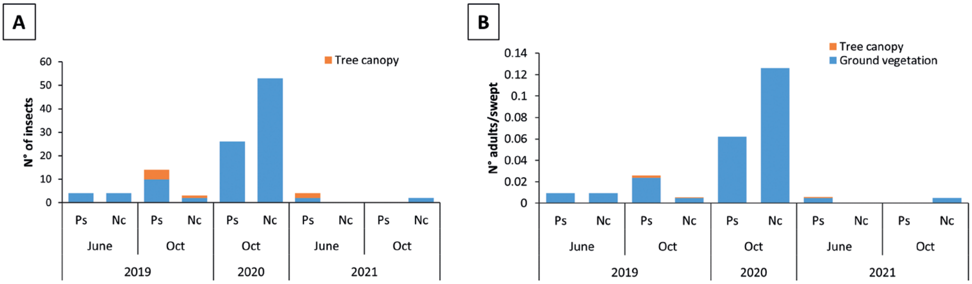

Figure 8. *Philaenus spumarius* (Pc) and *Neophilaenus campestris* (Nc) samples in 2019, 2020 and 2021 in the 100 m area around the sentinel plantation **A** the total number of collected adult insects **B** number of adults per sweep.

The sentinel plants

Molecular tests carried out over four years have never detected any bacteria in the collected samples of the sentinel plants.

Nevertheless, first symptoms on *S. alba* already started to appear in June of the first year (2018) with some slight necrosis at the leaf margins of some of the plants. In July of that year, 78% (21/27 plants) of the willows had slight symptoms, while in October, 96% (26/27 plants) presented leaf necrosis starting from the tip, sometimes followed by chlorosis (Fig. 9A, B). Regarding *P. domestica*, slight chlorosis followed by necrosis at leaf margins started to appear in July 2018 on five of the plants (Fig. 9E). In October of the same year, ten plants had slight symptoms and two had moderate symptoms of chlorosis and necrosis of leaf margins. Finally, concerning *Q. petraea*, first typical necrosis on leaf margins started to appear in September of the first year. In October, these symptoms were more widespread affecting 30% of the plants (8/27 plants) and consisted of typical necrosis of leaf margins with a chlorotic halo (Fig. 9C), while two plants completely died. The following years, the same symptoms started to appear on the new growing leaves, mainly on *S. alba* and *Q. petraea*. On *P. domestica*, typical leaf symptoms were less frequent; however, this species presented

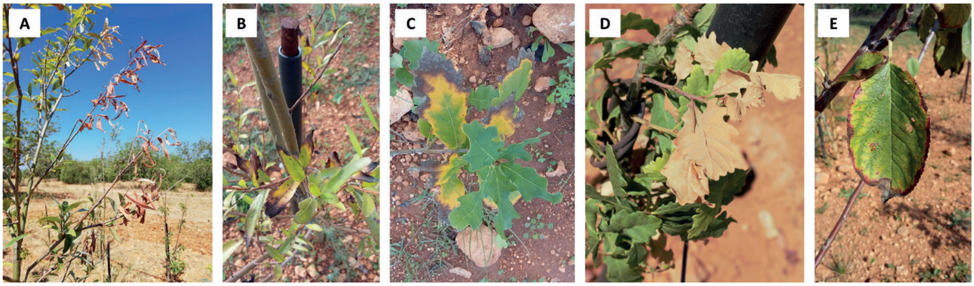

Figure 9. *Xylella*-like symptoms on the plants of the sentinel plantation in October of different years **A** on *Salix alba* in 2019 **B** on *Salix alba* in 2018 **C, D** on *Quercus petreae* in 2018 **E** on *Prunus domestica* in 2018.

more defoliation. The second year, the extremity of the principal stem of five plum trees and five willows started to die; for the three species, stem sprouts started to grow on 1–2 plants per species.

The summer of 2021 was declared the warmest recorded in Europe in the last 30 years, with severe heatwaves in the Mediterranean (Copernicus 2022). While the sentinel plants were already weakened by the last three hot summers despite the irrigation system, many of them died completely or partially this last year. Death was assigned after scraping the bark from several parts of the trunk. In total, 14 *S. alba* plants were completely dead and 13 had their main stem completely desiccated, but had developed sprouts at the bottom that were still living. The remaining leaves showed all symptoms of necrotic and chlorotic leaf margins. Three *Q. petraea* died and almost every remaining individual presented symptomatic leaves, while two of them had their main stem completely dead, but with living sprouts. Finally, two *P. domestica* died and about twenty of them had symptomatic leaves, which consisted of leaves turning red from the margins with a degraded colour, except for some leaves where the discoloured margins were quite delimited. About fifteen plants had between a quarter and a half of their main stem completely dead starting from the tip. Finally, the stem of two of them had completely died, leaving a second plant to grow from the variety Myrobolan, as the Opal variety was grafted on to this one. The size measured each year was not reported here because it was biased by the death, or partial death, of the main stem.

Concerning *Q. petraea*, damage caused by the herbivore *Lachnaia sexpunctata* Scopoli (Fig. 10A) in May-July 2018 forced us to put their foliage under a net (Fig. 10B) until mid-July to keep them alive, but this also resulted in their inaccessibility to *X. fastidiosa* insect vectors. A pesticide (Cypermethrin 10 ml/l) also had to be applied. The following years, the situation was better and the foliage could be exposed to the environment for all seasons. During the monitoring, fungal-like agents were also observed on the leaf surface of many individuals.

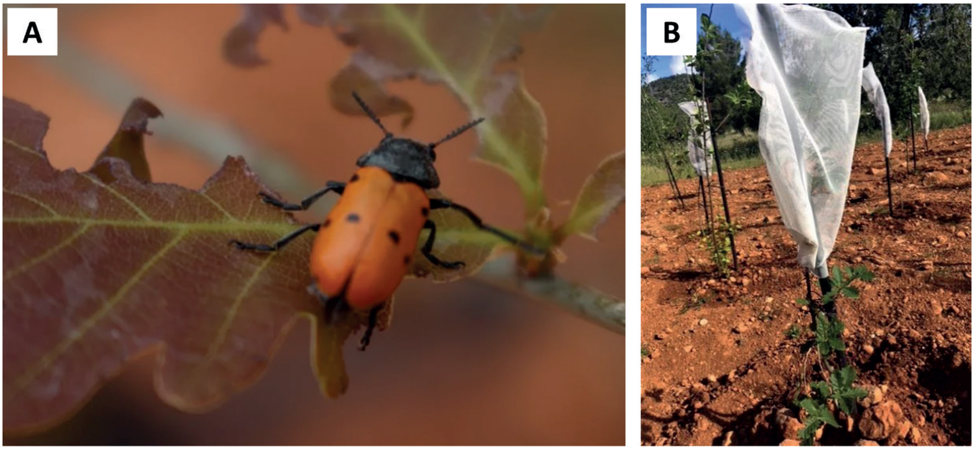

Figure 10. Herbivore damage on *Quercus petraea* **A** *Lachnaia sexpunctata* feeding on *Q. petraea* in the sentinel plantation **B** net on *Q. petraea* to avoid the herbivores eating their foliage.

Discussion

During the four-year sampling and monitoring, *X. fastidiosa* was never detected in our sentinel plants nor in the collected insects. While it is rather positive not to have any infection of this quarantine pathogen, the duration of the plantation establishment did not allow us to answer the question of the potential host range of *X. fastidiosa.* In fact, besides the low infectivity pressure that had been observed on the plot, the absence of detected interaction between the bacterium and the sentinel plants does not mean that interaction could never occur (Mansfield et al. 2019) mainly given the highly specific conditions required by this plant pathogen. Instead, this study was an experimental work to learn how to combine sentinel plantation and research on *X. fastidiosa*, by exploring the constraints that were encountered to improve or redirect the method for future sentinel projects. In addition, the establishment of this plantation has provided valuable data on insect abundance and infection rates near the UIB campus and has enabled the implementation of other parallel experiments, while establishing a lasting international cooperation between the two universities.

Complexity of sentinel plantations combined with *X. fastidiosa* research

Despite the publication of EPPO (2020) providing guidelines for sentinel studies, only two other assays that describe themselves as sentinel plantations have been reported in literature and both as part of the same project (Roques et al. 2015; Vettraino et al. 2015), while a third study can be characterised as one, even if it does not refer as such (Rathé et al. 2014). The sentinel plantations of Roques et al. (2015) and Vettraino et al. (2015) consisted of a four-year monitoring of five European tree species, including *Quercus* spp., which had been planted in China to investigate potential new host-pest/pathogen associations that could emerge in Europe through plant trade. While the experiments allowed the collection of valuable data and discovery of new associations, it already highlighted the complexity of the technique in terms of logistics and workload.

In our study, many constraints were faced and are reported in Table 2 with some perspectives on how the system could be improved to ease the implementation of the method. Our burdens started with permits and Italian administrations. In fact, the initial plan was to establish the plantation in the Apulian area where the first epidemic was declared. Ex-patria sentinel plantation studies require the movement and planting of non-native plants and they are, therefore, subjected to the host country's legislative and administrative procedures for importation and planting (EPPO 2020). After more than one year of back-and-forth e-mails to get approval from the Italian authorities, our request was transferred to our first correspondent. Therefore, the location of the plantation was changed to Majorca, where a good collaboration with UIB allowed us to obtain the agreement of the local authorities and the university, where the plantation was to be established, in about a month. A comparative view of the full procedural pathway between our first attempt in Apulia and Majorca can be viewed in Suppl. material 1. In addition, for administrative reasons, Roques et al. (2015) were unable to establish their plot in the initially optimal climatic zone where they wanted. In their study, many plants were lost due to the delays in Chinese authorisations and imposed quarantine measures. Due to the common external border, plants with a European passport can circulate in Europe without restrictions and sentinel plantation *intra*-Europe should, therefore, be easier to implement (Vettraino et al. 2020). Furthermore, Vettraino et al. (2020) classified Europe as having low bureaucratic complexity concerning sentinel plantations compared to other non-European countries in a ranking they established according to the country's bureaucratic procedures. Surprisingly, Italy was considered the least complex European responding country, in contrast to what was experienced here. However, the current sensitive issue of *X. fastidiosa* in Italy has certainly not helped to speed up the procedures. On the other hand, the government of the Balearic Islands immediately accepted our request under certain conditions, which were the compliance with the norms in force in the territory regarding *X. fastidiosa* and the prohibition of planting *Polygala myrtifolia*, initially chosen as a spy plant for its high susceptibility to the bacterium. Vettraino et al. (2020) reported that most of the countries have restrictions on the import of certain plant species or genera, for example, Roques et al. (2015) were prohibited from planting *Pinus* spp. for their sentinel plantations in China. Finally, it is worth noting that we were not able to import plants collected in semi-natural environments, such as cuttings of *S. alba*, because of the difficulty of obtaining a phytosanitary passport for this type of material and all imported plants had to be purchased from Belgian nurseries in order to be certified.

The second challenge of this plantation was to keep the plants alive. The fact they were grown in an environment with different conditions including temperature and soil, brought different biotic and abiotic stresses. The life of these plants depended once again on the good cooperation on site. For example, the delay in the irrigation system establishment in the first year led the local staff to water the plants by hand every two days, carrying more than 80 litres of water in cans to the plantation. Furthermore, if they had not placed mesh covering the foliage for the herbivore *L. sexpunctata* that devoured the oak leaves, the plants would have died during the first year. However, despite constant monitoring by local collaborators, plant mortality increased from year to year and stress often led, especially in willows, to death of the main stem and the growth of new shoots at the bottom of the plant. This may have an impact on the outcome of the experiment, as the death of the potentially contaminated plant parts would lead to the death of the bacteria itself.

Here, the hurdles faced in sentinel plantation assays were coupled with the difficulties often encountered in *X. fastidiosa* studies. In fact, this bacterium is known to be fastidious for research including in its detection (Wells et al. 1987). Its concentration in plants and insects could be below the detection threshold of the different methods (Cruaud et al. 2018; EPPO 2019) and it is irregularly distributed in plants so may be missed during sampling, especially in asymptomatic plants (EFSA PLH Panel 2015; EPPO 2019). On the other hand, symptoms are not always reliable as they can easily be confused with symptoms triggered by other factors, such as drought (EFSA PLH Panel 2015). Therefore, it is more than likely that other causes, such as drought or soil stress, were responsible for the typical chlorosis and necrosis of the leaf margins

Table 2. Constraints and perspective of using sentinel plantation for *Xylella*-research. Constraints encountered in establishing a sentinel plantation in the case of a *Xylella fastidiosa* survey and perspectives for improving the implementation of the method.

Constraints	Perspectives
Administrations	
- Administrative procedures: Apulia vs. Majorca. Probably impacted by the sensitive issue of *X. fastidiosa*.	- Need for strong, organised and well-informed partnership. - Despite EPPO guidelines (2020), need for more homogenisation of admin. procedures and interpretation of the regulations at European level (and at global level through other intercontinental organisation/conventions), requiring to improve consideration towards sentinel plantations by increasing awareness of their usefulness.
Legal framework of importing exotic plant material	
- Complexity of obtaining a European passport for material collected in semi-natural environments.	- Need to simplify the procedures at national level for obtaining passports for scientific research purposes, under verification conditions of the plant material innocuity.
Legal framework of *X. fastidiosa*, as a quarantine agent	
- No movement of plant material from infected zones.	- Need for a proper bio-molecular processing infrastructure on site.
- Removal of infected plants and vector control, decreasing infection pressure around the plantation.	- Choosing a containment site and not an eradication site. - Need for further PRA exploration if special permits could be obtained for not uprooting infected flora for scientist research purpose or for maintaining plants under certain conditions, for example by placing an insect-proof net on the plants to prevent spread by vectors.
- Routing tests of local host plants not advised.	- Plantation of own susceptible spy plant network.
- Restrictions of planting specific plant species (*Polygala myrtifolia*).	- Or obtaining special permits for research purpose after PRA exploration. Need to verify plant innocuity and to sample them regularly to remove them as soon as possible in case of infection to prevent participating in the spread of the disease locally.
Complexity to detect *X. fastidiosa*	
- Concentration below threshold of methods.	- Need to use several specific and sensitive detection methods (quantitative PCR, nested PCR…).
- Irregular distribution in plants and asymptomatic plants.	- Multiply sampling from all sides of the plant (leaves and twigs). If symptoms detected, prioritising sampling of symptomatic parts.
- Symptoms easily confused with ones due to other causes.	- Validation of bacterial presence only if detected with two different methods.
Length of establishment	
- Incubation period and length of establishment of *X. fastidiosa*.	- Long-term international financial and workload support. - Considering arboreta and botanical gardens studies.
- Plants submitted to the unpredictability of natural conditions, with high dependence on abundance, host preference and prevalence of *X. fastidiosa* insect vectors.	- Knowing the epidemiology of the exact sentinel location and choose a plot with high infective pressure (insect prevalence on site is measured on adults). - Considering targeted transmission experiments on sentinel plants with wild insects collected from naturally=infected areas.
Pathosystem polymorphism	
- Investigation only of potential pathosystems, based on local components.	- Choosing location according to the strains one wants to test (extrapolating on current knowledge on which bacterial subspecies affect which plant genus can help, but it is not always accurate).
- For Majorca, strains: ST1, ST81, ST7; insect vectors: *P. spumarius* or *N. philaenus*; local environmental conditions.	- If no preferential subspecies, choosing location with the most strains present or multiply experiments to several areas. - Choosing location with the closest conditions to country of origin (environmental or insect vector population type).
Abiotic and biotic stress for plants	
- Other symptoms masking those of interest	- Irrigation system, eventual fertiliser application.
- Plant mortality limiting the experiment	- Fitting environmental conditions of native area if possible (in case of northern European countries more complicated with *X. fastidiosa* only occurring in southern Europe, thus, considering arboreta and botanical garden studies or targeted transmission experiments in infected areas).

observed on all three species in this study, especially for such plants used to colder temperature and more humid soil, even with the irrigation provided. While an undetectable low bacterial concentration can be questioned, several studies reported that high symptomatic responses were correlated with high bacterial loads (Holland et al. 2014; Saponari et al. 2017) suggesting a greater probability of detection if symptoms were due to *X. fastidiosa* infection.

Another parameter to consider when studying host susceptibility of *X. fastidiosa* is that the incubation period can be measured over months and years (EFSA PLH Panel 2019), indicating that time is a key element. For example, the survival time of Majorcan almond trees from bacterial infection to tree decline has been estimated around 14 years (Olmo et al. 2021). Sentinel plantation studies are already by themselves long-term assays and superimposing the potential time required for infection of the bacteria gives us an idea of how long it takes to conduct this type of experiment. However, the longer incubation period does not necessarily mean lower susceptibility to the bacterium itself, since many external factors can influence it, for example, the vector population. In fact, as *X. fastisiosa* is an insect vector-borne pathogen, its circulation and infection will depend upon the abundance, host preference and prevalence of its insect vectors, which are adding complexity to the system compared to other sentinel studies that would, for example, measure the direct impact of herbivores on leaves. Moreover, a particularity of diseases caused by *X. fastidiosa* is the polymorphism of the pathosystems. In fact, different strains and bacterial subspecies will act differently with the various xylem-feeding insect species and the different host species or cultivars, leading to very specific epidemics around the world (Pierce's disease, Citrus variegated chlorosis, Olive quick decline…) to almost no symptoms or to an endophytic presence. While the choice of the region in relation to the strains one wants to study is essential, this means that an absence or an endophytic interaction does not mean that other strains cannot be aggressive on the same plant species and cultivar. This means that there will only be an answer for a potential pathosystem related to the chosen region, but there are multitudes of other possibilities. The identified pathosystem will keep the adjective "potential" until the disease is not actually observed in the country of origin, as local environmental conditions or the presence of an effective vector will also have an impact.

A final element to be taken into account in the case of sentinel plantations with *X. fastidiosa* is the European regulation as a quarantine agent (Council Directive 2000/29: EC 2000) and the European containment and eradication measures imposed in case of detection (Commission Implementing Regulation (EU) 2020/1201: EC 2020) with the establishment of a demarcated area delimiting an infected zone of at least 50 m and a buffer zone varying in terms of kilometres depending on the situation. In the infected zone, eradication measures have to be undertaken consisting of the removal of all specified host plants of *X. fastidiosa*. However, in areas in which the bacterium is considered widely established including Apulia, Corsica and Balearic Islands, lighter containment measures may be implemented as eradication is no longer considered feasible. Nevertheless, these measures still imply the removal of all the infected plants in the 50 m zone and an intensive surveillance within an area of at least 5 km radius together with vector control. These measures mean that, even in containment zones such as the Balearic Islands, the detection of an infected plant in the sentinel plantation would lead to the control of vector population in the area and to a decrease in the infection pressure around other plants of the plantation. Similarly, if the tested positive plant has to be removed immediately, the observation of symptom evolution and thus, the assessment of susceptibility is compromised, unless exceptional permits for scientific research are obtained. In this study, the problem did not arise because all plants tested negative. Nevertheless, we were still impacted by the consequences of the European legislation as, under the containment scenario in the Balearics, local government and UIB authorities did not advise systematic test of the host plants on the campus. In fact, a positive detection would have led to the uprooting of the campus vegetation, including, as mentioned before, our plantation if special permits were not issued. These measures are considered highly severe for an area where the bacterium is widespread and separated from other regions by the sea (Olmo et al. 2021). In areas infected by *X. fastidiosa*, the possibility of not having to remove infected plants in the field for scientific research purposes deserves further exploration in terms of PRA and bureaucratic procedure. Finally, for biosafety reasons related to quarantine organisms, plant samples cannot be moved and have to be processed on site, which again requires a good logistic, local collaboration and proper infrastructure.

Necessity of knowing the epidemiology of the exact sentinel location

The implementation of a sentinel plantation when studying a specific pest or pathogen requires knowing well the epidemiology of the exact spot of the establishment, as local environmental components have a great impact on the outcome of the experiment (Kenis et al. 2018). The location chosen for this study was probably not optimal, as it was later evidenced that *X. fastidiosa* infection pressure was low and, thus, this certainly constitutes the main reason for the lack of positive detections in insects, spy and sentinel plants in the plot. When the plantation was established on the UIB campus, the prevalence and the epidemiology of the outbreak on the island were not yet well known, which is still the case in several regions where *X. fastidiosa* has recently been detected. Positive detections were reported on the campus about a hundred metres from the plantation on one *R. officinalis* plant and two olive trees (M. A. Miranda, personal communication) and the health state of host plants including declining almond trees, one of the main crop affected by *X. fastidiosa* on the Island, led us to suspect that the place was infected. However, due to the lack of systematic sampling after the declaration of the contention scenario in the Balearics, the presence of the bacterium could not be confirmed by testing. In addition, the quantity of nymphs sampled when choosing the location was 1.9 nymphs/m^2 for *P. spumarius* and 4.2 nymphs/m^2 for *N. campestris* in March, which is actually higher than the mean observed in the ground vegetation sampled through the Island. López-Mercadal et al. (2021) reported an average of about 0.22 nymphs/m^2 for *P. spumarius* in the peak of March and 0.005

nymphs/m^2 for *N. campestris* with differences between plots and years. In our plot, the prevalence of these nymphs was null. However, this information was not relevant as the infectivity is lost with each moult (Purcell and Finlay 1979) and prevalence, therefore, has to be measured on adult insects to have robust data.

After deepest outbreak investigations, it appeared that the east side of the Island towards Manacor was probably the most infected part, while the plantation was located to the west side of the Island. In fact, Gutiérrez Hernández and García (2018) mapped the positive records of *X. fastidiosa* detected in the Balearics by the Plant Health Section of the Department of Environment, Agriculture and Fisheries of the Government of the Balearic Islands and showed that most of the positive samples were concentrated on the east side with the highest densities in agricultural and residential areas close to the main communication routes. They stressed, however, that the conducted sampling strategy could have biased this distribution, for instance, because the samples could have been collected preferentially in these more accessible areas. Based on direct field observations and using Google street view, Moralejo et al. (2020) also mapped the distribution of *Xylella*-symptomatic almond orchards and their mortality across the Island, tracking their evolution since 2012 (Fig. 11). They showed a gradient from east to west, showing a moderate incidence on the site of the plantation. However, molecular testing of infected almond trees did not reveal a clear spatial pattern (Moralejo et al. 2020). In addition, highly variable incidence was encountered in different orchards (Olmo et al. 2021), hence the need of knowing the incidence and prevalence of vectors at the precise location of a sentinel plantation.

The density and prevalence of insect vectors are one of the drivers of *X. fastidiosa* infection and impact the temporal dynamics of symptom appearance (EFSA PLH Panel 2019), as multiple and independent infections could lead to an injection of a higher bacterial load and a decrease in the incubation period (Daugherty and Almeida 2009). The damage in the Balearics are the consequence of almost 20 years of infection (Moralejo et al. 2020), suggesting that the infection pressure could be too low to conduct sentinel plantation experiments. In fact, the abundance of nymphs and sampled adults, as well as the prevalence of insects are lower than the values encountered in the infected areas of Apulia where the outbreak was more drastic. A prevalence of 23% was reported in Majorca (López-Mercadal et al. 2021) compared to up to 71% detected in an Apulian olive grove (Cornara et al. 2016a). Similarly, higher densities of vectors were measured in Apulia with 7 to 39 nymphs of *P. spumarius*/m^2 in olive orchards (Bodino et al. 2019), about 7 adults/olive trees and 0.5 adults/sweep in weeds recorded during the respective seasonal peaks (Cornara et al. 2016b), however, with heterogeneity identified amongst the orchards studied (Bodino et al. 2019). In our plot, the adult density varied according to the seasonal estivation and ground drying pattern of Mediterranean regions (Cornara et al. 2016b; López-Mercadal et al. 2021). It barely reached a maximum of 0.04 *P. spumarius*/sweep in May and 0.2 *P. spumarius*/tree in September 2018, while the average reported through the Island was below 0.1 adults/sweep in ground cover, tree canopy and border vegetation (López-Mercadal et al. 2021). In addition, *N. campestris* was not considered as a significant vector due to its

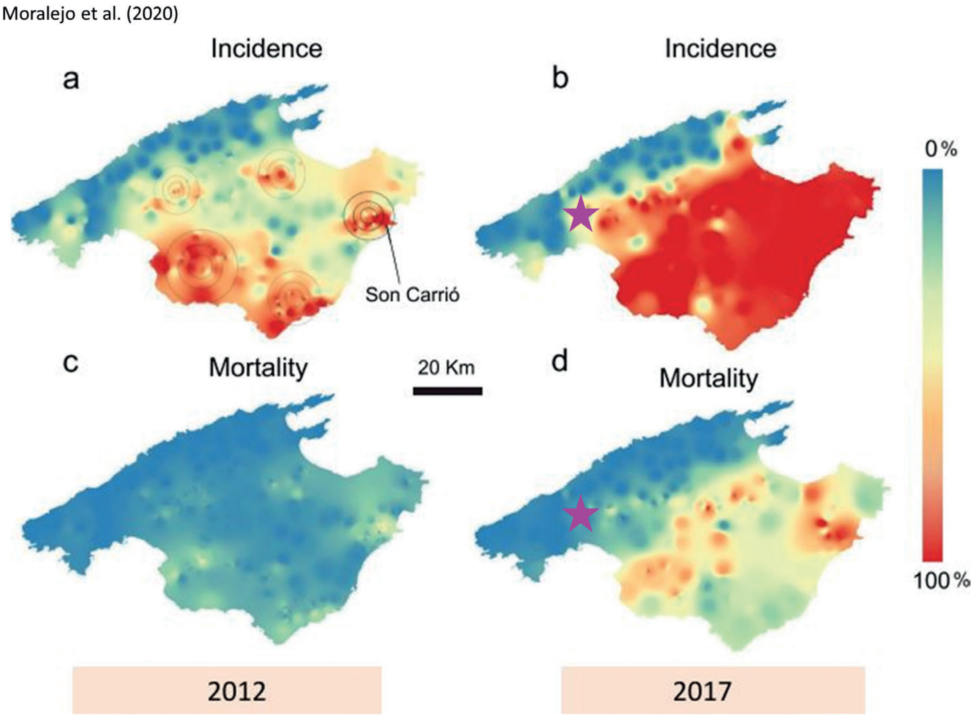

Figure 11. Almond leaf scorch incidence through Majorca. Map of the incidence of the almond leaf scorch disease and almond mortality within orchards across Majorca in 2012 and 2017 through field observation and Google view archives according to fig. 2 in Moralejo et al. (2020) (courtesy of E. Moralejo). The figure was adapted by adding the pink star at the localisation of the sentinel plantation.

very low presence on the tree canopy (López-Mercadal et al. 2021). Moreover, the soil around the plantation was ploughed almost every year, as common management on the Island, which, besides destroying several rosemary spy plants, probably decreased insect movement around the plot even with sowing of ground vegetation the second year. In fact, tillage is a technique of vector control reducing the number of vectors/m^2 (Bodino et al. 2019; EFSA PLH Panel 2019). In the study of Kenis et al. (2018), their plantation located at the edge of the forest took less time to be infested than another one situated in an agricultural-peri-urban area, highlighting again the impact of high local circulation of pests and pathogens on the time and outcome of the assay.

Sentinel plantations as an efficient tool for *X. fastidiosa* research in specific situations

Even in locations with high infection pressure, the efficiency of the sentinel plantation in the case of *X. fastidiosa* host range investigation is questioned due to the ratio results/time-workload. Yet the sentinel plantation method is currently being used in Apulia for the screening of olive cultivars coming from various Mediterranean olive-growing areas (Spain, Tunisia, Greece etc.) by exposing them to the natural pressure of inoculum in heavily-infected fields (XF-ACTORS 2017; Saponari et al. 2019b). The previous finding of the mild symptoms on the Leccino and FS17 olive cultivars adjacent to severely-affected orchards motivated the study (Boscia et al. 2017). Approximately 100 different genotypes were planted and are currently under evaluation in different plots, actually making the Apulian region home to one of the largest sentinel plantations of all time. This study is promising and is considered necessary for long-term management of *X. fastidiosa* in olive-growing regions as preliminary data show already differences in susceptibility in various cultivars (EFSA PLH Panel 2019; Saponari et al. 2019b). However, it highlights the long-term commitment required as the survey started in 2015 and is still ongoing. The project is part of a research programme funded by the European Union's Horizon 2020 Research and Innovation Programme, which explains how a project of this magnitude could be established and which underlines the need for long-term consistent international support for the implementation of such experiments. The success of this plantation, in addition to the selection of highly-infected plots, also comes from the fact that the tested potential hosts are related to the STs present in the environment. As the Apulian ST53 is highly aggressive on olives, it is obvious to carry out olive plant susceptibility in this area. However, other *X. fastidiosa* infected regions, such as Balearic Islands and Corsica, could be interesting to study the susceptibility to other STs, as three STs belonging to two subspecies coexist in Majorca, while only one in the Apulian Region.

Thus, the Apulia study proved the usefulness of sentinel plantations in the context of *X. fastidiosa*. However, it would be less relevant to conduct these studies in certain situations. There should be, for example, similarities between the climatic conditions of the two regions involved in the sentinel studies to minimise the impact of external factors. So far, the bacterium has only been found established in southern Europe, in regions with a Mediterranean type of climate and these studies would, therefore, be less suitable for northern European countries, as differences in environmental conditions could lead to weakening or even death of the plants and to misidentification of the cause of potential symptoms. Nevertheless, this tool remains very valuable and should be considered for studies on *X. fastidiosa*, as other techniques for screening potential hosts of this pathogen are also discussed. Amongst these techniques, mechanical inoculation shows a low rate of success, even in susceptible hosts (Prado et al. 2008; EFSA PLH Panel 2019) as this method artificially reproduces infection while in the environment and only xylem-specialised insect vectors have the capacity to infect plants (Almeida et al. 2005). Working with insect vectors is, therefore, a more relevant way of conducting experiments. However, besides the biosafety risk it could represent for *Xylella*-free regions and the need for proper infrastructure, the very act of infecting an insect is a challenge. Other experiments consisting in grafting more than 400 olive genotypes on infected trees were conducted in parallel with the sentinel plantation in Apulia to short incubation period and time imposed by insect traits (Saponari et al. 2019b). However, in addition to also being an artificial way of infection, it requires the availability of appropriate infected graft material. Therefore, sentinel plantations have their advantages and have to be considered as valuable complementary tools in certain situations.

In these situations, this study has provided a complete methodology to monitor the bacterium circulation through the sentinel plants. The use of spy plants is certainly useful if sampling of susceptible vegetation is not possible in the nearby area. In other cases, sampling of local flora may be sufficient, although it does not ensure real-time circulation of the bacteria, as the current state of the local flora could be the result of infection from the past (Moralejo et al. 2020). The use of small perennial plants may facilitate sampling, as bacteria are distributed irregularly in the plant. The species or mix of species must be adapted to local conditions, susceptible to the bacterial strains being investigated and favoured by local vectors. In this study, *R. officinalis* was chosen as it was reported infected with the European STs of subsp. *multiplex* and subsp. *pauca* (ST6, ST7, ST53, ST80, ST81 and ST87) and was found infected in Majorca with the ST81 (EFSA 2022). In addition, in America, the bacterium was detected on this plant species close to *X. fastidiosa* subsp. *fastidiosa*-infected vines (Freitag 1951).

Conducting sentinel studies differently to assess host range in northern countries

Sentinel studies can also be carried out differently to study host range in countries that cannot match closely the environmental conditions of the potential location. First, arboreta and botanical gardens are still an option for studying exotic host range in naturally-infected environments. However, as a detectable infection depends on the density and prevalence of *X. fastidiosa* insect vectors (Daugherty and Almeida 2009), the use of this method could also be discussed as these areas are often subjected to phytosanitary management. One advantage of these studies regarding *X. fastidiosa* would be that plants are grown in these sites for a long time, increasing the success concerning potential latent periods or low bacterial load potentially enabling detection. In addition, the study of Groenteman et al. (2015) has shown promising results for *X. fastidiosa* research by sampling in botanical gardens. They managed to discover 28 New Zealand plant species infected by *X. fastidiosa*, including several visited by the insect vector *Homalodisca vitripennis* Germar, in Californian botanical gardens where the disease is well established. They also found parasites capable of controlling the vector on these plant species with the aim of a biocontrol early-response strategy in case *H. vitripennis* invade New Zealand.

A second way would be to carry out transmission experiments in an insect-proof greenhouse with naturally-infected vectors in contaminated regions to bypass the problems of biosecurity imposed by *Xylella*-free areas and the difficulty of infecting insects. Compared to standard sentinel plantations, these experiments allow us to reduce the dependence on vector density and on insect feeding preferences. In fact, although *P. spumarius* is considered a polyphagous species and was observed feeding on the three studied sentinel plants in their area of distribution, it is possible that, in the sentinel country, these insects are more interested in native vegetation. Native plants could, therefore, compete with the exotic sentinel ones, potentially resulting in fewer vector feeding events decreasing the bacterial transmission probability. Even if vector preferences are biased and that natural conditions are, therefore, not fully met, these experiments can still be considered as sentinel studies since they consist in ex-patria plants sent to study the impact of exotic organisms in areas in which they occur. This has been done in Majorca as a complementary experiment where 20 new cuttings of *S. alba* and of *P. tremula* have been sent from Belgium to the UIB campus. There, transmission experiments with naturally-infected *P. spumarius* were conducted in an insect-proof greenhouse and revealed positive infection on *S. alba*, proving the higher efficiency of the technique compared to sentinel plantation.

Finally, in-patria sentinel plantings (Eschen et al. 2019) or sentinel nurseries (sensu Vettraino et al. 2017) consist of planting native traded plants without phytosanitary treatments on its own land to monitor pests and pathogens which could be spread through international trade (Vettraino et al. 2017). They obviously do not have the same objective as ex-patria plantations that inform PRA of organisms that are not yet present in a given area. Rather, they consist of surveillance for a known pathogen for which possible entry and dispersal pathways have been identified (Mansfield et al. 2019) and they still represent valuable sentinel assays to be conducted with the aim of early detection of *X. fastidiosa* in new regions. The major difference with a standard commercial nursery is that no pest control measures are implemented on these plants (EPPO 2020), so that it is possible for the vectors to reach the plants and for the plants to become infected if *X. fastidiosa* is introduced in the area. For this strategy to be effective, these plantations have to be established in strategic locations where the bacterium is the most likely to enter. The "plant for planting" pathway being the main entrance for exotic organisms including *X. fastidiosa* (Liebhold et al. 2012; EFSA PLH Panel 2018), their locations in/close to nurseries or other plant commercial places, would be relevant. In addition, these plantations must consist of known host plants that have a high probability to be the first infected when the bacterium enters an area and, if possible, to be highly susceptible for the infection to be visible and easily detectable. For example, the Auckland Botanic Garden has set up a sentinel plot of myrtle plants to detect the potential arrival of the myrtle rust (*Puccinia psidii* Winter) as early as possible in New Zealand, as the fungus was prevalent in Australia at the time (Barham et al. 2015). Similarly, one can imagine planting a network of *P. myrtifolia* near nurseries, previously tested for innocuity, which are regularly monitored for potential contamination by *X. fastidiosa*. Obviously, these susceptible plants should be tested carefully and regularly to provide the benefits of early detection while preventing them from serving as inoculum for disease establishment (Mansfield et al. 2019).

Conclusion

In conclusion, this study is an experimental work highlighting that sentinel plantations are not easy to implement in the case of *X. fastidiosa*, but that they are complementary to other studies and that they could provide valuable information on host interactions when some conditions are met. This work proposes a methodology to monitor future sentinel plantations and it suggests other ways of conducting sentinel experiments for screening host range or for early detection of *X. fastidiosa* in new areas.

Acknowledgements

We would like to acknowledge all the people who helped in the establishment of the sentinel plantation and the irrigation system and who contributed to maintain the plants alive, especially the UIB gardeners and technicians, Amélie Emond, Maria Antònia Tugores, Pau Mercadal, Carlos Barceló and Noelia Barros. We also want to thank all the people who irrigated manually with bravery the plantation during the first year. We are thankful to Sofia Delgado and Claudia Paredes for their laboratory support. Finally, we are grateful to UIB authorities and to the Government of the Balearic Islands for the authorisation to carry out these assays.

The research that yielded these results, was funded by the Belgian Federal Public Service of Health, Food Chain Safety and Environment through the contracts RF 19/6331 (XFAST project) and RT/7 XYLERIS 1 (XYLERIS project). NC was supported by the Foundation for Training in Industrial and Agricultural Research (FRIA, FNRS), and SH by the Belgian Federal Public Service of Health, Food Chain Safety and Environment.

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Supplementary material 1

Administrative burdens and delays, a comparative view

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Data type: PDF document

Explanation note: A comparative view of the full procedural pathway between the first attempt of implementing the sentinel plantation in Apulia and the second attempt in Majorca.

Link: https://doi.org/10.3897/neobiota.84.90024.suppl1

NeoBiota 84: 81–105 (2023)
doi: 10.3897/neobiota.84.89826
https://neobiota.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
NeoBiota
Advancing research on alien species and biological invasions

Cosmopolitan Scolytinae: strong common drivers, but too many singularities for accurate prediction

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Academic editor: Marc Kenis | Received 1 July 2022 | Accepted 2 November 2022 | Published 18 May 2023

Citation: Grégoire J-C, Jactel H, Hulcr J, Battisti A, Inward D, Petter F, Grousset F (2023) Cosmopolitan Scolytinae: strong common drivers, but too many singularities for accurate prediction. In: Jactel H, Orazio C, Robinet C, Douma JC, Santini A, Battisti A, Branco M, Seehausen L, Kenis M (Eds) Conceptual and technical innovations to better manage invasions of alien pests and pathogens in forests. NeoBiota 84: 81–105. https://doi.org/10.3897/neobiota.84.89826

Abstract

Many scolytine beetle species have been expanding in new territories, travelling with wood and plants for planting, sometimes with a high impact on plant health. Here, we attempt to quantify the mobility of these species and to identify the biological drivers of mobility and impact. Mobility was estimated by counting the numbers of landmasses (contiguous pieces of land, surrounded by ocean or sea) colonised by each species. A series of potential drivers (taxonomic tribes; feeding regimes; polyphagy; reproductive strategy; host taxa; aggregation pheromones and long-range primary attractants), as well as impact on host health were recorded. A total of 163 species were identified, out of 5546 counted in the whole subfamily. The cosmopolitan taxa amongst the subfamily showed significant disharmony with regards to invasion frequency. Four tribes (Xyleborini; Ipini; Crypturgini; Hylastini) were significantly over-represented and two others (Corthylini; Hexacolini) were under-represented. Some 53% of the 163 species are inbreeding, a very significant excess as compared to the whole subfamily (29%). The inbreeders colonised more landmasses than the outbreeders. There is a significant relationship between the number of host families attacked by a species and the number of colonised landmasses. Most of the invasive species are recorded to respond to long-range host primary attractants, only one quarter respond to pheromones. All very mobile species respond to long-range primary attractants and none is known to respond to pheromones. Very mobile species are all associated with a substantial or moderate impact. The most mobile species belong to a limited number of subtribes. They are often inbreeding, polyphagous and respond to long-range primary attractants, but do not produce pheromones. However, there are many counter-examples. The outbreeding *Scolytus multistriatus* attacks only three host families, producing aggregation pheromones and has established in thirteen landmasses, with a high impact. Due to these many exceptions, species-based risk prediction relying on the few traits routinely analysed in literature suffers from important uncertainties.

Keywords
ambrosia beetles, bark beetles, entry, establishment, fungi, geographic distribution, impact, inbreeding, landmasses, polyphagy, quarantine, risk assessment, spread, semiochemicals

Introduction

Very few species are studied in depth before they become noticeable pests. Consequently, most attempts to assess the risk of potentially invasive species rely on limited information. Invasive species assessments now use multiple methodologies ranging from consensus-seeking horizon scans to climate match modelling. However, nearly all these methodologies suffer from one fundamental problem – lack of information about the interactions between a specific species and its potential new environment or hosts.

The typical solution is to take a broader taxonomic perspective and assume that the ecology of a species can be derived from the ecology of related species for which there is more knowledge or to assume that species within a genus are ecologically similar. The invasive species modelling literature is rich with examples of assessments of genera or even entire families or even guilds (see, for example, Mech et al. 2019; Barwell et al. 2020; Schulz et al. 2021).

Some of the most damaging forest pests in the world are bark and ambrosia beetles belonging to the weevil (Curculionidae) subfamily Scolytinae. Global climate change and intense silviculture enabled species, such as *Dendroctonus ponderosae* Hopkins and *Ips typographus* L., to multiply to epidemic proportions in North America and Europe, respectively (Grégoire et al. 2015; Hicke et al. 2016) with a total of 455.7 million m^3 of pine killed by *D. ponderosae* in British Columbia between 2000 and 2015 (British Columbia Government 2019) and 148 million m^3 of spruce killed by *I. typographus* between 1950 and 2000 in Europe (Schelhaas et al. 2003), with dramatically increasing damage during the last few years (Hlásny et al. 2021). In addition to these species which are currently spreading within their native continents only, many others have been expanding their territorial range worldwide, especially travelling with international trade. Bark and ambrosia beetles may additionally cause damage as vectors of pathogenic fungi. The redbay ambrosia beetle, *Xyleborus glabratus* Eichhoff, of Asian origin, was first reported in North America in 2002 (Rabaglia et al. 2006). It vectors the fungal symbiont, *Raffaelea lauricola* T.C. Harr., Fraedrich and Aghayeva, causing “laurel wilt”. At least 300 million *Persea borbonia* L. Spreng. (redbay trees) have been killed by laurel wilt in the USA (Hughes et al. 2017) and several other tree species of the Lauraceae family, including avocado (*Persea americana* Mill.) are also affected by the disease. Another example is the polyphagous shot hole borer, *Euwallacea fornicatus*

Eichhoff (Stouthamer et al. 2017; Smith et al. 2019), a Scolytinae which, together with a symbiotic *Fusarium* sp. fungus, attacks a large number of plants, mostly in cultivated settings in its area of origin, Asia, as well as, more recently, in areas of introduction[1] in North America (Rabaglia et al. 2006), Israel (Mendel et al. 2012) and South Africa (Paap et al. 2018). The North American species *Dendroctonus valens* LeConte was reported in China at the end of the 1990s and, by 2005, it had spread over 500,000 ha of pine forest in three provinces, killing more than 10 million *Pinus tabuliformis* Carr. (Yan et al. 2005). Other harmful Scolytinae species killing living trees and recently introduced into Europe include the Asian ambrosia beetle, *Xylosandrus crassiusculus* (Motschulsky), the black twig borer, *X. compactus* (Eichhoff) and the walnut twig beetle, *Pityophthorus juglandis* Blackman. This latter species vectors the pathogenic fungus *Geosmithia morbida* Kolarík, Freeland, Utley and Tisserat, causing thousand cankers disease of walnuts, *Juglans* spp. (EPPO 2015; Seybold et al. 2019).

The observed spread of these species and many others continues. At the same time, dozens of bark- and ambrosia beetle species have been introduced into non-native regions without any detectable impact. Most bark beetle "tramp species" are harmless.

So far, at least 163 species out of the ~ 6,000 described scolytine species (Hulcr et al. 2015) are known to have established outside of their native areas (Table 1 and Suppl. material 1). The remaining ninety-eight percent of scolytine species are, thus, still potentially able to colonise new territories and their potential impact is still mostly unknown.

Other species that have not spread to date and which are not recognised as harmful, might start expanding their range, benefiting from the trade of new commodities or from commercial movements along new routes. These beetles, alone or together with pathogens, may also colonise new hosts that may prove to be more susceptible than their native hosts or form new associations with local pathogens as suggested by Rassati et al. (2019a). For both known and unrecognised spreading species, the possibility that they can be successfully introduced into new areas and their subsequent potential economic or environmental impact are two major components of phytosanitary risk.

"Horizontal" regulations globally addressing the host plants of non-native pests are locally implemented. For example, all non-European Scolytinae-attacking conifers are targeted in the European Union by phytosanitary requirements applying to the importation of coniferous wood[2] (EU 2019), but equivalent requirements do not exist for the trade of non-coniferous wood. A recent EPPO study focused on twenty-six representative Scolytinae and Platypodinae ambrosia- and bark beetle species associated with non-coniferous wood (EPPO 2020; Grousset et al. 2020). Sixteen life-history traits and other factors were qualitatively weighed with expert knowledge against invasion success. Inbreeding, polyphagy (number of host families) and the lack of aggregation pheromones were common features of species with a successful introduction

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[1] In this context, 'the entry of a pest resulting in its establishment', following the terminology of the Glossary of Phytosanitary Terms of the International Plant Protection Convention (FAO 2019).

[2] 'Commodities such as round wood, sawn wood, wood chips and wood residue, with or without bark, excluding wood packaging material, processed wood material and bamboo and rattan products' (FAO 2021).

history. Association with pathogenic fungi, the use of aggregation pheromones and the capacity to attack and kill new host species were identified as factors contributing to high impact. One of the important conclusions of this EPPO study was that traits related to species with a past invasion history had a strong influence on invasion risks. However, it was found that the main factors that are driving successful establishment and impact vary from species to species and are not always fully identified. One important recommendation of this study was that horizontal phytosanitary measures similar to those for conifer wood better address the risk than regulation of individual species. In another recent study, Lantschner et al. (2020) similarly reviewed 123 Scolytinae species with a history of invasion, focusing on biological characteristics (feeding regime and mating strategy), cumulative trade between world regions, size of source species pools, forest area and climatic matching between the invaded and source regions. They identified sib-mating as a major factor favouring the movement of Scolytinae species into new territories, but also found that a non-biological trait, cumulative trade between world regions, is a primary driver of scolytine invasion.

At a broader taxonomic scale, Mech et al. (2019) and Schulz et al. (2021) focused on the impact of non-native herbivorous insects established in North America. They found that the evolutionary proximity between the native and novel host plants, life history traits of the novel hosts and the presence of native close congeners with a long-term association with the novel host were better predictors of impact than were traits of the invading insects themselves.

In this study, we tried to demonstrate that even relatively closely related species can differ in their capacity to colonise new territories and in their impact. We used presence in at least two landmasses (defined below) as a criterion to select 163 “mobile” Scolytinae species and quantified further their mobility by counting the number of colonised landmasses, according to the literature. We also ranked their impact on plant health. Finally, we attempted to identify biological and ecological features (feeding regimes, inbreeding, polyphagy, aggregation pheromones, primary attractants, conifer/non-conifer hosts or both), associated with differences in mobility and impact.

Methodology

The counting of colonised territories served as a proxy to estimate mobility. A dataset of Scolytinae species known to have spread beyond geographical barriers (across seas or oceans in this study) was constructed (Suppl. material 1), including any species distributed across at least one barrier (hereafter designated as “*Scolytinae with an invasion history*” - SIH), irrespective of its area of origin which is often difficult to delimit (see, for example, Lin et al. 2021). The list includes all the Scolytinae species from the EPPO study (EPPO 2020; Grousset et al. 2020), as well as the species introduced into North America, New Zealand and Europe, listed respectively by Haack (2001, 2006), Brockerhoff et al. (2006) and Kirkendall and Faccoli (2010). This initial set was expanded using information mostly from Wood and Bright (1992), Lantschner et al.

(2020), Atkinson (2021) and from other publications (full list of references in Suppl. material 1). The dataset was completed in December 2020 and, therefore, does not include several important studies (in particular Bright 2021) published after this date.

Amongst the biological features taken into account, the association with pathogens was not considered as a predictor because, in addition to previously known species, species so far harmless on their native hosts (e.g. *R. lauricola*, *G. morbida*) become pathogenic when their vectors colonise new host trees. Besides, scolytines species considered as harmless are sometimes found associated with aggressive pathogens (Wingfield and Gibbs 1991), making pathogens a dubious predictor of impact. Climatic requirements, dispersal capacity and voltinism were also not considered, because of the wide knowledge gap regarding these potential drivers (but see EPPO 2020 and Grousset et al. 2020).

Feeding regimes

We retained the following general categories (Kirkendall et al. 2015): phloeophagy (feeding in inner bark; this category corresponds to the bark beetles *stricto sensu*); xylomycetophagy (fungus farming; this category corresponds to the ambrosia beetles, which live in the xylem of woody plants, where they cultivate symbiotic fungi on which they feed); spermatophagy (feeding in seeds) and herbiphagy (feeding in non-woody plants).

Inbreeding

In some species, the females are fertilised by a brother, with extreme situations where the males are flightless and do not even leave their natal gallery. Only the species with full inbreeding were considered here. The outbreeding species that show some level of inbreeding (e.g. *Orthotomicus erosus*, *Tomicus piniperda*, *Hylurgus ligniperda*, *Ips grandicollis*, *Ips pini*) were not considered as inbreeding in this study. Unless specified in Suppl. material 1, the information comes from Kirkendall et al. (2015).

Polyphagy

Polyphagy was measured, as in EPPO (2020) and Grousset et al. (2020), by the number of host-plant families colonised. Unless specified otherwise in Suppl. material 1, host-plant data come from Wood and Bright (1992) or Atkinson (2021).

Aggregation pheromones (categories: 0/1/2)

We relied on published information, with the understanding that some species might use pheromones that have not been identified so far, for example, short-distance sex pheromones. The source for this field is El-Sayed (2018) unless specified otherwise. Three categories were considered: 0 (no pheromone identified or unknown for the genus); 1 (pheromones known for at least one other species in the genus); 2: (pheromone(s) identified in the species).

Long-range primary attractants (0/1/2)

Here too, we relied on published information, with the understanding that some species might respond to long-range primary attractants that have not been identified so far or only weakly respond to known attractants. Unless specified by a footnote in Suppl. material 1, the information regarding primary attractants (e.g. ethanol and/or alpha-pinene, emitted by the host or by other organisms within the host) comes from Atkinson (2021). The three categories considered are the same as for pheromones.

Host plants: conifers vs. non-conifers (1/2/3)

Three categories were considered: 1 (species attacking only conifers); 2 (species attacking only non-conifers); 3 (species attacking both conifers and non-conifers).

Impact on plant health (0/1/2)

Only direct impact on living trees and seeds or economic impact on traded products (e.g. logs, seeds) were considered. Other ecological impact mechanisms, such as those affecting native arthropods, wood decomposition and other ecosystem processes and patterns, can occur, but these are too poorly known in invasive Scolytinae. Even for the most obvious impact mechanisms, very few quantitative measurements are available in literature and only for a few species in a restricted number of areas. In addition, qualitative estimates vary greatly between assessors. We were, thus, led to rank impact according to three categories (0-1-2): 0 (no impact documented in the literature); 1 (moderate impact: some indication of impact, with some uncertainties because of discrepancies in literature); 2 (known substantial impact documented sometimes quantitatively by several sources). The criteria for damage by spermatophages were the reported colonisation of fruits (none – moderate – massive) and/or impact on regeneration (none – moderate – massive).

Landmasses

We use the term *landmass* to define a contiguous piece of land (a continent or an island, irrespective of its size) surrounded by ocean or sea. This approach admittedly creates large biases. Even if a continent is very large, we consider it as a single landmass. The movements of a species within a landmass are not considered because they are often incompletely documented. However, continents that are not fully separated by oceans (North, Central and South America; Europe, Asia and Africa) are considered as distinct landmasses because of the distances and ecoclimatic differences between them. Some archipelagos (e.g. Cape Verde, Fiji, Galápagos, Hawaii, Micronesia) were considered each as one unit. Islands comprising several countries (e.g. Republic of Ireland + Northern Ireland; Haiti + Dominican Republic) were considered as single units. The size of the geographic barriers between landmasses and of the landmasses themselves has not been considered. Great Britain and the European mainland would, thus, be considered as separate landmasses, although the Channel that separates them is locally less than 35 km broad. On the other hand, South America, which is more than 7000 km long, is considered as a single landmass. Despite these many inconsistencies, we believe that this approach provides a useful, if probably conservative, metric to consider pest mobility. Suppl. material 1 provides a listing and a counting of the discrete landmasses occupied by each species. The acronyms used to designate the different landmasses are listed in Suppl. material 2. When possible, ISO alpha-3 codes (https://www.iso.org/obp/ui/#search) were used. Codes for locations absent from this list because they refer to intra-national territories (e.g. an island belonging to a larger country) were taken from the International Working Group on Taxonomic Databases For Plant Sciences (TDWG) (https://github.com/tdwg/wgsrpd) or were created for the purpose of this analysis.

Statistical analyses

Disharmony with regards to invasion frequency amongst the different SIH tribes; feeding regimes vs. reproductive strategies

2 × 2 Chi-Square tests were used, with Yate's correction for continuity for expected values inferior to 5.

Multivariate analyses on impact

A factorial discriminant analysis (FDA) was performed as a supervised classification method to discriminate amongst three categories of beetle species *a priori* classified, as in the Methodology and in Suppl. material 1, according to their level of damage (impact), as having no impact (0), moderate impact (1) or substantial impact (2), using ecological characteristics as predictor variables (Suppl. material 1). The dataset consisted of 163 species characterised by one quantitative functional trait, polyphagy, expressed as the number of known host plant families and five qualitative functional traits transformed into dummy variables, namely whether bark beetle species exhibited the following characteristics: xylomycetophagy (ambrosia beetles), inbreeding, using aggregation pheromones, using long-range primary attractants and host specialisation ("specialists": attacking either conifers or non-conifers; "generalists": attacking both) .

Covariance analyses on mobility

A Spearman correlation analysis was performed between the number of colonised land masses and the functional traits of the 163 scolytine species. Two variables were identified as significantly correlated with beetle cosmopolitanism, one quantitative, the degree of polyphagy (expressed in terms of number of known host plant families) and one qualitative, the use (or not) of long-range primary attractants for host plant colonisation. We then used an analysis of covariance (Ancova, with and without interaction) to assess the magnitude of the effects of these two factors. All statistical analyses were made with XLSTAT.

Results

Scolytinae with an invasion history - overall features

Disharmony with regards to invasion frequency amongst the SIH tribes

Five tribes, the Xyleborini, Trypophloeini, Ipini, Crypturgini and Hylastini are significantly more frequent amongst the invasive Scolytinae than amongst the Scolytinae as a whole. Two tribes, the Corthylini and Hexacolini are significantly less frequent (Table 1).

Tribes over-represented amongst the invasive Scolytinae are in bold, followed by (+); tribes under-represented are in bold, followed by (-). World figures taken from Hulcr et al. (2015), except for the Trypophloeini, Cryphalini, Corthylini and Ernoporini, for which the revision by Johnson et al. (2020a) was used. The number of non-SIH species is calculated by subtracting the number of SIH in a tribe from the total number of species in the tribe.

The small tribes Amphiscolytini (1 sp.), Cactopinini (21), Carphodicticini (5), Hyorrhynchini (19) and Phrixosomatini (25) are absent from the SIH list, as well as the larger tribes Diamerini (132), Micracidini (298) and Xyloctonini (78).

Feeding regimes

Amongst the 163 SIH species, 79 (48.5%) are phloeophagous, 60 (36.8%) are xylomycetophagous, twelve (7.4%) are herbiphagous and twelve are spermatophagous. The majority (82.3%) of the phloeophages amongst the SIH are outbreeding, whilst

Table 1. Tribes represented amongst the Scolytinae with an invasion history (SIH).

Tribes	SIH species		Non-SIH species		Total	Chi^2	
	N_{SIH}	Weight of tribe within category (%)	$N_{non\text{-}SIH}$	Weight of tribe within category (%)	N	$\text{Chi}^2_{(1, N)}$	p
Xyleborini (+)	**56**	**34.4**	**1112**	**20.6**	**1168**	**17.0422**	**0.000024**
Trypophloeini (+)	**18**	**11.0**	**246**	**4.6**	**264**	**14.7696**	**0.000121**
Dryocoetini	14	8.6	460	8.5	474	0.0004	0.984373
Ipini (+)	**14**	**8.6**	**216**	**4.0**	**230**	**8.3351**	**0.003889**
Crypturgini (+)	**8**	**4.9**	**47**	**0.9**	**55**	**22.2837**	**< 0.00001**
Scolytini	8	4.9	201	3.7	209	0.6013	0.438087
Hypoborini	7	4.3	202	3.7	209	0.1281	0.720387
Hylastini (+)	**6**	**3.7**	**49**	**0.9**	**55**	**9.7088**	**0.001834**
Hylurgini	6	3.7	124	2.3	130	0.7786	0.377564
Corthylini (-)	**5**	**3.1**	**1237**	**22.9**	**1242**	**35.8508**	**< 0.00001**
Cryphalini	5	3.1	247	4.6	252	0.8257	0.363514
Phloeosinini	4	2.5	223	4.1	227	1.1493	0.283696
Polygraphini	3	1.8	151	2.8	154	0.2465	0.619527
Hylesinini	2	1.2	162	3.0	164	1.1856	0.276221
Phloeotribini	2	1.2	108	2.0	110	0.1747	0.675995
Bothrosternini	1	0.6	130	2.4	131	1.5137	0.218568
Hexacolini (-)	**1**	**0.6**	**241**	**4.5**	**242**	**5.6591**	**0.017365**
Scolytoplatypodini	1	0.6	52	1.0	53	0.0022	0.962393
Xyloterini	1	0.6	21	0.4	22	0.0344	0.852906
Ernoporini	1	0.6	177	3.3	178	2.8113	0.093603
Total	163	100	5406	100	5569		

Table 2. Feeding regimes of the Scolytinae with an invasion history.

Feeding regime	Outbreeding			Inbreeding			Total		Chi^2_1	
	N	% of total	% of regime	N	% of total	% of regime	N	% of total	Chi^2_1	p
Xylomycetophagy (+)	4	2.5	6.7	56	34.4	93.3	60	36.8	60.9222	< 0.00001
Phloeophagy (-)	65	39.9	82.3	14	8.6	17.7	79	48.5	78.3002	< 0.00001
Herbiphagy	5	3.1	41.7	7	4.3	58.3	12	7.4	0.128	0.720506
Spermatophagy (-)	2	1.2	16.7	10	6.1	83.3	12	7.4	4.6719	0.03066
Total	**76**	46.6		**87**	53.4		**163**			

the majority of the xylomycetophages (93.3%) and of the spermatophages (83.3%) are inbreeding. The mating habits of the herbiphages are equally balanced (Table 2).

Biological features influencing risks of introduction and impact

Mating strategy

Amongst the 163 species in our study, 87 (53.4%) are inbreeding (Table 3). This proportion of inbreeding species is significantly larger than that (27.8%) of the non-SIH inbreeders in the world (1544 species - Kirkendall et al. 2015) amongst the known species belonging to tribes with SIH species (5569 species - Hulcr et al. 2015; Johnson et al. 2020a): $Chi^2_{(1;\ N=5569)} = 47.42$; $p < 0.00001$. The Xyleborini and Trypophloeini, over-represented in Table 1, are all inbreeding and the under-represented Corthylini and Hexacolini are all outbreeding. However, the over-represented Crypturgini and Hylastini are all outbreeding (Table 3).

Table 3. Mating strategies of the Scolytinae tribes with an invasion history.

Tribes	Outbreeding (% of tribe)	Inbreeding (% of tribe)	Total
Xyleborini	0	56	56
Trypophloeini	0	18	18
Cryphalini	5	0	5
Dryocoetini	4 (28.6%)	10 (71.4%)	14
Ipini	12 (85.7%)	2 (14.3%)	14
Crypturgini	8	0	8
Scolytini	8	0	8
Hypoborini	7	0	7
Hylastini	6	0	6
Hylurgini	5 (83.3%)	1 (16.7%)	6
Corthylini	5	0	5
Phloeosinini	4	0	4
Polygraphini	3	0	3
Hylesinini	2	0	2
Phloeotribini	2	0	2
Bothrosternini	1	0	1
Hexacolini	1	0	1
Scolytoplatypodini	1	0	1
Xyloterini	1	0	1
Ernoporini	1	0	1
Total	**76 (46.6%)**	**87 (53.4%)**	**163**

Overall, the inbreeding (*stricto sensu*) SIH colonised a much larger set of landmasses than the outbreeding species (Fig. 1). Strikingly, with the exception of *Hypocryphalus mangiferae* (Stebbing) (17 landmasses), all the species colonising the larger numbers of landmasses are inbreeding.

Host nature and condition

The capacity to colonise living hosts appears to favour establishment. In our dataset, species with a recorded impact on their hosts colonised the larger numbers of landmasses (Fig. 2).

Host specificity

Amongst the 36 species in Suppl. material 1 attacking only conifers, 33 species attack only one family and two species attack two families. The Scolytinae attacking only non-conifers or attacking both non-conifers and conifers have a much wider and diverse range of host trees. Conifer specialists colonise fewer landmasses (median: 5) than non-conifer specialists (median: 6) and species attacking both types of hosts (median: 9) (see Fig. 3).

The genus *Hypothenemus*, representing 11% of the 163 species in the list, includes the most polyphagous species in the list with *H. eruditus*, reported from 65 plant families and *H. crudiae* and *H. seriatus*, each reported from 57 plant families. These species are reported from 37, 21 and 22 landmasses, respectively.

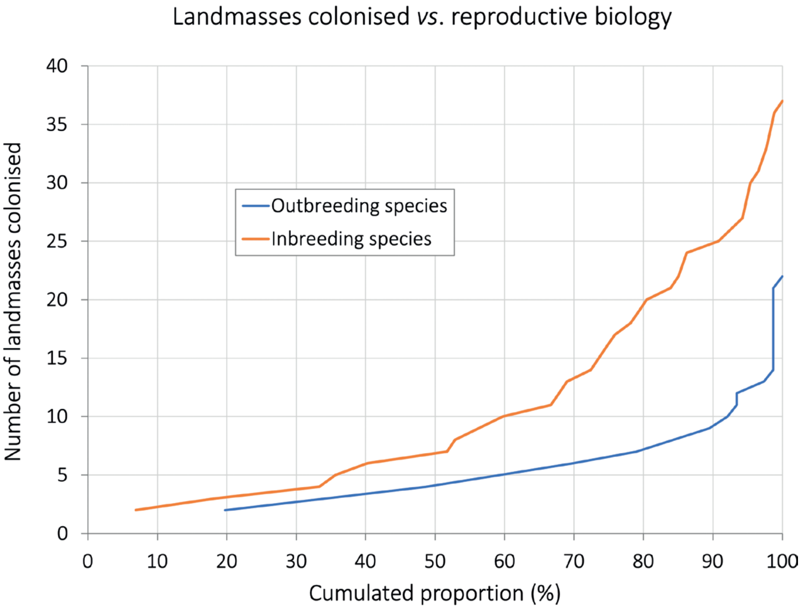

Figure 1. Cumulative proportion of landmasses colonised by either outbreeding or inbreeding species amongst the Scolytinae with an invasion history.

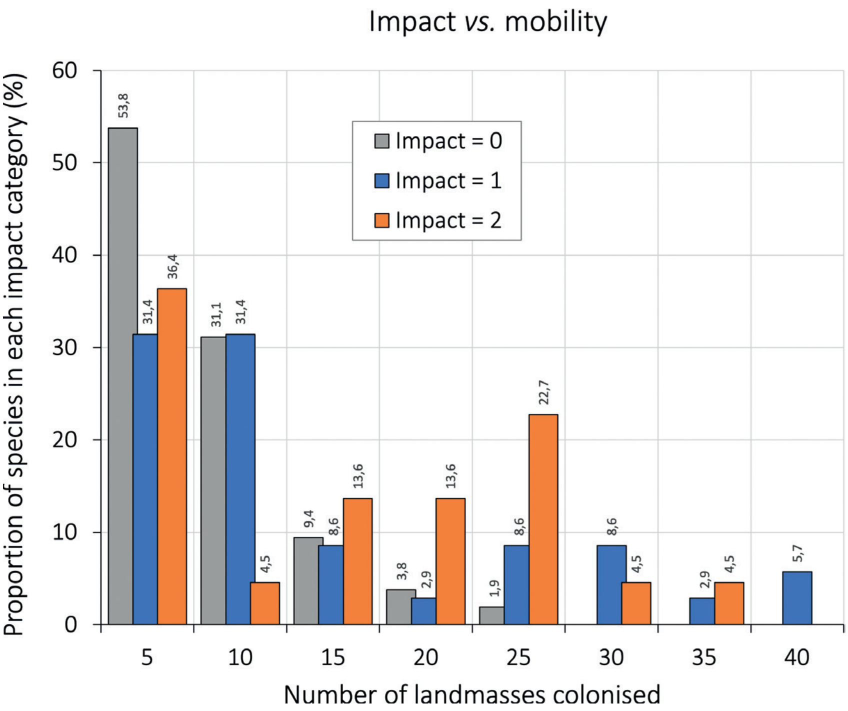

Figure 2. Impact versus mobility amongst scolytines with an invasion history.

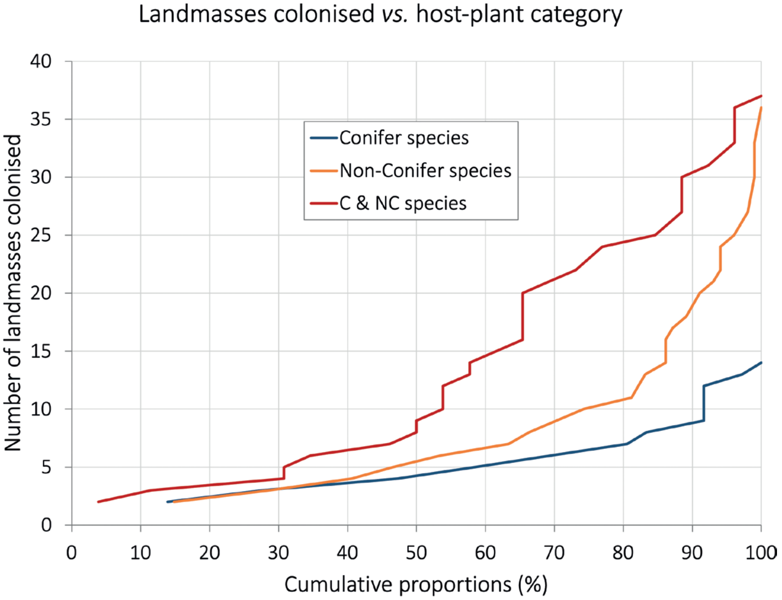

Figure 3. Host-plant category (conifer vs. non-conifer) influences the number of landmasses colonised by Scolytinae with an invasion history.

Aggregation pheromones and long-range primary attractants

Aggregation pheromones

Pheromone-mediated mass attacks are known amongst the SIH species, i.e. for *Orthotomicus erosus*, *Gnathotrichus materiarius* (Fitch), *Ips calligraphus* (Germar), *I. cembrae* (Herr), *I. grandicollis*, *Pityogenes bidentatus* (Herbst), *P. calcaratus* (Eichhoff), *P. chalcographus* (L.), *Pityokteines curvidens* (Germar), *Pityophthorus juglandis*, *Polygraphus poligraphus* (L.), *P. proximus* Blanford, *P. rufipennis* (Kirby), *Scolytus amygdali* Guerin-Meneville, *S. multistriatus* (Marsham), *T. domesticum* and many others.

Long-range primary attractants

94 SIH species out of 163 are known to respond to primary attractants and an additional 47 are likely to use these chemical clues as well.

Twenty species are not known to respond to primary attractants and do not produce pheromones either: five *Aphanarthrum* spp.; *Dendroctonus micans*; *Dryoxylon onoharaense*; *Kissophagus hederae*; six *Liparthrum* spp.; *Microborus boops*; two *Microperus* spp; *Pagiocerus frontalis*; *Scolytoplatypus tycon*; *Thamnurgus characiae*.

Multivariate analyses

Impact

The factorial discriminant analysis showed significant effects of functional traits on impact (Wilks' lambda test, P < 0.0001). The separation between the three impact levels was mainly explained by the FDA canonical function F1 (percentage variance explained 81.8%, P < 0.0001; while F2 explained 18.2%, P = 0.09). F1 was mainly driven by the degree of polyphagy (P = 0.001), use of aggregation pheromones (P = 0.002), host specialisation (P = 0.004) and, to a lesser extent, use of primary attractants (P = 0.089). The confusion matrix (Table 4) showed 100% correct classification for the category of non-damaging beetles (no impact; 107 species). The beetle species with no impact were characterised by a low degree of polyphagy, lack of aggregation pheromone, host specialisation on broadleaves or conifers and non-use of primary attractants. Only 11.4% of scolytine

Table 4. Confusion matrix for the factorial discriminant analysis (FDA) of the three categories of impact by the 163 beetle species studied.

a priori \ a posteriori	No impact	Low impact	Substantial impact	Total	% correct
No impact	107	0	0	107	100%
Moderate impact	29	4	2	35	11.4%
Substantial impact	15	4	2	21	9.5%
Total	151	8	4	163	69.3%

species with moderate impact and 9.5% with substantial impact were correctly classified, the other species of these categories being mainly misclassified as non-damaging. However, it should be noted that four *Euwallacea* species combined traits of polyphagy and lack of host specialisation, using aggregation pheromone and primary attractant: *E. piceus*, *E. interjectus*, *E. similis* and *E. validus* and they all had a significant impact.

The complete list of well-classified and misclassified species is available as supplementary material (Suppl. material 3).

Mobility

The Ancova analysis showed a significant effect of the degree of polyphagy ($P < 0.0001$) and use of primary attractant ($P = 0.023$) on the number of landmasses colonised, but the interaction of these two factors was not significant ($P = 0.58$), with an overall determination coefficient of $R^2 = 0.41$. Beetle species not using primary attractants ($n = 22$) colonised significantly fewer land masses (3.5 ± 0.4, mean ± standard error) than those ($n = 141$) attracted by the host plant (9.6 ± 0.7). The number of colonised landmasses increased with the degree of polyphagy (number of known host plant species) by the same magnitude for the two categories of beetle species (using or not primary attractants, Fig. 4).

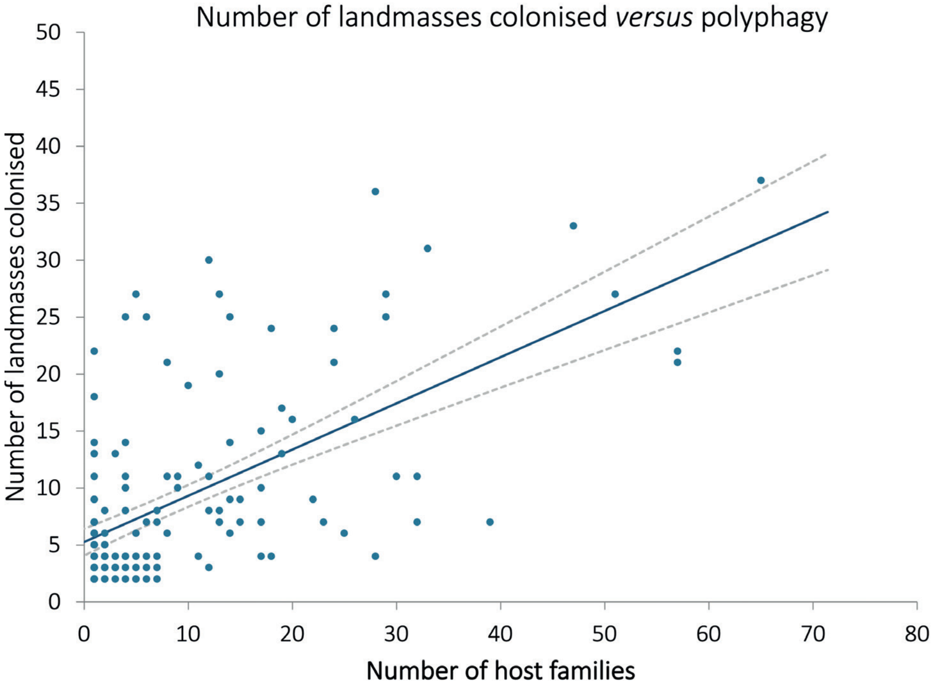

Figure 4. Number of colonised land masses versus degree of polyphagy (number of host-plant families) for the 163 scolytine species studied (independently of their use of primary attractants). Dashed lines represent the confidence interval of the linear regression line.

Discussion and conclusion

Disharmony with regards to invasion frequency amongst SIH tribes

Disharmony with regards to invasion frequency appears common amongst non-native insect orders worldwide and has been ascribed to the preference of certain orders for the main commercial pathways (Liebhold et al. 2016). Disharmony has been more finely recorded within Coleopteran (Liebhold et al. 2021) and Lepidopteran (Mally et al. 2022) non-native families.

Mating strategy

The SIH include a higher proportion of inbreeders than the world Scolytinae fauna. This is an asset for prompt establishment in strange lands. The females leaving the tree are already fertilised and can create a new colony on their own. In theory, the Allee population threshold (the minimal number of individuals below which a population cannot grow) for such species could be one single female.

Inbreeders are also often haplodiploid. Unfertilised females parthenogenetically produce haploid males and then mate with their sons (Jordal et al. 2000 and references therein). This further facilitates colonisation as females do not even have to be fertilised before dispersal and finding a host. For example, all the Xyleborini and most of the *Coccotrypes* spp. are haplodiploid (EPPO 2020; Grousset et al. 2020; Jordal et al. 2000).

Amongst the supposedly outbreeding species that crossed a geographic barrier, *Orthotomicus erosus* (Wollaston) (Mendel 1983) and *Tomicus piniperda* (Linnaeus) (Janin et al. 1988) show a proportion of females already mated upon emergence, possibly with a sibling or mated during maturation feeding on twigs or during overwintering at the base of trees previous to colonising a new host. Similarly, *Hylurgus ligniperda* (Fabricius) (Fabre and Carle 1975) and *Ips grandicollis* (Eichhoff) (Witanachchi 1980) have been observed to mate prior to emergence. As in the inbreeding species *stricto sensu*, these early mated females may be able to start a new colony alone. Wilkinson (1964) showed that *I. grandicollis* females induced alone to oviposit on pine logs produced a progeny. However, species with no invasive history are also capable of early mating. Lissemore (1997) found that three out of eight pre-emergent, overwintering *Ips pini* (Say) females collected in the spring in the litter around attacked trees were fertilised and able to start a new gallery alone. The North American species *Ips pini* has never expanded outside of its range, where it is widely distributed (Atkinson 2021). Similarly, Bleiker et al. (2013), examining 1510 emergent female *Dendroctonus ponderosae* Hopkins from two different locations in Alberta, found 3–5% of pre-emergent matings.

Host nature and condition

Many different relationships to the hosts are observed amongst bark- and ambrosia beetles, making it difficult to predict the risks associated with new insect-host associations or even the long-term risks associated with long-standing associations. Bark- and ambrosia beetle species attack a wide range of trees, from apparently healthy individuals to dead and even decaying ones (Raffa et al. 2015; Hulcr et al. 2017). Other SIH species colonise a wide range of plant parts and, therefore, commodities in trade, including seeds, fine twigs and roots (Kirkendall et al. 2015 and see section 1.2). The nature and condition of the host allow a certain level of prediction regarding the entry, establishment and impact of a particular species or, after an event has occurred, provide clues for retrospective scenarios.

Entry

Xylophagous and xylomycetophagous species living in the sapwood are protected from mechanical damage and, when the wood has not been dried, from desiccation. Many phloeophagous bark beetles (e.g. *H. ligniperda*) and xylomycetophagous ambrosia beetles (e.g. *Xylosandrus germanus*) (Blandford) have travelled in wood packaging material or in wood or wood product shipments. The coffee berry borer, *Hypothenemus hampei* (Ferrari) is transported in the coffee seed trade (Johnson et al. 2020b). Plants for planting provide another pathways for species living in the stems of living hosts, such as *Xylosandrus compactus* (Eichhoff). *Coccotrypes dactyliperda* Fabricius, which live in dates, is likely to have spread around the world in commercial shipments. *C. rhizophorae* (Hopkins), which specifically lives in the propagules of the red mangrove, *Rhizophora mangle* Linnaeus, might have moved from Asia where it originates to North America in host propagules floating long distance across the ocean (Atkinson and Peck 1994).

Establishment and impact

Species capable of attacking living trees are more likely to find suitable hosts in the locations of entry. Hulcr et al. (2017) proposed to search for ambrosia beetle-fungus associations colonising live trees in their native habitats to identify future exotic tree-killing pests. Living trees, however, can vary in vigour and resistance to pests. Often, apparently healthy trees have been previously exposed to various forms of stress factors, including flooding, drought, wind break, snow break, freezing, ozone exposure, graft incompatibility, site and stand conditions, nutrients supply disorders, diseases or animal pest damage (Ranger et al. 2010; Ploetz et al. 2013; Hulcr and Stelinski 2017;) and this generally makes them more vulnerable to beetle attacks. Thirty-five SIH species may kill stressed hosts; twenty-one species out of 163 are able to kill apparently healthy, living trees (Suppl. material 1).

Importantly, the impact in a new area cannot always be predicted from the relationship of a beetle-fungus association with its native host trees. *X. glabratus* and its symbiont *R. lauricola* colonise stressed or injured Lauraceae all over the world. Whilst they exert little noticeable damage in their native areas, they massively kill *P. borbonia* in the USA because of the hypersensitive response of the New World Lauraceae and the changes in behaviour they induce in the beetles (Hulcr et al. 2017; Martini et al. 2017). *Anisandrus dispar* (Fabricius), which attacks weakened or dead trees in Europe is an important pest of young chestnut trees stressed by excess water or late frost in north-western USA and western Canada (Kühnholz et al. 2001). Similarly, *D. valens*, which usually settles on the stumps of freshly cut pines or more rarely establishes in low numbers on stressed pines in North America, killed millions of *Pinus tabuliformis* since its introduction into China during the late 1990s (Yan et al. 2005). The causes of this increased aggression in China are unclear, but have been related to exceptionally dry years following introduction (the outbreak subsided after the drought) and, possibly to some degree, to the association with a new, naïve host, with more aggressive strains of symbiotic fungi (Sun et al. 2013). Sometimes, even in their native range, species usually restricted to dead or dying hosts start attacking apparently healthy trees. *Trypodendron domesticum* (Linnaeus) and *T. signatum* (Fabricius) started infesting thousands of standing, live beech *Fagus sylvatica* L. in Belgium in the early 2000s, in connection with exceptional early frosts (La Spina et al. 2013). In Canada, *T. retusum* (LeConte) which is usually restricted to wind-broken or weakened trees was observed to attack apparently healthy aspen, *Populus tremuloides* Michaux (Kühnholz et al. 2001).

Scolytinae are not only a threat to forestry. For example, *H. hampei* is a major pest of coffee worldwide (Johnson et al. 2020b) and *C. dactyliperda* causes major damage on date production (Rodriguez et al. 2014).

Host specificity

Polyphagy and the ability to attack new hosts in new locations are advantageous for entry, establishment (higher probability of finding a suitable host) and impact (EPPO 2020).

Polyphagy

Bark beetles usually have a narrow host range and are often monophagous (all hosts belong to the same genus) or oligophagous (all hosts selected within one family). Ambrosia beetles often have a broader range of hosts, as their host is mainly a substrate for the fungi they grow and feed on (Beaver 1979; Jordal et al. 2000; Seybold et al. 2016). Many species specialise in either conifers or non-conifers, although some exceptionally polyphagous species attacks both.

There is no direct relationship between polyphagy and impact. Some less polyphagous ambrosia beetles have a substantial impact in newly-invaded territories, as illustrated by *X. glabratus* (4 host-plant families) after its introduction in the USA. On the contrary, very polyphagous species may cause limited damage in new areas, as well as in their native range. *Hypothenemus eruditus* (65 host families), which usually colonises dead hosts, is normally considered harmless (Kambestad et al. 2017).

New hosts

Many scolytines, even some not known as polyphagous, have been recorded on new host species when introduced into new areas (EPPO 2020; Grousset et al. 2020). Encounters with new hosts do not always result in damage, but are an important component of the potential impact. There are striking example of encounters with new very susceptible hosts, leading to extensive damage, such as *X. glabratus* on *Persea borbonia* in the USA (EPPO 2020) or *D. valens* on *P. tabuliformis* in China (Yan et al. 2005).

Aggregation pheromones and long-range primary attractants

Aggregation pheromones

The need for mass-attacks can be unfavourable to establishment, but mass attacks, once the species is established and the epidemic threshold is reached, can result in higher impact (EPPO 2020). Some bark- or ambrosia beetles use aggregation pheromones to mass-attack standing hosts and overcome their defences (D.L. Wood 1982). The mass-colonisation of undefended, fallen trees is more likely the result of collective foraging, also mediated by aggregation pheromones (Toffin et al. 2018). As large numbers of individuals are required for a mass-attacking species to colonise a new tree, the Allee threshold is necessarily high, making establishment in a new area more difficult. On the contrary, solitary colonisers (e.g. *Hypothenemus* spp.; *Xylosandrus* spp.) have displayed high success in establishment (see section 2.1).

Long-range primary attractants

Physiologically stressed trees emit a range of volatile compounds, such as ethanol, which attract many bark- and ambrosia beetles colonising weakened hosts (Byers 1992; Miller and Rabaglia 2009; Ranger et al. 2010; Rassati et al. 2019b). Monoterpenes emitted by conifers also serve as clues for conifer-inhabiting species (Byers 1992), but reduce the response of species attacking non-conifers to ethanol or other primary attractants (Ranger et al. 2011). *H. hampei* is attracted to ripe coffee berries by conophthorin and chalcogran, but deterred by conifer monoterpenes (Jaramillo et al. 2013). Beetle response to primary attractants can be extremely accurate. In South Africa, Tribe (1992) showed that adults of the European species *Hylastes angustatus* (Herbst) and *Hylurgus ligniperda* were capable of finding *Pinus radiata* logs buried horizontally under 40 cm of soil. This accuracy is perhaps one component of the invasive success of these two species. However, working with native secondary species in Canada, Saint-Germain et al. (2007) showed that primary attractants allow bark beetles to locate a patch inhabited by susceptible hosts, but that, at closer range, host selection is governed by different processes, including random landing.

As they are not very specific (e.g. ethanol is produced by tissue fermentation of both conifers and non-conifers and monoterpenes, such as alpha-pinene, are produced by most conifers), long-range primary attractants can particularly facilitate host location and, thus, establishment amongst polyphagous species.

Conclusions

Throughout this review, several biological traits, particularly inbreeding and polyphagy, appear correlated with higher introduction potential and impact in new areas. However, as with the results obtained in EPPO (2020) and Grousset et al. (2020) for a narrower range of species, none of these traits, alone or combined, explains the success of all the SIH species and there are obvious outliers. For example, the over-represented tribes Crypturgini and Hylastini (Table 1) are outbreeders. The moderately polyphagous *X. glabratus* (4 host families) has a much higher impact than *H. eruditus* (65 host families). More generally, 59 SIH species attack hosts in only one plant family, suggesting many exceptions to the influence of polyphagy on introduction. Whilst aggregation pheromones do not appear to favour establishment, there is the exception of *E. fornicatus*.

To summarise, some of the identified drivers are widespread amongst SIH species, but none is shared by the whole group, making it difficult to characterise univocally the potentially successful invaders amongst the bark- and ambrosia beetles of the world. In addition, the non-biological risk factors, as identified in EPPO (2020) and Lantschner et al. (2020), also play an important role. As concluded in EPPO (2020), the main factors that are driving successful establishment and impact vary from species to species and are not always fully identified. Still, one single feature common to most of the SIH species has been implicitly identified in this study on species crossing geographical barriers: their capacity to travel by trade, either on wood commodities and wood packaging material or on plants for planting or on fruits, depending on the species. The major conclusion of the present study is, thus, that, because of the lack of drivers that could allow for robust predictions regarding the invasive potential of any scolytine species, it is safer to consider the establishment of horizontal measures for trade of commodities.

Acknowledgements

HJ and AB participated in this study as part of the HOMED project (http://homed-project.eu/), which received funding from the European Union's Horizon 2020 Research and Innovation Programme, under grant agreement no. 771271. We are grateful to Sandy Liebhold and Miloš Knížek for useful comments on an earlier draft.

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Supplementary material 1

Main characteristics of the 163 Scolytinae with an invasion history

Authors: Jean-Claude Grégoire, Hervé Jactel, Jiri Hulcr, Andrea Battisti, Daegan Inward, Françoise Petter, Fabienne Grousset

Data type: table (docx file)

Explanation note: Main characteristics of the 163 Scolytinae with an invasion history.

Link: https://doi.org/10.3897/neobiota.84.89826.suppl1

Supplementary material 2

Landmasses

Authors: Jean-Claude Grégoire, Hervé Jactel, Jiri Hulcr, Andrea Battisti, Daegan Inward, Françoise Petter, Fabienne Grousset

Data type: table (docx file)

Explanation note: Landmasses (islands and continents).

Link: https://doi.org/10.3897/neobiota.84.89826.suppl2

Supplementary material 3

Well-classified and misclassified species

Authors: Jean-Claude Grégoire, Hervé Jactel, Jiri Hulcr, Andrea Battisti, Daegan Inward, Françoise Petter, Fabienne Grousset

Data type: table (docx file)

Explanation note: Well-classified and misclassified species identified by the factorial discriminant analysis (FDA) of the three categories of impact by the 163 beetle species studied.

Link: https://doi.org/10.3897/neobiota.84.89826.suppl3

NeoBiota 84: 107–135 (2023)
doi: 10.3897/neobiota.84.90829
https://neobiota.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
NeoBiota
Advancing research on alien species and biological invasions

Jewels on the go: exotic buprestids around the world (Coleoptera, Buprestidae)

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Academic editor: Marc Kenis | Received 29 July 2022 | Accepted 10 March 2023 | Published 18 May 2023

Citation: Ruzzier E, Haack RA, Curletti G, Roques A, Volkovitsh MG, Battisti A (2023) Jewels on the go: exotic buprestids around the world (Coleoptera, Buprestidae). In: Jactel H, Orazio C, Robinet C, Douma JC, Santini A, Battisti A, Branco M, Seehausen L, Kenis M (Eds) Conceptual and technical innovations to better manage invasions of alien pests and pathogens in forests. NeoBiota 84: 107–135. https://doi.org/10.3897/neobiota.84.90829

Abstract

Buprestidae (Coleoptera: Buprestoidea) is one of the three wood-borer beetle groups of major phytosanitary interest worldwide, together with Cerambycidae and Scolytinae (Curculionidae). As in other beetle families, some buprestid species have been unintentionally or intentionally introduced around the world, in some cases causing significant environmental and economic damage in the invaded territories. Despite the phytosanitary relevance of the Buprestidae, information regarding the identity of exotic buprestids, their biogeographic areas of origin, introduction pathways, and larval host plants, have remained scattered in the literature. Our objective was to summarize much of the existing knowledge on these topics in the present paper. Our analysis resulted in a list of 115 exotic buprestids worldwide, representing introductions both within and between biogeographic realms and corresponding to less than 1% of the known buprestid species worldwide. Invasiveness does not seem to be linked to their larval host plant preferences, as introduced species utilize 158 plant genera in 70 plant families and are equally represented in all feeding guilds (monophagous, oligophagous, and polyphagous). As trade of plants or plant parts can serve as a pathway for future introductions, the information reported in this review can help in pest risk assessment.

Keywords

Biodiversity, exotic species, invasive alien species, jewel beetles

Introduction

Buprestidae Leach, 1815 (Coleoptera: Buprestoidea), commonly known as jewel beetles, include more than 15,000 described species distributed in all continents except Antarctica (Bellamy 2008). The family includes six subfamilies, namely Agrilinae, Buprestinae, Chrysochroinae, Galbellinae, Julodinae, and Polycestinae, (Bellamy 2003).

All Buprestidae are phytophagous and generally oligophagous (i.e., associated with a single plant family) as both adults and larvae (Curletti 1994). Buprestid larvae develop in both living and dead plant tissues; most species are internal feeders, boring or mining in roots, stems, branches, and leaves of both woody plants and herbaceous plants (Bellamy and Volkovitsh 2005), while only Julodinae possess soil-dwelling larvae that feed externally on roots (Kolibáč 2000).

Many buprestids, especially the wood-boring species, select dead, dying, or stressed plants for oviposition (Chamorro et al. 2015); however, some species are capable of infesting or even prefer healthy living hosts (Carlson and Knight 1969). This last group can have an important economic impact on human activities because it includes pests in orchards and tree plantations (Bonsignore et al. 2008; Hashim et al. 2018; Dawadi et al. 2019). Furthermore, buprestids can have substantial negative impacts on the natural ecosystems during outbreaks (Coleman et al. 2012; Muilenburg and Herms 2012; Sallé et al. 2014; Vuts et al. 2016; Haack and Petrice 2019).

The cryptic nature of most buprestid larvae, being hidden in woody tissues and, for some species, their slow larval development due to feeding in nutrient-poor xylem (Haack and Slansky 1987), has allowed multiple species to be transported in wood products and introduced to areas far from their place of origin. Much of this dispersal has been human-mediated and related to trade (Wu et al. 2017). One of the earliest accounts deals with the introduction of *Chalcophora detrita detrita* (Klug, 1829) from the Middle East to Southern Italy by the Etruscans or the Maritime Republics (from 1000 to 2000 years ago; Biagioni et al. 2015). However, since the end of the nineteenth century the introduction rate of exotic buprestids worldwide has substantially increased in similar fashion to many other invasive forest insects (Aukema et al. 2010; Chamorro et al. 2015; Hoebeke et al. 2017; Bozorov et al. 2018; Jendek et al. 2018; Roques et al. 2020; Volkovitsh et al. 2020).

Buprestidae have taken advantage of globalization with the opening of new trade routes and the increase in the number and speed of movement of goods and people (Pyšek and Richardson 2010). In some cases, species such as *Agrilus planipennis* Fairmaire, 1888 (hosts: *Chionanthus* and *Fraxinus* [main host]), *A. mali* Matsumura, 1924 (hosts: *Cydonia*, *Emmenopterys*, *Malus* [main], *Prunus*, *Pyrus*, *Sorbus*), and *Aphanisticus cochinchinae seminulum* Obenberger, 1929 (hosts: *Saccharum*, *Tripsacum*) have become invasive, causing significant damage in urban and natural forests and agriculture, and often requiring significant investments for monitoring and control (Hespenheide 2007; Bauer et al. 2008; Jones et al. 2013; Volkovitsh et al. 2020). Consequently, Buprestidae is one of the Coleoptera families of major silvicultural interest worldwide (Maynard et al. 2004; Inghilesi et al. 2013; Haack et al. 2014; MacQuarrie et al. 2020).

Given this condition, great efforts have been made in the last few decades to identify the main entry pathways, and to develop and implement early detection programs, effective monitoring strategies, and new tools for species identification (Meurisse et al. 2019; Poland and Rassati 2019). To date, however, little has been summarized about the main patterns of buprestid introductions worldwide, their taxonomic affinities, and their biogeographic origins.

The purpose of this article is to provide a comprehensive review of natural and human-assisted translocation of buprestid species among and within various biogeographic realms, describe the contribution of each realm and buprestid subfamily to this exchange of species, and provide the first comprehensive list of all introduced Buprestidae worldwide from the mid-1800s to present. Furthermore, a list of host plant associations at the genus and family level is provided, with an indication of the host range of each buprestid species. Our general aim is to provide information that can be used in pest risk assessment and invasion ecology.

Methods

In order to compile and then review the literature on exotic Buprestidae, we performed reiterated research in Google Scholar through the use of keywords such as "Buprestidae," "introduced," "exotic," and "alien" and then integrated with the Boolean operators AND, OR, NOT and the use of " " for specific word combinations. We also obtained a considerable amount of literature that was not available in Google Scholar thanks to the support of many colleagues and buprestid specialists. Screening of the literature collected was done following the PRISMA approach and only the papers retained are cited in the Suppl. material 1 and were used for the analysis (Moher et al. 2009). The resulting reference library included papers in Chinese, English, French, German, and Italian.

In the analysis, we considered only those publications where buprestids were identified to species or subspecies level, and for those records published between 1850 and December 2020. In the taxonomic discussion, we did not consider the rank of subgenus. In particular, the non-native status of a given species was evaluated for its consistency throughout the reviewed literature; in case there was only a single reference publication and in the absence of any further information, the non-native status of a species was considered as valid. For each species included in the present research, we considered the most recent and comprehensive publication highlighting and explaining the non-native status as a key reference. For those buprestid species for which the literature was limited, we referred to the original faunistic record published. A full list of the Buprestidae species, associated with the reference literature, is provided in Suppl. material 1.

Where the origin of a given taxon could not be assigned to a single biogeographic region, every possible area of origin was considered. The world's biogeographic areas considered in this paper generally follow the interpretation and categorization provided by Löbl and Löbl (2016).

At times it was difficult to know if an insect was firmly established in a new area or was simply intercepted at a port of entry, because papers varied in terminology and detail. In our dataset, when considering the species status, we have generally adopted the following categorization: A) Neonative: species native to a continent but introduced into regions other than the native ones either through natural spread indirectly favored by human activities (climate change, habitat change) or through accidental human-mediated introductions; B) Established: non-native species that sustain self-replacing populations over several life cycles (inclusive of single specimens collected in the wild away from potential entry points); C) Invasive: a non-native species established in natural or semi-natural ecosystems or habitat, which has impact and threatens native biological diversity; D) Intercepted: insects detected during inspection procedures or similar situations where no reproducing population is known to occur; E) Intentionally introduced: species that have been actively introduced in areas other than their native range with a specific purpose, such as biological control of invasive plants; F) Unclear: all species for which the status is unclear (e.g., apparently extinct adventive populations, species described in areas where that specific genus does not occur, species record vague without any specific detail, mislabeling and misidentification).

Data collected were organized in an Excel spreadsheet including the following information, organized by columns: subfamily, tribe, genus, species (full name plus author), biogeographic region of origin, biogeographic region of detection, status, and host plants. Detection region and host plant were associated with a specific column called references, which included all relevant information used to recover the data. Each species could have multiple entries (rows) in cases of multiple introduction events in different biogeographic areas, or in situations where the origin of the species was not reducible to a single biogeographic region. In the case of single introductions of widely distributed species in which it was clear the biogeographic region of origin of the insects, we considered only the record for that specific region. The taxonomy of plant genera and families used in the paper is based on the information available on the "Plants of The World Online" database (https://powo.science.kew.org/). Analyses and graphics were realized using the R software (version 4.1.2).

Host plant preference was defined in the categories: monophagous (for buprestids feeding only on plant species of the same genus), oligophagous (buprestids feeding on different plant genera within the same host family), polyphagous (buprestids feeding on plant species from different host families).

Results

Faunistic part

Our literature review identified 162 events of buprestid introductions among and within biogeographic regions that involved 115 distinct taxa (Suppl. material 1). The taxa included 44 species in the subfamily Agrilinae (tribes Agrilini, Aphanisticini, Coraebini, and Tracheini) (Table 1), 51 species of Buprestinae (tribes Anthaxiini, Buprestini, Chrysobothrini, Curidini, Melanophilini, and Nascionini) (Table 2), 16 species of Chrysochroinae (Chalchophorini, Chrysochroini, Dicercini, Sphenopterini, Paraleptodemini, and Poecilonotini) (Table 3), and 6 species of Polycestinae (tribes Acmaeoderini, Polycestini, Prospherini and Ptosimini) (Table 4). No species of the subfamilies Galbellinae and Julodinae were recorded as introduced. The revision of all published records revealed that the buprestid species involved in introductions either within or between biogeographical realms constitute only 0.76% of all known buprestid species worldwide.

The analysis showed that the introduction of exotic Buprestidae included all biogeographic realms (with the obvious exclusion of the Antarctic realm), including introductions both among and within the realms (Fig. 1). In addition, our analysis revealed that the Nearctic and Palearctic bioregions contributed the greatest number of introduced species (90 in total) and also the most distinct introduction events (72.4% combined). The realm that was the source for the highest number of buprestids introduced elsewhere was the Palearctic, with 52 out of approximately 2,500 native species (2.1%), followed by the Nearctic (38 out of ~800; 4.8%), the Indomalayan (13 out of ~2,800; 0.5%), the Neotropical (13 out of ~3,700; 0.4%), the Australasian (10 out of ~1,600; 0.6%), the Afrotropical (7 out of ~3,800; 0.2%), and the Oceanian (2 out of ~70; 2.9%). The analyses between the number of buprestid species per biogeographic realm and the number of species introduced elsewhere from each realm did not show any significant statistical relation (t = -0.10389, df = 5, p-value = 0.9213).

Palearctic and Nearctic were the two regions with the highest number of introduced species (Fig. 1) but, despite somewhat similar environments, climate, and flora, there were substantial differences in the patterns of inter- and intra-biogeographic realm introductions (Table 5). In the case of intra-realm introductions, Palearctic and Nearctic showed a similar number of species (23 vs 20) despite the fact that the genera contributing to more than 50% of total introductions were, at least in part, different: *Agrilus* (9 species) and *Buprestis* (4 species) in the Palearctic, and *Chrysobothris* (9) and *Agrilus* (6) in the Nearctic.

By contrast, when considering introductions between the two realms, it was possible to observe a strong imbalance with 9 exotic species recorded in the Palearctic compared with 25 in the Nearctic. Furthermore, Agrilinae represented the majority of the exotic buprestids in the Nearctic, while Buprestinae were dominant in the Palearctic.

With respect to all buprestid species considered introduced worldwide, we found 41 cases where the species were considered established, 43 cases as interceptions at entry points, 32 cases where the status was unclear, and 22 cases of neonative species. We also classified 13 introductions where the species became invasive, and 6 cases where species were intentionally introduced.

For the 41 cases of establishment, Buprestinae was the most represented subfamily, with 23 records subdivided among the genera *Anthaxia* (1 species), *Buprestis* (8 species), *Belionota* (1 species), *Chrysobothris* (6 species), and *Trachypteris* (1 species). Agrilinae accounted for 14 establishments, represented by 10 species of *Agrilus*, 1 *Diphucrania*, and 2 *Trachys*. The subfamilies Chrysochroinae and Polycestinae were involved in only a limited number of establishments, i.e., 1 *Steraspis*, 1 *Prospheres* and 2 *Acmaeodera*.

Table 1. Subfamily Agrilinae: species list, biogeographic realms concerned, status, and larval host plants. * species confused with *Agrilus coxalis* Waterhouse, 1889 in the literature.

Species	Biogeographic realm		Status	Plant host genera
	origin	introduction		
Agrilus angustulus (Illiger, 1803)	Palearctic	Palearctic	Unclear	*Corylus*, *Ostrya* (Betulaceae); *Fagus*, *Castanea*, *Quercus* (Fagaceae)
Agrilus anxius Gory, 1841	Nearctic	Nearctic	Neonative	*Betula* (Betulaceae)
Agrilus auriventris Saunders, 1873	Australasian, Indomalayan	Oceanian	Invasive	*Citrus* (Rutaceae)
Agrilus auroguttatus Schaeffer, 1905*	Nearctic	Nearctic	Invasive	*Quercus* (Fagaceae)
Agrilus bilineatus (Weber, 1801)	Nearctic	Palearctic	Established	*Castanea*, *Quercus* (Fagaceae)
Agrilus biguttatus (Fabricius, 1776)	Palearctic	Australasian	Intercepted	*Fagus*, *Castanea*, *Quercus* (Fagaceae); *Tilia* (Malvaceae); *Populus* (Salicaceae); *Ulmus* (Ulmaceae)
Agrilus cavatus Chevrolat, 1838	Nearctic	Neotropical	Unclear	*Rhus* (Anacardiaceae); *Acaciella* (Fabaceae)
Agrilus convexicollis Redtenbacher, 1849	Palearctic	Palearctic	Neonative	*Euonymus* (Celastraceae); *Philadelphus* (Hydrangeaceae); *Fraxinus*, *Ligustrum*, *Olea*, *Syringa* (Oleaceae)
Agrilus cuprescens (Ménétriés, 1832)	Palearctic	Nearctic	Established	*Rosa*, *Rubus* (Rosaceae)
Agrilus cyanenoniger Saunders, 1873	Palearctic	Palearctic	Neonative	*Croton* (Euphorbiaceae); *Quercus* (Fagaceae)
Agrilus cyanescens (Ratzeburg, 1837)	Palearctic	Palearctic, Nearctic	Unclear, Established	*Lonicera*, *Symphoricarpos* (Caprifoliaceae); *Rhamnus* (Rhamnaceae)
Agrilus derasofasciatus Lacordaire, 1835	Palearctic	Nearctic	Non-native	*Vitis* (Vitaceae)
Agrilus difficilis Gory, 1841	Nearctic	Nearctic	Established	*Gleditsia* (Fabaceae); *Zanthoxylum* (Rutaceae)
Agrilus extraneus Fisher, 1933	Oceanian	Oceanian	Established	*Argemone* (Papaveraceae)
Agrilus fleischeri Obenberger, 1925	Palearctic	Nearctic	Intercepted	*Populus*, *Salix* (Salicaceae)
Agrilus furcillatus Chevrolat, 1835	Nearctic, Neotropical	Nearctic	Intercepted	*Pinus* (Pinaceae); *Zea* (Poaceae); Coffea (Rubiaceae); *Salix* (Salicaceae)
Agrilus graminis Kiesenwetter, 1857	Palearctic	Palearctic	Neonative	*Alnus*, *Corylus*, *Ostrya* (Betulaceae); *Euonymus* (Celesteraceae); *Castanea*, *Quercus* (Fagaceae); *Acer* (Sapindaceae); *Viburnum* (Viburnaceae)
Agrilus hyperici (Creutzer, 1799)	Palearctic	Australasian, Nearctic	Intentionally introduced	*Hypericum* (Hypericaceae)
Agrilus kaluganus Obenberger, 1940	Palearctic	Palearctic	Neonative	*Corylus* (Betulaceae)
Agrilus livens Kerremans, 1892	Indomalayan	Palearctic	Unclear	*Citrus* (Rutaceae)
Agrilus mali Matsumura, 1924	Palearctic	Palearctic	Neonative	*Cydonia*, *Malus*, *Prunus*, *Pyrus*, *Sorbus* (Rosaceae); *Emmenopterys* (Rubiaceae)
Agrilus nicolanus Obenberger, 1924	Palearctic	Palearctic	Neonative	*Quercus* (Fagaceae); *Ulmus* (Ulmaceae)
Agrilus occipitalis (Eschscholtz, 1822)	Australasian, Indomalayan, Palearctic	Oceanian	Invasive	*Citrus* (Rutaceae)
Agrilus pilosovittatus Saunders, 1873	Palearctic	Nearctic	Established	*Wisteria* (Fabaceae)
Agrilus planipennis Fairmaire, 1888	Palearctic	Nearctic, Palearctic	Invasive, Neonative	*Chionanthus*, *Fraxinus* (Oleaceae)
Agrilus prionurus Chevrolat, 1838	Nearctic	Nearctic	Neonative	*Chionanthus* (Oleaceae); *Sapindus* (Sapindaceae)
Agrilus pulchellus Bland, 1865	Nearctic	Nearctic	Intercepted	*Chrysothamnus* sp., *Erigeron* (Asteraceae); *Amsinkia* (Boraginaceae); *Celtis* (Cannabaceae); *Quercus* (Fagaceae); *Sphaeralcea* (Malvaceae); *Allionia*, *Boerhavia* (Nyctaginaceae)
Agrilus ribesi Schaefer, 1946	Palearctic	Nearctic	Invasive	*Ribes* (Grossulariaceae)
Agrilus sinuatus (Olivier, 1790)	Palearctic	Nearctic	Established	*Crataegus*, *Malus*, *Prunus*, *Pyrus*, *Sorbus* (Rosaceae)
Agrilus smaragdifrons Ganglbauer, 1890	Palearctic	Nearctic	Established	*Ailanthus* (Simaroubaceae)
Agrilus sulcicollis Lacordaire, 1835	Palearctic	Nearctic	Established	*Fagus*, *Castanea*, *Quercus* (Fagaceae)
Agrilus subrobustus Saunders, 1873	Indomalayan, Palearctic	Nearctic	Established	*Albizia* (Fabaceae)
Aphanisticus antennatus Saunders, 1873	Palearctic	Indomalayan, Neotropical	Unclear	Not available

Species	Biogeographic realm		Status	Plant host genera
	origin	introduction		
Aphanisticus cochinchinae seminulum Obenberger, 1929	Indomalayan	Nearctic, Neotropical, Oceanian	Invasive	*Saccharum*, *Tripsacum* (Poaceae)
Coraebus andrewesi Obenberger, 1922	Indomalayan, Palearctic	Neotropical	Unclear	Not available
Coraebus rubi (Linnaeus, 1767)	Palearctic	Palearctic	Neonative	*Rosa*, *Rubus* (Rosaceae)
Coraebus undatus (Fabricius, 1787)	Palearctic	Palearctic	Intercepted	*Diospyros* (Ebenaceae); *Castanea*, *Fagus*, *Quercus* (Fagaceae)
Diphucrania viridipurpurea Carter, 1924	Australasian	Palearctic	Established	Not available
Hylaeogena jureceki Obenberger, 1941	Neotropical	Afrotropical, Australasian	Intentionally introduced	*Dolichandra* (Bignoniaceae)
Leiopleura carbonata (LeConte, 1860)	Neotropical	Neotropical	Unclear	Not available
Leiopleura otero (Fisher, 1935)	Neotropical	Neotropical	Unclear	Not available
Lius poseidon Napp, 1972	Neotropical	Oceanian	Intentionally introduced	*Miconia*, *Chaetogastra* (Melastomataceae)
Trachys minutus (Linnaeus, 1758)	Palearctic	Nearctic	Established	*Corylus* (Betulaceae); *Sorbus* (Rosaceae); *Salix* (Salicaceae), *Ulmus* (Ulmaceae)
Trachys troglodytiformis Obenberger, 1918	Palearctic	Nearctic	Established	*Althea*, *Hibiscus*, *Malva* (Malvaceae)

Table 2. Subfamily Buprestinae: species list, biogeographic realms concerned, status, and larval host plants.

Species	Biogeographic realm		Status	Plant host genera
	origin	introduction		
Anthaxia godeti Gory & Laporte, 1839	Palearctic	Palearctic	Neonative	*Picea*, *Pinus* (Pinaceae)
Anthaxia laticeps Abeille de Perrin, 1900	Palearctic	Palearctic	Neonative	*Pinus* (Pinaceae)
Anthaxia proteus Saunders, 1873	Palearctic	Palearctic	Unclear	*Pinus* (Pinaceae)
Anthaxia salicis (Fabricius, 1776)	Palearctic	Nearctic	Established	*Castanea*, *Quercus* (Fagaceae); *Salix* (Salicaceae); *Acer* (Sapindaceae)
Cobosina willineri (Cobos, 1972)	Neotropical	Neotropical	Neonative	Not available
Buprestis apricans Herbst, 1801	Nearctic	Neotropical	Established	*Pinus* (Pinaceae)
Buprestis aurulenta Linnaeus, 1767	Nearctic	Australasian, Neotropical, Palearctic, Oceanian	Intercepted, Established, Unclear, Established	*Thuja*, *Juniperus* (Cupressaceae); *Abies*, *Picea*, *Pinus*, *Pseudotsuga* (Pinaceae)
Buprestis dalmatina Mannerheim, 1837	Palearctic	Nearctic, Palearctic	Intercepted Neonative	*Pinus* (Pinaceae)
Buprestis decora Fabricius, 1775	Nearctic	Neotropical, Palearctic	Established	*Pinus* (Pinaceae)
Buprestis haemorrhoidalis Herbst, 1780	Palearctic	Afrotropical, Australasian, Nearctic, Neotropical, Palearctic	Unclear, Intercepted, Established, Unclear, Unclear	*Callitris* (Cupressaceae); *Abies*, *Picea*, *Pinus* (Pinaceae)
Bruprestis humeralis Klug, 1829	Palearctic	Palearctic	Neonative	*Pinus* (Pinaceae)
Buprestis lineata Fabricius, 1781	Nearctic	Australasian, Nearctic, Neotropical, Palearctic	Intercepted, Neonative, Established, Unclear	*Pinus* (Pinaceae)
Buprestis maculativentris Say, 1825	Nearctic	Australasian	Intercepted	*Abies*, *Picea*, *Pinus* (Pinaceae)
Buprestis maculipennis Gory, 1841	Nearctic	Neotropical	Established	*Taxodium* (Cupressaceae); *Pinus*, *Tsuga* (Pinaceae)
Buprestis novemmaculata Linnaeus, 1767	Palearctic	Afrotropical, Indomalayan, Nearctic, Neotropical, Palearctic	Unclear, Unclear, Intercepted, Established, Established	*Larix*, *Picea*, *Pinus* (Pinaceae)
Buprestis salisburyensis Herbst, 1801	Nearctic	Nearctic	Established	*Pinus* (Pinaceae)

Species	Biogeographic realm		Status	Plant host genera
	origin	introduction		
Trachykele blondeli Marseul, 1865	Nearctic	Australasian, Palearctic	Intercepted, Non-native	*Calocedrus*, *Chamaecyparis*, *Cupressus*, *Juniperus*, *Thuja* (Cupressaceae)
Belionota prasina (Thunberg, 1789)	Australasian, Indomalayan	Afrotropical, Australasian, Nearctic, Neotropical Palearctic	Established, Intercepted, Established, Established, Intercepted	*Anacardium*, *Mangifera* (Anacardiaceae); *Delonix*, *Pithecellobium* (Fabaceae); *Casuarina* (Casuarinaceae); *Hopea* (Dipterocarpaceae); *Ceiba* (Malvaceae)
Merimna atrata (Gory & Laporte, 1837)	Australasian	Oceanian	Intercepted	*Eucalyptus* (Myrtaceae)
Chrysobothris adelpha Gemminger & Harold, 1869	Nearctic	Oceanian	Intercepted	*Prospis* (Fabaceae); *Carya* (Juglandaceae); *Amelanchier* (Rosaceae)
Chrysobothris acutipennis Chevrolat, 1835	Nearctic, Neotropical	Neotropical	Established	*Ebenopsis*, *Leucaena* (Fabaceae)
Chrysobothris affinis (Fabricius, 1794)	Palearctic	Australasian	Intercepted	*Pistacia* (Anacardiaceae); *Alnus*, *Betula*, *Carpinus*, *Corylus*, *Ostrya* (Betulaceae); *Cornus* (Cornaceae); *Arbutus* (Ericaceae); *Cercis*, *Gleditsia*, *Robinia* (Fabaceae); *Castanea*, *Fagus*, *Quercus* (Fagaceae); *Punica* (Lythraceae); *Juglans* (Juglandaceae); *Tilia* (Malvaceae); *Ficus*, *Morus* (Moraceae); *Eucalyptus* (Myrtaceae); *Fraxinus* (Oleaceae); *Cedrus* (Pinaceae); *Platanus* (Platanaceae); *Crataegus*, *Malus*, *Prunus*, *Pyrus*, Rosa, *Sorbus* (Rosaceae); *Populus*, *Salix* (Salicaceae); *Acer* (Sapindaceae); *Ulmus* (Ulmaceae)
Chrysobothris analis LeConte, 1860	Nearctic	Nearctic	Established	*Rhus* (Anacardiaceae); *Celtis* (Cannabaceae); *Diospyros* (Ebenaceae); *Cercis*, *Ebenopsis*, *Haematoxylum*, *Leucaena*, *Mimosa*, *Parkinsonia*, *Prosopis* (Fabaceae); *Carya*, *Juglans* (Juglandaceae); *Coccoloba* (Polygonaceae); *Prunus* (Rosaceae); *Citrus* (Rutaceae); *Sapindus* (Sapindaceae); *Ulmus* (Ulmaceae)
Chrysobothris cavifrons Deyrolle, 1864	Australasian	Palearctic	Intercepted	Not available
Chrysobothris cerceripraeda Westcott & Thomas, 2015	Nearctic	Nearctic	Unclear	Not available
Chrysobothris chrysonota Deyrolle, 1864	Australasian	Palearctic	Intercepted	Not available
Chrysobothris costata Kerremans, 1895	Oceanian	Oceanian	Invasive	*Intsia* (Fabaceae); *Citrus* (Rutaceae)
Chrysobothris costifrons Waterhouse, 1887	Nearctic	Nearctic	Neonative	*Quercus* (Fagaceae)
Chrysobothris dorsata (Fabricius, 1787)	Afrotropical, Palearctic	Palearctic	Unclear	*Acacia*, *Ceratonia* (Fabaceae)
Chrysobothris ellyptica Deyrolle, 1864	Australasian	Palearctic	Intercepted	Not available
Chrysobothris femorata (Olivier, 1790)	Nearctic	Australasian, Oceanian, Palearctic	Intercepted	*Liquidambar* (Altingiaceae); *Carpinus* (Betulaceae); *Celtis* (Cannabaceae); *Diospyros* (Ebenaceae); *Cercis* (Fabaceae); *Castanea*, *Quercus* (Fagaceae); *Carya*, *Juglans* (Juglandaceae); *Tilia* (Malvaceae); *Fraxinus* (Oleaceae); *Platanus* (Platanaceae); *Amelanchier*, *Crategus*, *Cydonia*, *Malus*, *Prunus*, *Sorbus* (Rosaceae); *Populus*, *Salix* (Salicaceae); *Acer* (Sapindaceae); *Ulmus* (Ulmaceae)
Chrysobothris igniventris Reitter, 1895	Palearctic	Nearctic	Intercepted	*Larix*, *Pinus* (Pinaceae)
Chrysobothris indica Castelnau & Gory, 1837	Indomalayan	Oceanian	Established	*Terminalia* (Combrentaceae); *Shorea* (Dipterocarpaceae); *Acacia* (Fabaceae); *Myristica* (Myristicaceae); *Mimusops* (Sapotaceae)
Chrysobothris knulli Nelson, 1975	Nearctic	Nearctic	Established	*Acacia* (Fabaceae)

Species	Biogeographic realm		Status	Plant host genera
	origin	introduction		
Chrysobothris mali Horn, 1886	Nearctic	Nearctic	Intercepted	*Alnus*, *Betula*, *Corylus* (Betulaceae); *Arbutus*, *Arctostaphylos* (Ericaceae); *Pickeringia*, *Prosopis*, *Wisteria* (Fabaceae); *Fagus*, *Quercus* (Fagaceae); *Ribes* (Grossulariaceae); *Juglans* (Juglandaceae); *Persea* (Lauraceae); *Liriodendron* (Magnioliaceae); *Ficus* (Moraceae); *Eucalyptus* (Myrtaceae); *Platanus* (Platanaceae); *Ceanothus*, *Rhamnus* (Rhamnaceae); *Adenostoma*, *Cercocarpus*, *Cotoneaster*, *Crataegus*, *Cydonia*, *Malus*, *Oemleria*, *Photinia*, *Prunus*, *Pyracantha*, *Pyrus*, *Rhaphiolepis*, *Rosa*, *Rubus*, *Sorbus* (Rosaceae); *Populus*, *Salix* (Salicaceae); *Acers*, *Aesculus* (Sapindaceae); *Ulmus* (Ulmaceae)
Chrysobothris octocola LeConte, 1858	Nearctic	Oceanian	Established	*Acacia*, *Parkinsonia*, *Prosopis* (Fabaceae); *Prunus* (Rosaceae); *Salix* (Salicaceae)
Chrysobothris pupureoplagiata Scheaffer, 1904	Nearctic	Nearctic	Intercepted	*Canotia* sp. (Celasteraceae), *Psorothamnus* (Fabaceae)
Chrysobothris quadriimpressa Gory & Laporte, 1837	Nearctic	Nearctic	Neonative	*Liquidambar* (Altaginaceae); *Quercus* (Fagaceae); *Juglans* (Juglandaceae); *Sapindus* (Sapindaceae)
Chrysobothris rotundicollis Gory & Laporte, 1837	Nearctic	Neotropical	Unclear	*Ebenopsis* (Fabaceae); *Larix*, *Pinus* (Pinaceae)
Chrysobothris rugosiceps Melsheimer, 1845	Nearctic	Nearctic	Neonative	*Castanea*, *Quercus* (Fagaceae)
Chrysobothris sexpunctata, Fabricius 1801	Neotropical	Neotropical	Established	Not available
Chrysobothris superba Deyrolle, 1864	Australasian	Palearctic	Intercepted	Not available
Chrysobothris tranquebarica (Gmelin, 1790)	Neotropical	Nearctic	Unclear	*Casuarina* (Casuarinaceae); *Conocarpus* (Combrentaceae); *Cassia* (Fabaceae); *Pinus* (Pinaceae); *Rhizophora* (Rhizophoraceae)
Chrysobothris trinervia (Kirby, 1837)	Nearctic	Nearctic	Intercepted	*Larix*, *Picea*, *Pinus*, *Pseudotsuga* (Pinaceae)
Anilara hoscheki Obenberger, 1916	Australasian	Palearctic	Intercepted	Not available
Melanophila consupta LeConte, 1857	Nearctic	Oceanian	Non-native	*Calocedrus* (Cupressaceae); *Eucalyptus* (Myrtaceae); *Pinus Pseudotsuga* (Pinaceae)
Phaenops cyanea (Fabricius, 1775)	Palearctic	Nearctic	Intercepted	*Abies*, *Larix*, *Pinus* (Pinaceae)
Phaenops drummondi (Kirby, 1837)	Nearctic	Nearctic, Palearctic	Intercepted	*Abies*, *Cedrus*, *Larix*, *Picea*, *Pseudotsuga* (Pinaceae)
Trachypteris picta decostigma (Fabricius, 1787)	Palearctic	Neotropical	Established	*Populus*, *Salix* (Salicaceae)
Nascio vetusta (Boisduval, 1835)	Australasian	Australasian	Intercepted	*Eucalyptus*, *Metrosideros* (Myrtaceae); *Xanthorrhoea* (Asphodelaceae)

With respect to the 43 cases where the buprestids were apparently only intercepted, the Buprestinae had the highest number of interceptions worldwide (28), which included 24 species. The most commonly intercepted genus was *Chrysobothris* (14 species), followed by *Buprestis* (6 species). There were 6 cases of intercepted Agrilinae, involving 4 species of *Agrilus* and 1 *Coraebus*. For both Chrysochroinae and Polycestinae there were multiple single species interceptions. For 28 species among Agrilinae, Buprestinae, Chrysochroinae and Polycestinae it was not possible to assign their status to any of the existing categories; therefore, they were classified as "unclear." We recognize that many more species of Buprestidae have been intercepted at ports throughout the world, but in almost all cases these datasets are not available to the public and therefore could not be considered in our paper.

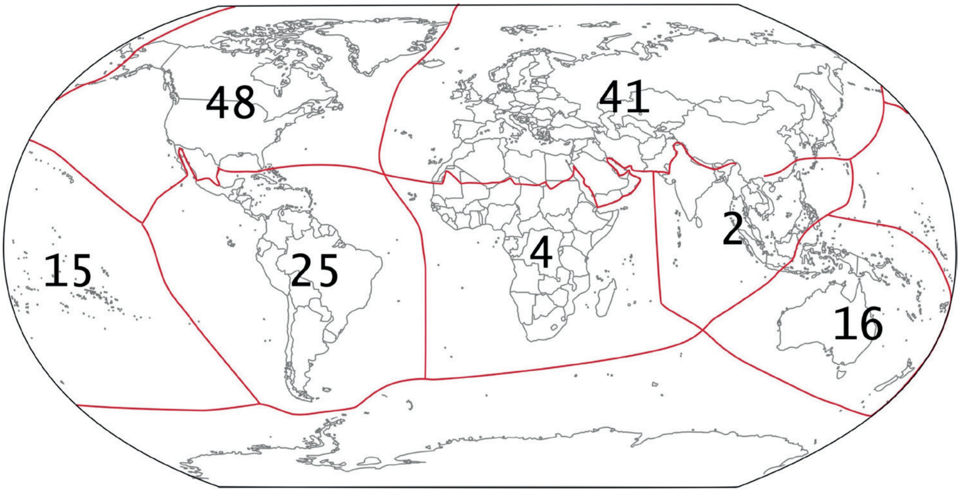
Table 3. Subfamily Chrysochroinae: species list, biogeographic realms concerned, status, and larval host plants.

Species	Biogeographic realm		Status	Plant host genera
	origin	introduction		
Chalcophora angulicollis (LeConte, 1857)	Nearctic	Nearctic, Palearctic	Unclear	*Abies*, *Pinus*, *Pseudotsuga* (Pinaceae)
Chalcophora japonica (Gory, 1840)	Palearctic	Nearctic	Intercepted	*Pinus* (Pinaceae)
Chalcophora virginiensis (Drury, 1770)	Nearctic	Neotropical, Palearctic	Unclear	*Taxodium* (Cupressaceae); *Pinus* (Pinaceae)
Cyphogastra foveicollis (Boisduval, 1835)	Australasian	Palearctic	Intercepted	Not available
Dicerca moesta (Fabricius, 1794)	Palearctic	Nearctic, Palearctic	Intercepted, Unclear	*Abies*, *Pinus*, *Picea* (Pinaceae)
Dicerca furcata (Thunberg, 1787)	Palearctic	Australasian	Intercepted	*Betula* (Betulaceae)
Dicerca tuberculata (Laporte & Gory, 1837)	Nearctic	Neotropical	Non-native	*Tsuga* (Pinaceae)
Euchroma gigantea (Linnaeus, 1758)	Neotropical	Neotropical	Unclear	*Ceiba*, *Pachira*, *Pseudobombax* (Malvaceae)
Lampetis bahamica Fisher, 1925	Neotropical	Neotropical	Intercepted	Not available
Lampetis corruscans (Carter, 1924)	Australasian	Australasian	Unclear	Not available
Lampetis fastuosa (Fabricius, 1775)	Australasian	Australasian	Unclear	*Areca* (Arecaceae); *Acacia* (Fabaceae); *Eucalyptus* (Myrtaceae); *Tectona* (Lamiaceae)
Lamprodila festiva (Linnaeus, 1767)	Palearctic	Palearctic	Neonative	*Callitris*, *Chamaecyparis*, *Cupressus*, *Juniperus*, *Platycladus*, *Tetraclinis*, *Thuja* (Cupressaceae); *Ziziphus* (Rhamnaceae); *Tamarix* (Tamaricaceae)
Lamprodila vivata (Lewis, 1893)	Palearctic	Nearctic	Intercepted	*Cryptomeria*, *Chamaecyparis*, *Juniperus* (Cupressaceae)
Sphenoptera jugoslavica Obenberger, 1926	Palearctic	Nearctic	Intentionally introduced	*Centaurea* (Asteraceae)
Steraspis squamosa (Klug, 1829)	Afrotropical, Palearctic	Palearctic	Established, Neonative	*Tamarix* (Tamaricaceae)

Table 4. Subfamily Polycestinae: species list, biogeographic realms concerned, status, and larval host plants.

Species	Biogeographic realm		Status	Plant host genera
	origin	introduction		
Acmaeodera bipunctata (Olivier, 1790)	Palearctic	Palearctic	Neonative	*Euphorbia* (Euphorbiaceae); *Juniperus*, *Thuja* (Cupressaceae); *Ficus* (Moraceae); *Abies*, *Cedrus*, *Larix*, *Pinus* (Pinaceae)
Acmaeodera flavomarginata (Gray, 1832)	Nearctic, Neotropical	Neotropical	Established	*Acacia*, *Prosopis* (Fabaceae); *Diospyros* (Ebenaceae)
Acmaeodera neoneglecta Fisher, 1949	Nearctic	Nearctic	Intercepted	*Acacia*, *Ebenopsis*, *Leucaena*, *Prosopis*, *Sophora* (Fabaceae); *Carya* (Juglandaceae); *Ulmus* (Ulmaceae)
Prospheres aurantiopictus (Laporte & Gory, 1837)	Australasian	Australasian	Established	*Araucaria* (Araucariaceae); *Pinus* (Pinaceae)
Ptosima undecimmaculata (Herbst, 1784)	Palearctic	Nearctic	Intercepted	*Mangifera* (Anacardiaceae); *Ceratonia* (Fabaceae), *Crataegus*, *Malus*, *Prunus*, *Pyrus* (Rosaceae); *Citrus* (Rutaceae); *Vitis* (Vitaceae)

Among all the taxa investigated, 22 species were considered as neonatives. There were 10 Agrilinae (9 *Agrilus* and 1 *Coraebus*); 9 Buprestinae (2 *Anthaxia*, 1 *Cobosina*, 3 *Buprestis*, and 3 *Chrysobothris*); 2 Chrysochroinae (1 *Steraspis* and 1 *Lamprodila*), and 1 Polycestinae (1 *Acmaeodera*). Neonative species were recorded almost exclusively in the Northern Hemisphere, with 15 species in the Palearctic and 6 in the



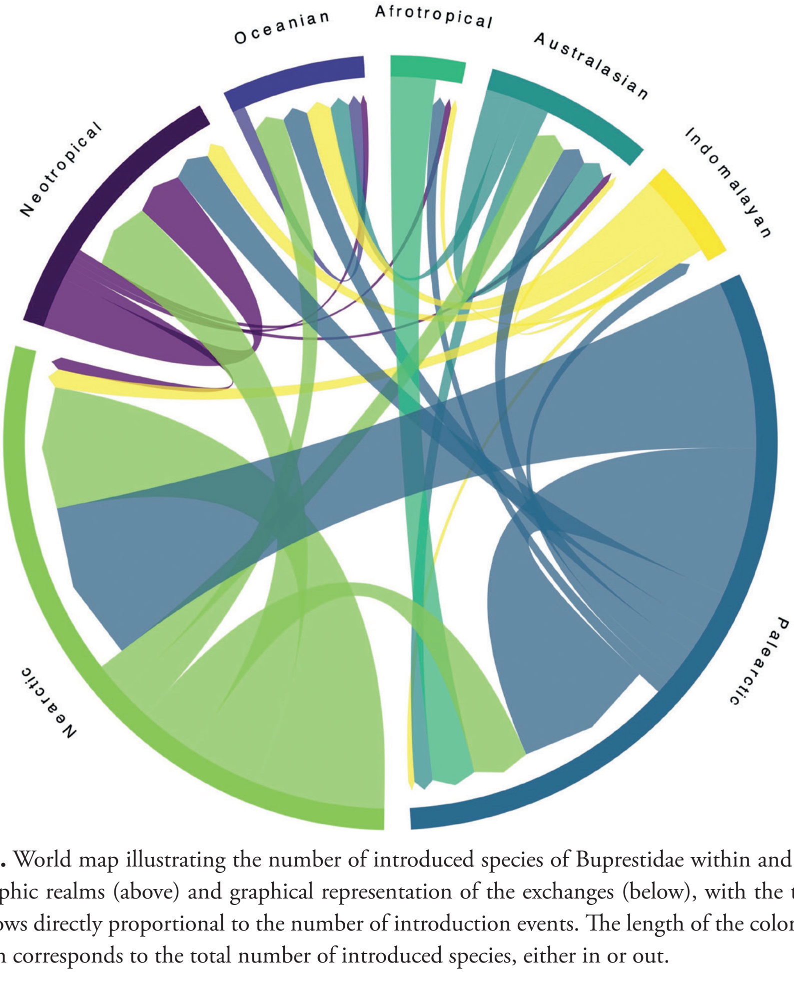


Figure 1. World map illustrating the number of introduced species of Buprestidae within and between biogeographic realms (above) and graphical representation of the exchanges (below), with the thickness of the arrows directly proportional to the number of introduction events. The length of the colored arc of each realm corresponds to the total number of introduced species, either in or out.

Table 5. Comparison between buprestid introductions within and between the Nearctic and Palearctic realms, with details on the number of species within each genus.

within Palearctic	within Nearctic	Palearctic to Nearctic	Nearctic to Palearctic
9 *Agrilus*	9 *Chrysobothris*	12 *Agrilus* (one species intentionally introduced)	3 *Buprestis*
4 *Buprestis*	6 *Agrilus*	3 *Buprestis*	2 *Chalcophora*
3 *Anthaxia*	2 *Buprestis*	2 *Trachys*	1 *Agrilus*
2 *Coraebus*	1 *Acmaeodera*	1 *Anthaxia*	1 *Chrysobothris*
1 *Acmaeodera*	1 *Chalcophora*	1 *Chalcophora*	1 *Phaenops*
1 *Chrysobothris*	1 *Phaenops*	1 *Chrysobothris*	1 *Trachykele*
1 *Dicerca*		1 *Dicerca*	
1 *Steraspis*		1 *Lamprodila*	
1 *Lamprodila*		1 *Phaenops*	
		1 *Ptosima*	
		1 *Sphenoptera* (intentionally introduced)	

Nearctic realm. *Agrilus* was the most represented genus in the Palearctic with 7 species, while *Chrysobothris* was the most represented genus in the Nearctic with 3 species. A single species of *Cobosina* was the only example of a neonative taxon in the Neotropic realm.

All 13 cases of invasive buprestids are species of Agrilinae and Buprestinae. These species became invasive once introduced to the Nearctic, Oceanian and Neotropical realms. There were 6 species of invasive Agrilinae (5 *Agrilus* and 1 *Aphanistichus*), and only two invasive Buprestinae in the genera *Belionota* and *Chrysobothris*.

Six cases of intentionally introduced taxa were found, representing 4 species in the genera *Agrilus* (Agrilini), *Sphenoptera* (Sphenopterini), *Hylaeogena* and *Lius* (Tracheini). These species were introduced into the Nearctic, Afrotropical, and Australasian realms.

Larval host plants

The analysis of larval host plants for all Buprestidae introduced worldwide identified 158 different plant genera within 70 families (3 Gymnosperms and 67 Angiosperms), with only a few introduced buprestids without host information (Tables 1–4). The exotic Buprestidae included sets of species with wide variation in the range of their larval hosts, varying from highly polyphagous on non-phylogenetically related plant families to monophagous on a single plant genus. Larval host specificity (i.e., monophagous, oligophagous and polyphagous) of introduced Buprestidae is equally distributed among the subfamilies (Kruskal-Wallis chi-squared = 1.2007, df = 2, *p*-value = 0.5486) (Table 6).

The larval host families most represented were Pinaceae (60 host records), Rosaceae (52), Fabaceae (49), Fagaceae (36), and Cupressaceae (24), which together accounted for 52% of all host records (Table 7). Considering introductions within and between biogeographic realms, it emerged that the most common genera of host plants varied greatly among world biogeographic realms, both in abundance and diversity (Table 8).

Table 6. Number of introduced species with different levels of larval host-use specialization by buprestid subfamilies.

	Monophagous	Oligophagous	Polyphagous	Unknown
Agrilinae	13	9	17	5
Buprestinae	11	11	20	8
Chrysochroinae	5	4	3	3
Polycestinae	0	0	6	0
Total	29	24	46	16

Table 7. Summary table of the main plant families and genera exploited as larval host plants by introduced Buprestidae by subfamily. Numbers between parenthesis refers to the number of records, not distinct species.

Plant Families	Plant Genera	Buprestid subfamilies	Buprestid genera
Pinaceae (60)	*Pinus* (27), *Abies* (8), *Picea* (8), *Larix* (7)	Buprestinae (45), Chrysochroinae (9), Polycestinae (4), Agrilinae (1)	*Buprestis* (21), *Chrysobothris* (10), *Phaenops* (8), *Chalcophora* (5)
Rosaceae (52)	*Prunus* (9), *Malus* (7), *Sorbus* (7), *Pyrus* (5)	Buprestinae (30), Agrilinae (15), Polycestinae (7)	*Chrysobothris* (30), *Agrilus* (10), *Ptosima* (4), *Acmaeoderella* (3)
Fabaceae (49)	*Acacia* (9), *Prosopis* (6), *Ebenopsis* (4), *Leucaena* (3)	Buprestinae (31), Polycestinae (14),	*Chrysobothris* (29), *Acmaeodera* (7), *Acmaeoderella* (6), *Agrilus* (3)
Fagaceae (35)	*Quercus* (18), *Castanea* (11), *Fagus* (6)	Agrilinae (20), Buprestinae (13), Polycestinae (2)	*Agrilus* (17), *Chrysobothris* (11), *Coraebus* (3)
Cupressaceae (23)	*Juniperus* (5), *Thuja* (4)	Chrysochroinae (11), Buprestinae (10), Polycestinae (2)	*Lamprodila* (10), *Trachykele* (5), *Buprestis* (2), *Acmaeodera* (2)
Betulaceae (18)	*Corylus* (6), *Betula* (4), Alnus (3)	Buprestinae (9), Agrilinae (8), Chrysochroinae (1)	*Chrysobothris* (9), *Agrilus* (7)
Salicaceae (16)	*Salix* (9), *Populus* (7)	Buprestinae (10), Agrilinae (5), Polycestinae (1)	*Chrysobothris* (7), *Agrilus* (4), *Trachypteris* (2)

Discussion

The low introduction rate, 0.76% compared for example to the 2.17% out of ~ 6000 taxa of Curculionidae Scolytinae (Lantschner et al. 2020), indicates a general low propensity for Buprestidae to be introduced by humans, either directly or indirectly. In support of this contention is the high number of single buprestid introductions (i.e., one species introduced only once and only in a single biogeographic realm), with respect to the total number of introduction events. In addition, the invasiveness does not seem to be linked to larval host plant preferences, as introduced species are included in all feeding guilds (monophagous, oligophagous, and polyphagous).

The genera *Agrilus* (Agrilinae: Agrilini), *Buprestis* (Buprestinae: Buprestini), and *Chrysobothris* (Buprestinae: Chrysobothrini) would seem to be more predisposed to introduction events than other genera, possibly owing to both their morphological and biological traits. *Agrilus* are generally small in size and univoltine (Solomon 1995; Chamorro et al. 2015). They infest mostly live plants and signs of their presence are difficult to detect prior to adult emergence and host dieback. Therefore, several *Agrilus* species have likely been moved over time through trade of live plants, such as ornamentals or nursery stock, as well as through domestic and international movements

Table 8. Summary table of the most common plant genera exploited as larval host plants by buprestid species introduced either within or between biogeographic realms.

Origin – Introduction realm	Most common larval host plant genera exploited by those species with a narrow host range
Afrotropical – Palearctic	Angiosperms: *Acacia*, *Ceratonia*, *Tamarix*
Australasian – Australasian	Angiosperms: *Eucalyptus*
Australasian – Oceanian	Angiosperms: *Citrus*
Australasian – Palearctic	Angiosperms: *Anacardium*, *Casuarina*, *Ceiba*, *Delonix*, *Hopea*, *Mangifera*, *Pithecellobium*
Indomalayan – Afrotropical	Angiosperms: *Anacardium*, *Casuarina*, *Ceiba*, *Delonix*, *Hopea*, *Mangifera*, *Pithecellobium*
Indomalayan – Australasian	Angiosperms: *Anacardium*, *Casuarina*, *Ceiba*, *Delonix*, *Hopea*, *Mangifera*, *Pithecellobium*
Indomalayan – Palearctic	Angiosperms: *Citrus*
Indomalayan – Nearctic	Angiosperms: *Albizia*, *Anacardium*, *Casuarina*, *Ceiba*, *Delonix*, *Hopea*, *Mangifera*, *Pithecellobium*, *Saccharum*, *Tripsacum*
Indomalayan – Neotropical	Angiosperms: *Anacardium*, *Casuarina*, *Ceiba*, *Delonix*, *Hopea*, *Mangifera*, *Pithecellobium*, *Saccharum*, *Tripsacum*
Indomalayan – Oceanian	Angiosperms: *Citrus*
Nearctic – Australasian	Gymnosperms: *Pinus*
Nearctic – Nearctic	Angiosperms: *Acacia*, *Juglans*, *Prosopis*, *Sapindus*, *Ulmus* Gymnosperms: *Pinus*, *Pseudotsuga*
Nearctic – Oceanian	Angiosperms: *Amelanchier*, *Carya*, *Prosopis*, *Prunus*, *Salix* Gymnosperms: *Pinus*, *Pseudotsuga*
Nearctic – Palearctic	Gymnosperms: *Abies*, *Pinus*, *Pseudotsuga*
Nearctic – Neotropical	Gymnosperms: *Pinus*
Neotropical – Afrotropical	Angiosperms: *Dolichandra*
Neotropical – Australasian	Angiosperms: *Dolichandra*
Neotropical – Nearctic	Gymnosperms: *Pinus*
Neotropical – Neotropical	Angiosperms: *Acacia*, *Ceiba*, *Diospyros*, *Ebenopsis*, *Leucaena*, *Pachira*, *Prosopis*, *Pseudobombax*
Neotropical – Oceanian	Angiosperms: *Miconia*, *Tibouchina*
Palearctic – Afrotropical	Gymnosperms: *Picea*, *Pinus*
Palearctic – Australasian	Angiosperms: *Castanea*, *Fagus*, *Populus*, *Quercus*, *Tilia Ulmus*
Palearctic – Indomalayan	Gymnosperms: *Larix*, *Picea*, *Pinus*
Palearctic – Nearctic	Angiosperms: *Salix* Gymnosperms: *Abies*, *Larix*, *Picea*, *Pinus*
Palearctic – Neotropical	Gymnosperms: *Picea*, *Pinus*
Palearctic – Oceanian	Angiosperms: *Citrus*
Palearctic – Palearctic	Angiosperms: *Castanea*, *Quercus* Gymnosperms: *Abies*, *Picea*, *Pinus*
Oceanian – Oceanian	Angiosperms: *Argemone*, *Citrus*, *Intsia*

of recently cut logs and manufactured wood products, especially when not debarked. The example of the emerald ash borer, *A. planipennis*, is remarkable in the number of pathways (e.g., logs, firewood, nursery stock) by which it has moved in North America (Herms and McCullough 2014; Haack et al. 2015).

By contrast to *Agrilus*, most *Buprestis* and *Chrysobothris* species have longer larval developmental periods; they can infest both living, stressed, and dead plants; and they typically tunnel in host xylem, including both sapwood and heartwood (Solomon 1995; Evans et al. 2004). As a consequence of this multi-year developmental period deep inside wood, infestations are generally difficult to detect until adult emergence. Although most species oviposit in bark cracks or under the bark, a few species can oviposit directly on exposed wood (xylem). Moreover, once larvae have entered the xylem, the presence of bark is no longer required. Therefore, introductions of these species can result from movement of logs and milled wood products either with or without bark.

Given the relatively low number of exotic buprestids investigated and the heterogeneity of the sources consulted, it has not been possible to delineate an exact temporal trend for worldwide buprestid introductions, although it seems evident that most species were likely introduced before the 1970s, with very few ever intercepted during port surveys. This condition likely reflects the lack of strict phytosanitary regulations in the early 1900s (Eschen et al. 2015). In addition, international trade among European countries and their overseas colonies likely facilitated the movement of some species early on, as well as later during the two world wars. Examples come from *Buprestis aurulenta* Linnaeus, 1767 and *Buprestis novemmaculata* Linnaeus, 1767, two species introduced in all biogeographic realms edging the Atlantic Ocean, including Azores and Canary Islands, two important bridgeheads in the trade routes between Europe and the Americas (Steckley 1972; Crosby 1984; de Avilez Rocha 2019). Similarly, sugar cane cultivation is associated with the worldwide spread of *Aphanisticus cochinchinae seminulum* Obenberger, 1929 (Zack et al. 2009).

In more recent times, many examples of intracontinental spread of buprestids have been reported, especially for certain species of *Agrilus*, *Anthaxia*, and *Chrysobothris* (Westcott 2005; Fägerström et al. 2009; Izzillo 2013; Orlova-Bienkowskaja and Volkovitsh 2015; Westcott et al. 2018; Curletti and Ranghino 2020). Rapid intracontinental spread probably reflects greater connectivity among trading partners as well as increased speed of transport, especially in the European Union and North America. Range expansion of some neonative species has apparently resulted from human-caused climate and environmental changes, such as for *Agrilus graminis* Kiesenwetter, 1857; *Agrilus nicolanus* Obenberger, 1924; *Buprestis dalmatina* Mannerheim, 1837; *Lamprodila festiva* (Linnaeus, 1767). In the USA, the southward and westward spread of the native birch specialist *Agrilus anxius* Gory, 1841 has been attributed to the widespread planting of ornamental birch trees in many areas outside the native range of North American birch species (Muilenburg and Herms 2012).

It is interesting to note that most neonatives have caused little damage, although there are a few exceptions often associated with the inadvertent movement of infested live plants. For example, the introduction of *Agrilus planipennis* from Eastern Asia to the Moscow area resulted in severe mortality of ash (*Fraxinus*) trees in European Russia (Orlova-Bienkowskaja 2014); however, it is also plausible that *Agrilus planipennis* could have been introduced in Moscow on ash nursery stock imported from North America (Haack et al. 2015). Another example is *Lamprodila festiva* (Linnaeus, 1767), a southern European – circum-Mediterranean species, which has expanded its distribution northward and eastward, benefiting from extensive plantings of its host plants (Cupressaceae) as ornamental plants in private and public gardens (Nitzu et al. 2016; Rabl et al. 2017; Volkovitsh and Karpun 2017; Ruicănescu and Stoica 2019). Similarly, *Agrilus mali* Matsumura, 1924, an eastern Palearctic species, has taken advantage of expanding cultivation of Rosaceae fruit trees and patches of natural forest as a springboard to spread westward in the Palearctic (Volkovitsh et al. 2020; Zhang et al. 2021; Lu et al. 2022).

Only four buprestid species have been intentionally introduced as biological control agents against invasive weeds in North America, South Africa, and Australia. *Sphenoptera jugoslavica* Obenberger, 1926 has been intentionally introduced and successfully established in the western USA where it is used to control the invasive plant *Centaurea diffusa* Lam. (Asteraceae) (Lang et al. 1998); *Agrilus hyperici* (Creutzer, 1799) was introduced in the USA and Australia where it provides efficient control of invasive *Hypericum* species (Hypericaceae); while *Hylaeogena jureceki* Obenberger, 1941 was introduced and established with different rates of success in South Africa and Australia to control the invasive plant *Dolichandra unguis-cati* (L.) L.G.Lohmann (Bignoniaceae) (King et al. 2011; Snow and Dhileepan 2014). The Neotropical *Lius poseidon* Napp, 1972 was instead intentionally introduced to Hawai'i to control the invasive *Miconia crenata* (Vahl) Michelang (Melastomataceae); however, in Hawai'i the species naturally became a biocontrol agent of another invasive plant *Chaetogastra herbacea* (DC.) P.J.F.Guim. & Michelang. (Melastomataceae) (Culliney and Nagamine 2000; Conant and Hirayama 2001; Conant et al. 2013).

Conclusion

The family Buprestidae is highly diverse with a global distribution defined by multiple abiotic and biotic factors, including human-mediated introductions. Although some biological and ecological traits, such as apparent obligate outbreeding and obligate maturation feeding for all buprestids, can serve as barriers to successful establishment, the opening of new continental and intercontinental trade routes as well as the ever-increasing volume and types of goods and plants traded increases the risk of future introductions or passive diffusion of more buprestid species. With respect to climate change and the widespread practice of introducing exotic plants for ornamental, agricultural, and forestry purposes around the world, it will be important to identify possible new introduction pathways for exotic Buprestidae along with pest risk assessments. In this regard, more research is needed on buprestid taxonomy and ecology, together with training and funding of more buprestid specialists. The development of new technologies for rapid species identification, either morphological or molecular, would be very useful for the management of this important group of plant pests, which are becoming of increasing economic importance worldwide.

Acknowledgements

The present paper resulted from activities framed into the HOMED project (HOlistic Management of Emerging forest pests and Diseases), which was funded from the European Union's Horizon 2020 research and innovation programme under grant agreement no. 771271.

The authors thank Eduard Jendek (Faculty of Forestry and Wood Sciences, Czech University of Life Sciences) for providing important literature useful for this contribution.

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Supplementary material 1

Systematic list of all Coleoptera Buprestidae introduced around the world between 1850 and 2020

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Data type: table (excel document)

Link: https://doi.org/10.3897/neobiota.84.90829.suppl1

NeoBiota 84: 137–167 (2023)
doi: 10.3897/neobiota.84.95864
https://neobiota.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
NeoBiota
Advancing research on alien species and biological invasions

Pining away and at home: global utilisation of *Pinus radiata* by native and non-native insects*

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Academic editor: M. Branco | Received 1 October 2022 | Accepted 7 February 2023 | Published 18 May 2023

Citation: Brockerhoff EG, Gresham BA, Meurisse N, Nahrung HF, Perret-Gentil A, Pugh AR, Sopow SL, Turner RM (2023) Pining away and at home: global utilisation of *Pinus radiata* by native and non-native insects. In: Jactel H, Orazio C, Robinet C, Douma JC, Santini A, Battisti A, Branco M, Seehausen L, Kenis M (Eds) Conceptual and technical innovations to better manage invasions of alien pests and pathogens in forests. NeoBiota 84: 137–167. https://doi.org/10.3897/neobiota.84.95864

Abstract

Pinus radiata (radiata pine or Monterey pine) is threatened in its native range in California and, at the same time, one of the most widely-planted tree species worldwide, especially in the southern hemisphere. It is affected by a wide range of plant-feeding insects both in its native range and in regions where it is planted as an introduced tree. In addition, there are many invasive insects that have colonised *P. radiata*, in some cases causing major damage. Here, our objectives were to provide a complete and up-to-date overview of all insect species recorded from *P. radiata* worldwide, to summarise where these insects are native and which countries or regions they have invaded, to categorise them according to their impacts as damaging species or as vectors of plant pathogens, and to examine border interceptions to determine whether pathways exist that would allow these species to enter and potentially invade additional regions. Our compilation of insects feeding on *P. radiata* provides a list of 649 species (and an additional 11 species identified at the genus level only). Coleoptera is the most represented order in the list (299 species), followed by Lepidoptera (224 species) and Hemiptera (65 species). We classified 28 species as high-impact, including 12 true bark beetles (Coleoptera: Curculionidae: Scolytinae), eight Lepidoptera, five other Coleoptera,

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* This paper is dedicated to the late John Bain, eminent forest entomologist at the New Zealand Forest Research Institute, who devoted much of his career to studying insects on *Pinus radiata*.

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two Hymenoptera and one Hemiptera. These species can cause substantial direct damage or act as vectors of highly-damaging plant pathogens. Other species cause only occasional damage, rarely requiring management (classified as 'low-medium impact') or they are generally benign ('negligible impact'). Hemiptera and Scolytinae have a high proportion of species established outside their native range. The Nearctic and Neotropic regions have been invaded by the most high-impact species, mainly by species native to Europe. Border interceptions of 185 species (29% of those on our list) were recorded during import inspections between 1995–2021, indicating considerable potential for further invasions. The findings of our study can be used to identify potential high-impact invaders and the pathways that may require more phytosanitary attention. Furthermore, our analyses provide useful insights into the insect-plant interactions resulting from the global distribution of a tree species and the native and non-native insects feeding on it.

Keywords

Biological invasions, establishment, impact assessment, insect herbivores, interceptions, Monterey pine, pest risk analysis, Pinaceae, plantation forest, radiata pine

Introduction

Pinus radiata D. Don (Monterey pine or radiata pine) is one of the most extensively-planted tree species worldwide (Lavery and Mead 1998; Mead 2013). Although the native area of *Pinus radiata* is less than 6000 ha in coastal California and islands off the coast of Baja California, its fast growth rate, usefulness for a wide range of purposes, and suitability across a range of temperate climatic conditions, have led to it being a preferred choice for plantation forestry, especially in the southern hemisphere (Lavery and Mead 1998). It is planted on a large scale as an introduced (non-native) species mainly in Chile (ca. 1.9 million ha (CONAF 2021)), New Zealand (ca. 1.5 million ha (NZFOA 2021)), Australia (ca. 0.7 million ha (Legg et al. 2021)) and South Africa (ca. 40,000 ha (Forestry Economics Services CC 2020), but formerly ca. 0.1 million ha (Lavery and Mead 1998)). In addition, it has been planted in Spain (0.2 million ha (Mead 2013)) and in other European countries including Italy and France (CABI 2019, Mead 2013), as well as in China (e.g., Bi et al. 2003, 2013) and, to a lesser extent, in several other countries (CABI 2019).

Given the importance of *P. radiata* for forestry, there is considerable interest in insects and pathogens affecting tree health. In its native range, *P. radiata* suffers from a number of important insect pests (e.g., Ohmart 1982a) and pathogens (Gordon et al. 2001). In other parts of the northern hemisphere where other pine species are native, the introduced *P. radiata* is severely affected by native pests of pines (e.g., Cobos-Suarez and Ruiz-Urrestarazu 1990, Castedo-Dorado et al. 2016). By contrast, in the planted areas in the southern hemisphere, its pest burden is comparatively low because the lack of native pines or other Pinaceae south of the equator means there are few native insects that cause substantial damage to pines (e.g., White 1974; Berndt et al. 2004; Wingfield et al. 2008a). Non-native plants without close relatives in their introduced range are usually less affected by native phytophagous insects than those with close relatives in the native flora (e.g., Harvey et al. 2012; Branco et al. 2015). Therefore, pine plantations planted well outside the native range of pines in the southern hemisphere were in a largely enemy-free space, consistent with the enemy-release hypothesis (Mitchell and Power 2003; Colautti et al. 2004). However, these pine plantations are highly susceptible to invasion by insect pests left behind in their native range (i.e., reconnection of "old associations"), pine pests from other regions, and by polyphagous insects and those feeding on closely related plants in their introduced range (i.e., "new associations"). The risk of invasions is confirmed by the steady increase in the number of established non-native insects that affect plantations of non-native pines and other trees (e.g., Hurley et al. 2016; Brockerhoff and Liebhold 2017; Nahrung and Carnegie 2020).

The arrival of highly damaging non-native pests in southern hemisphere plantations of *P. radiata* began with the woodwasp *Sirex noctilio* which was detected in New Zealand in 1900 (Bain et al. 2012) and subsequently invaded most southern hemisphere regions where pines are grown (Slippers et al. 2015). Other notable invasive insect pests of *P. radiata* are the eastern five-spined engraver bark beetle (*Ips grandicollis*) first recorded in Australia in the 1940s (Neumann 1987), the European pine shoot moth (*Rhyacionia buoliana*) first found in Chile in 1985 (Alvarez and Ramirez 1989), and the Monterey pine aphid (*Essigella californica*) detected in Europe, Australia, New Zealand and South America between the 1980s and early 2000s (Watson et al. 2008; Eyles et al. 2011). However, most of the more damaging insect species feeding on *P. radiata* still have a limited distribution and many have not yet invaded the southern hemisphere countries with large *P. radiata* plantations. Consequently, there was and is much interest in risk assessments and surveys for insect pests of *P. radiata* to identify potential invaders and to prevent their invasion (e.g., Allen 1973; Ohmart 1980; Carter and Griffith 1989; Mead 2013; Brockerhoff and Bulman 2014; Brockerhoff et al. 2016; Lawson et al. 2018). However, there has not been a comprehensive assessment of the insects feeding on *P. radiata* since the 1980s when Clifford P. Ohmart studied insects associated with it in its native region and all main areas where it was planted (Ohmart 1980, 1982a, b). In addition, the role of insects as vectors of pathogens has received more consideration since then (e.g., Hoover et al. 1996; Kirisits 2004; Wingfield et al. 2008a).

The objectives of the present study are:

(1) to provide a complete and up-to-date overview of all insect species recorded from *P. radiata* in its native and introduced ranges,

(2) to summarise where these insects are native and which countries or regions they have invaded,

(3) to categorise these species according to their impacts as damaging species or as vectors of plant pathogens,

(4) to examine whether border interceptions have been recorded, which would indicate that pathways exist that would allow these species to enter and potentially invade additional regions, and

(5) to provide additional information on these points specific to New Zealand and Australia because more detailed records and data are available for these countries.

Methods

Sources of insect records from *Pinus radiata*

We compiled world-wide records of insect species recorded on *Pinus radiata* that incorporated the original lists of Ohmart (1980, 1981, 1982a, b) and additional records from New Zealand and other countries which had been continuously added to and curated by John Bain (Scion (New Zealand Forest Research Institute)). Beginning in 2018, this list was thoroughly reviewed and updated with a literature search using the Scopus database (see below) as well as forward and backward searches in relevant publications. In 2020, a list independently compiled by Helen Nahrung (University of the Sunshine Coast, Queensland, Australia) with records from Australia was incorporated. Additional records were added between 2020 and 2022 by further interrogating the literature, Scion's Forest Health Database and other available databases. The main criterion for inclusion in our pine pest list was that species feed on any tissue of *P. radiata* (see below for more information on the impact classification). The full species list with references is available as Suppl. material 1: table S1 and at the online repository Zenodo (Brockerhoff et al. 2023).

Taxonomy and geographic distribution

Using the scientific name of each insect as the search term, the current taxonomy, synonyms and distribution in native and introduced ranges were retrieved for all species, initially by systematic searches using Scopus (https://www.scopus.com), Google Scholar (https://scholar.google.com), the CABI Invasive Species Compendium (https://www.cabi.org/ISC), the Global Biodiversity Information Facility (GBIF, https://www.gbif.org), NZOR – New Zealand Organisms Register (https://www.nzor.org.nz), the Atlas of Living Australia (https://www.ala.org.au), the Australian Faunal Directory (https://biodiversity.org.au/afd/home), and the Australian Plant Pest Database (https://www.appd.net.au) as well as Google (https://www.google.com) and Wikipedia (https://en.wikipedia.org). Other databases (some taxon-specific) and literature records were accessed as required, and in some cases, experts were consulted directly (see references in Suppl. material 1: table S1). Establishment data were cross-checked against the 'International non-native insect establishment data' database (Turner et al. 2021b).

The species list was standardised taxonomically using the GBIF taxonomic database (GBIF Secretariat 2021) and the "taxize" package in R (Chamberlain and Szöcs 2013). For any names not recognised by GBIF, standardisation was performed manually via searches of other databases and literature. Coleoptera family names were based on the framework in Bouchard et al. (2011), and Lepidoptera families as per Mally et al. (2022).

For each species, native and non-native occurrences were grouped by biogeographic regions defined as shown below. Our biogeographic regions are mostly aligned with those of Udvardy (1975) but not strictly because our information sources were specific to countries of occurrence, whereas the borders of Udvardy's biogeographic realms often pass through countries (i.e., one country can be in more than one region).

Our regions are defined as follows:

- Western Palearctic ("W Palearctic"): Europe, North Africa and Near East;
- Eastern Palearctic ("E Palearctic"): Northern and eastern Asia and including the Indo-Malayan region;
- South West Pacific ("SW Pacific"): Australasia and Pacific Islands (excluding Hawaii);
- Afrotropic: Sub-Saharan Africa;
- Nearctic: North America including all of Mexico and Hawaii;
- Neotropic: South and Central America (excluding all of Mexico) and the Caribbean.

Using the information on occurrences of native species and establishments of non-native species, we compiled for each biogeographic region (i) the number of native species feeding on *P. radiata*, (ii) the number of established non-native species feeding on *P. radiata*, and (iii) the number of species originating from each region that became established in another region or in another country in the same region.

Interception data

Three datasets with border interceptions were analysed to determine which of the species on our list have been intercepted during border inspections of imports, vessels and containers, and in some cases international mail and passenger baggage. Post-border interceptions were not considered.

Unless otherwise stated, analyses with border interception data were conducted using an international dataset. This recent dataset is a collection of international border interceptions between 1995 and 2021 in New Zealand, Australia, South Africa, South Korea, Japan, Canada, the United States, the United Kingdom and the European and Mediterranean (EPPO) region. The international interception dataset is comprised of the border interceptions described in Turner et al. (2021a). In addition, we queried South African border interceptions from Saccaggi et al. (2021), additional border interceptions from Japan between 1996–2019 extracted from http://www.pps.go.jp/TokeiWWW/Pages/report/index.xhtml (Plant Protection Station, The Ministry of Agriculture, Forestry and Fisheries of Japan), and updated EPPO border interceptions for the 2011–2021 period from the Europhyt annual interception reports. Included in the international dataset was the New Zealand data subset which spans the period from 2000–2017 (Turner et al. 2021a) which was used for a country-specific analysis.

Additional statistics were drawn from two older border interception databases. Firstly, the Scion BUGS database for New Zealand 1950–2000 which contains border interceptions of species relevant for trees, and secondly the USDA 1949–2008 interceptions of Scolytinae and Cerambycidae (Brockerhoff et al. 2014).

Impact classification

Each species on the list was assigned one of three impact ratings relating to evidence of pest status on *P. radiata*: 'negligible impact' – species where no interventions, management or damage records were found; 'low-medium impact' – species with evidence of damage, management or control but this was either short-term, localised or minor; and 'high impact' – species that required ongoing management and/or had significant economic effects, such as severe damage to forest or amenity trees and/or are important vectors of highly damaging pathogens of *P. radiata*. Species causing severe impacts on human or veterinary health (e.g. from urticating hairs of caterpillars) were also considered 'high impact'. In some cases, we combined species in the low-medium and high impact categories as species of 'non-negligible impact'. Impacts related to market access were excluded in our study because these are often associated with species that do not damage live trees or cause no damage at all. Likewise, impacts of species whose recorded damage was exclusive to timber in service, such as borers in dry deadwood, were excluded because the focus of our assessment was on insects feeding on living trees. Consequently, species exclusively affecting market access or causing only damage to timber in service were classified as having negligible impact.

Our impact classification differs from the now widely used EICAT classification (IUCN 2020) because our impacts relate mainly to damage to *Pinus radiata* planted for commercial purposes outside their native range and in some cases also to trees in their native range, whereas EICAT focuses only on "impact to native taxa" (IUCN 2020, p. 8). However, our categories can be translated to approximately corresponding EICAT categories ('negligible impact' = 'minimal concern'; 'low-medium' = 'minor'; 'high impact' = 'moderate'). None of the insects considered in our list have a 'major' or 'massive' impact according to EICAT as both these involve at least local extinction of the affected species.

Analyses

The final dataset containing all insect species feeding on *Pinus radiata* was analysed and visualised in R version 4.1.2 (2022-05-20). When analysing by biogeographic region, we excluded seven cosmopolitan species with a widespread distribution across multiple biogeographic regions where it could not be determined which regions were part of the native or non-native range. When analysing non-native species, we included species which were successfully eradicated as these represent the establishment potential in the absence of a post-border biosecurity response. For example, four of the species invasive to New Zealand fell into this category (*Coptotermes acinaciformis*, *Coptotermes frenchi*, *Cryptotermes brevis* and *Teia anartoides*).

Comparisons were made among all insects on the pine pest list (i.e., any species feeding on *Pinus radiata*) as well as specifically among the "non-negligible" impact species (those in the combined low-medium or high impact categories).

To investigate relationships between border interceptions and establishments, the number of species was compared by taxon groups which were defined at the level of insect orders with the exception of four particularly species-rich and important families/subfamilies (Cerambycidae, Scolytinae, Geometridae and Tortricidae) which were analysed separately. If relationships between interceptions and establishments were independent of taxon group, we would expect the number of established species in each group to be relative to the number of intercepted species in each group and proportional to the ratio of established insect species per intercepted insect species (i.e. expected number of establishments in taxa group = (total number of established insects)/(total number of intercepted insects)*(number of intercepted insects in taxa group). We assume that the number of established species per group can then be described by a Poisson distribution and calculate a prediction interval for each of our taxa groups. The prediction interval bounds were calculated to show the region within which all 11 taxa groups would be expected to fall 95% of the time. When calculating the interval quantiles, a Bonferroni correction was used for multiple comparisons.

The relationship between the number of native and non-native insects per biogeographic region was visualised on a scatter plot. The effect of feeding guilds (i.e., borers, defoliators, sap-feeders and others) was visualised by adding ellipses showing the 95% confidence intervals for a multivariate t-distribution (Fox and Weisberg 2011).

Pearson's chi-square test was used to test for evidence of differences in proportions between groups (i.e., negligible vs non-negligible, intercepted vs not intercepted, feeding guilds), followed by pairwise comparisons of proportions using the Holm (1979) method of adjustment for multiple comparisons. In situations where expected counts were fewer than 5, Fisher's exact test was used instead.

Results

Species recorded on *Pinus radiata*

We found records of 649 insect species (in 438 genera, 83 families and nine orders) feeding on *P. radiata* (Table 1, Suppl. material 1: table S1). An additional 11 records were named at the genus level only; these were all of negligible or low impact, and as their identity could not be confirmed, they were excluded from the analyses (but are listed in Suppl. material 1: table S1). Coleoptera is the most represented order (299 species or nearly 50% of all species), followed by Lepidoptera (224 species), Hemiptera (65 species), Blattodea (i.e., termites), Hymenoptera and other orders. Twenty-eight species were categorised as 'high impact' and 168 species as 'low-medium impact' (Table 1). The remaining 453 species (nearly 70% of the species total) were considered to have negligible impacts on *P. radiata* as no records of damage were found for these species (Table 1). Of the 49 insects on our list that are known to vector diseases, evidence of detrimental impact exists for 37 species. In terms of feeding guilds, most species are either borers or defoliators while sap-feeders and other guilds such as root feeders and cone insects are less represented (Table 2).

Table 1. Overview of pine pest list species, their impact classification, interceptions and establishments, grouped by main taxa. Note: Interceptions are based on the 1995-2021 international dataset (see methods). Establishments include species established unintentionally anywhere outside their native range around the world, regardless of whether or not they were subsequently eradicated, and include cosmopolitan species. See Fig. 2 for results of statistical tests comparing proportions among taxa for establishments and interceptions. Taxa with shared letters within a column were not significantly different in terms of the proportions within the column based on a Fisher pairwise test with Holm adjustment for multiple comparisons.

Taxa	Number of species in taxon	Number (percent) high impact*	Number (percent) low-medium impact*	Number (percent) negligible impact*	Number (percent) established	Number (percent) intercepted
Blattodea: Isoptera	22	0 (0) ab	3 (14) ab	19 (86) abc	9 (41) abc	6 (27) abcd
Coleoptera: Cerambycidae	69	1 (1) b	8 (12) b	60 (87) a	14 (20) bc	20 (29) bcd
Coleoptera: Curculionidae: Scolytinae	55	12 (22) a	16 (29) ab	27 (49) bc	20 (36) ab	35 (64) a
Coleoptera: Curculionidae: other	90	3 (3) ab	20 (22) ab	67 (74) abc	15 (17) bc	17 (19) cd
Coleoptera: other	85	1 (1) b	17 (20) ab	67 (79) ab	13 (15) bc	22 (26) bcd
Hemiptera	65	1 (2) b	16 (25) ab	48 (74) abc	36 (55) a	32 (49) ab
Hymenoptera	14	2 (14) ab	6 (43) ab	6 (43) bc	4 (29) abc	3 (21) abcd
Lepidoptera: Geometridae	40	0 (0) ab	9 (22) ab	31 (78) abc	3 (8) bc	2 (5) d
Lepidoptera: Tortricidae	33	2 (6) ab	17 (52) a	14 (42) c	6 (18) bc	11 (33) abcd
Lepidoptera: other	151	6 (4) b	46 (30) ab	99 (66) abc	19 (13) c	25 (17) d
All other orders	25	0 (0) ab	10 (40) ab	15 (60) abc	7 (28) abc	12 (48) abc
Total	**649**	**28 (4)**	**168 (26)**	**453 (70)**	**146 (22)**	**185 (29)**

*only impacts on living trees were considered.

Table 2. Pine pest list grouped by feeding type in terms of impacts, interceptions and establishments. Note: Impact is based on evidence for impact on *P. radiata*. Establishments included those of species established unintentionally anywhere outside their native range around the world inclusive of cosmopolitan species, regardless of whether or not they were subsequently eradicated. Interception data used here is the 1995-2021 international dataset (see methods). The letters in each column indicate the results from pairwise comparison of proportions with Holm adjustment for multiple comparison. Taxa with the same letters were not significantly different in terms of the proportions within the column. For detailed statistics, see Suppl. material 2: table S2.

Feeding guild	Number in feeding guild	Number (percent) high impact	Number (percent) low-mid impact	Number (percent) established	Number (percent) intercepted
Borer	270	20 (7)	48 (18) b	67 (25) b	87 (32) a
Defoliator	278	7 (3)	93 (33) a	31 (11) c	53 (19) b
Sap-feeder	67	1 (1)	17 (25) ab	37 (55) a	33 (49) a
Other	34	0 (0)	10 (29) ab	11 (32) ab	12 (35) ab
Total	**649**	**28 (4)**	**168 (26)**	**146 (22)**	**185 (29)**

High-impact species

The 28 species classified as high-impact comprised 17 Coleoptera (12 of which are true bark beetles (Scolytinae)), eight Lepidoptera, two Hymenoptera and one Hemiptera (Tables 1, 3). Twenty of these high-impact species are borers, seven are defoliators, and one is a sap-feeder (Table 3), with significant differences in proportions between groups (Table 2, Fisher's Exact Test, P=0.016). Seventeen of the 28 high-impact species are known vectors of serious pathogens affecting *P. radiata*, especially the pitch canker fungus *Fusarium circinatum* (Table 3). Other species are high-impact pests in their own right such as the European six-toothed bark beetle *Ips sexdentatus* which can occasionally cause substantial tree mortality.

Table 3. High-impact species feeding on *Pinus radiata*, their native and established regions, number of interceptions internationally, and important plant pathogens vectored (where applicable). Note that the East Palearctic includes records from the Indo-Malayan region.

Scientific name	Common name(s)	Feeding guild	Native region	Invaded regions	Interceptions	Vector of pathogens
Conophthorus radiatae Hopkins	Monterey pine cone beetle	Borer	Nearctic	–	0	*Fusarium circinatum*
Dioryctria sylvestrella (Ratzeburg)	New pine knot-horn, maritime pine borer	Defoliator	W Palearctic, E Palearctic	–	0	–
Ernobius punctulatus (LeConte)	–	Borer	Nearctic	–	4	*Fusarium circinatum*
Essigella californica (Essig)	Monterey pine aphid	Sap-feeder	Nearctic	W Palearctic, Neotropic, SW Pacific	0	–
Hyalarcta huebneri (Westwood)	Common leaf case moth	Defoliator	SW Pacific	–	0	–
Hylastes angustatus (Herbst)	–	Borer	W Palearctic	Afrotropic	1	*Fusarium circinatum*, *Leptographium procerum*
Hylastes ater (Paykull)	Black pine bark beetle	Borer	W Palearctic	Neotropic, SW Pacific	778	*Ophiostoma* spp., *Leptographium* spp.
Hylobius abietis (Linnaeus)	Large brown pine weevil, large pine weevil	Borer	W Palearctic, E Palearctic	–	11	–
Ips grandicollis (Eichhoff)	Eastern five-spined engraver, five-spined bark beetle, southern pine engraver	Borer	Nearctic	SW Pacific, E Palearctic	25	*Ophiostoma ips*
Ips mexicanus (Hopkins)	Monterey pine engraver	Borer	Nearctic, Neotropic	–	2	*Fusarium circinatum*
Ips paraconfusus Lanier	California five-spined Ips, California five-spined engraver	Borer	Nearctic	–	0	*Fusarium circinatum*
Ips plastographus maritimus (Lanier)	–	Borer	Nearctic	–	1	*Fusarium circinatum*
Ips sexdentatus (Boerner)	Six-toothed bark beetle	Borer	W Palearctic	–	453	*Fusarium circinatum*, *Leptographium* spp., *Ophiostoma* spp.
Lymantria dispar (Linnaeus)	Spongy moth, gypsy moth	Defoliator	W Palearctic, E Palearctic	Nearctic	465	–
Lymantria monacha (Linnaeus)	Black arches, nun moth	Defoliator	W Palearctic, E Palearctic	–	0	–
Monochamus galloprovincialis (Olivier)	Black pine sawyer beetle	Borer	W Palearctic	–	40	*Bursaphelenchus xylophilus*
Neodiprion sertifer (Geoffroy)	European pine sawfly, fox-coloured sawfly	Defoliator	W Palearctic, E Palearctic	Nearctic	1	–
Ormiscodes cinnamomea (Feisthamel)	–	Defoliator	Neotropic	–	0	–
Orthotomicus erosus (Wollaston)	Mediterranean pine beetle	Borer	W Palearctic, E Palearctic	Afrotropic, Nearctic, Neotropic	136	*Ophiostoma ips*, *Verticicladiella alacris*
Pissodes castaneus (De Geer)	Small banded pine weevil, banded pine weevil, lesser banded pine weevil	Borer	W Palearctic	Neotropic	3	*Leptographium* spp., *Armillaria* spp., *Sporothrix inflata*. Carrier but not confirmed vector of *F. circinatum*
Pissodes nemorensis Germar	Deodar weevil, northern pine weevil	Borer	Nearctic	Afrotropic	0	*Fusarium circinatum*, *Diplodia pinea*
Pityophthorus carmeli Swaine	–	Borer	Nearctic	–	0	*Fusarium circinatum*
Pityophthorus setosus Blackman	–	Borer	Nearctic	–	0	*Fusarium circinatum*
Rhyacionia buoliana (Denis & Schiffermuller)	European pine shoot moth	Borer	W Palearctic	Nearctic, Neotropic	0	–
Rhyacionia frustrana (Comstock)	Nantucket pine tip moth	Borer	Nearctic	–	0	–
Sirex noctilio Fabricius	Sirex woodwasp	Borer	W Palearctic, E Palearctic	Afrotropic, Nearctic, Neotropic, SW Pacific	29	*Amylostereum areolatum*
Thaumetopoea pityocampa (Denis & Schiffermuller)	Pine processionary moth	Defoliator	W Palearctic	–	0	–
Tomicus piniperda (Linnaeus)	Common pine shoot beetle, pine shoot beetle, larger European pine shoot beetle, larger pine shoot beetle	Borer	W Palearctic, E Palearctic	Nearctic	65	*Leptographium wingfieldii*, *Leptographium guttulatum*, *Ophiostoma minus*

Native species by biogeographic region

Seven cosmopolitan species which occur in multiple regions and for which the native range could not be determined were excluded from the analysis of native or invaded ranges except for the specific analysis for New Zealand and Australia (see below). Most native species feeding on *P. radiata* were recorded in the SW Pacific region (42% of all non-cosmopolitan species, with 167 species being native to Australia and 107 species native to New Zealand), followed by the Nearctic (20%), the Afrotropic (16%), the W Palearctic (12%) and the Neotropic region (12%) (Fig. 1A). The fewest native species feeding on *P. radiata* were recorded in the E Palearctic (6%). Despite the large number of species recorded for the SW Pacific, this region has just one native high-impact species (the Australian psychid moth *Hyalarcta huebneri* (Table 3)). The three southern hemisphere regions have the highest proportions of species with negligible impact and an average proportion of low-medium impact species (Fig. 1A). The W Palearctic has a high proportion and the largest number of high-impact species recorded on *P. radiata* (15 species: 8 Coleoptera, 5 Lepidoptera and 2 Hymenoptera), followed by the Nearctic region (11 species: mainly Coleoptera) and the E Palearctic region (8 species: 3 Coleoptera, 3 Lepidoptera and 2 Hymenoptera). However, there is considerable overlap in the native regions of these species. For example, eight high-impact species native to W Palearctic are also native to E Palearctic. The Neotropic has two high-impact species, the bark beetle *Ips mexicanus* in the northern part of this region (in the native range of pines) and *Ormiscodes cinnamomea*, a polyphagous saturniid in Chile.

Establishments of species outside their native ranges

Establishments of non-native species (irrespective of impact)

Our compilation revealed almost one quarter (146 species) of insects feeding on *P. radiata* are established outside their native range, seven of which are considered cosmopolitan (Table 1). Beetles are the order with the most established non-native species with a total of 62 species including 20 bark and ambrosia beetles (Scolytinae), 15 other weevils (Curculionidae) and 14 longhorn beetles (Cerambycidae). This means that 22% of all beetles feeding on *P. radiata* are already established somewhere outside their native range (Table 1). With 36 established species, Hemiptera are also well represented among successful invaders. More than half (55%) of all the Hemiptera known from *P. radiata* are present outside their native range, significantly more than most other groups (Fig. 2A). Lepidoptera are another group of prominent invaders with 28 established species, but with a lower percentage of established species (12.5% of 224 Lepidoptera species known from *P. radiata*).

Borers were the dominant feeding guild among the established species, followed by sap-feeders and defoliators (Table 2). However, sap-feeders were the most successful invaders relative to the total number known in each feeding guild (i.e., 55% of all sap-feeders known to feed on *P. radiata* are already established somewhere. By contrast,

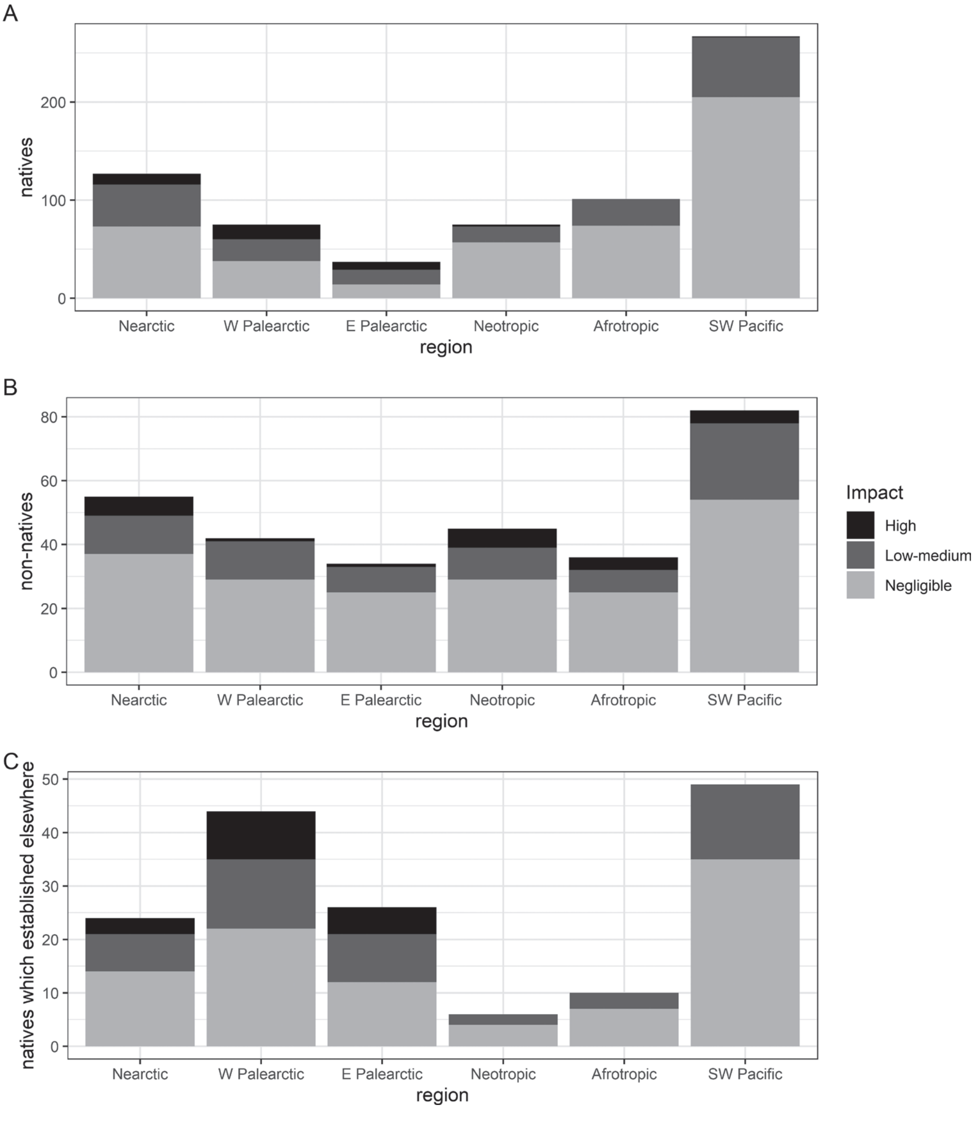

Figure 1. Impact levels of insect species feeding on *Pinus radiata* and their biogeographic ranges, excluding cosmopolitan species. (**A**) Species native to each biogeographic region. (**B**) Species non-native to each biogeographic region. (**C**) Species native to a biogeographic region (x-axis) which have established somewhere outside their native range (could be in the same biogeographic region e.g. from Australia to New Zealand). Note that the East Palearctic includes records from the Indo-Malayan region.

only 25% of known borers and 11% of known defoliators of *P. radiata* have successfully invaded somewhere (Table 2).

The SW Pacific region has the most known established non-native species (13% of all non-cosmopolitan species on the list), mainly due to a large number of species with negligible or low-medium impact (Fig. 1B). This is followed by the Nearctic (9%)

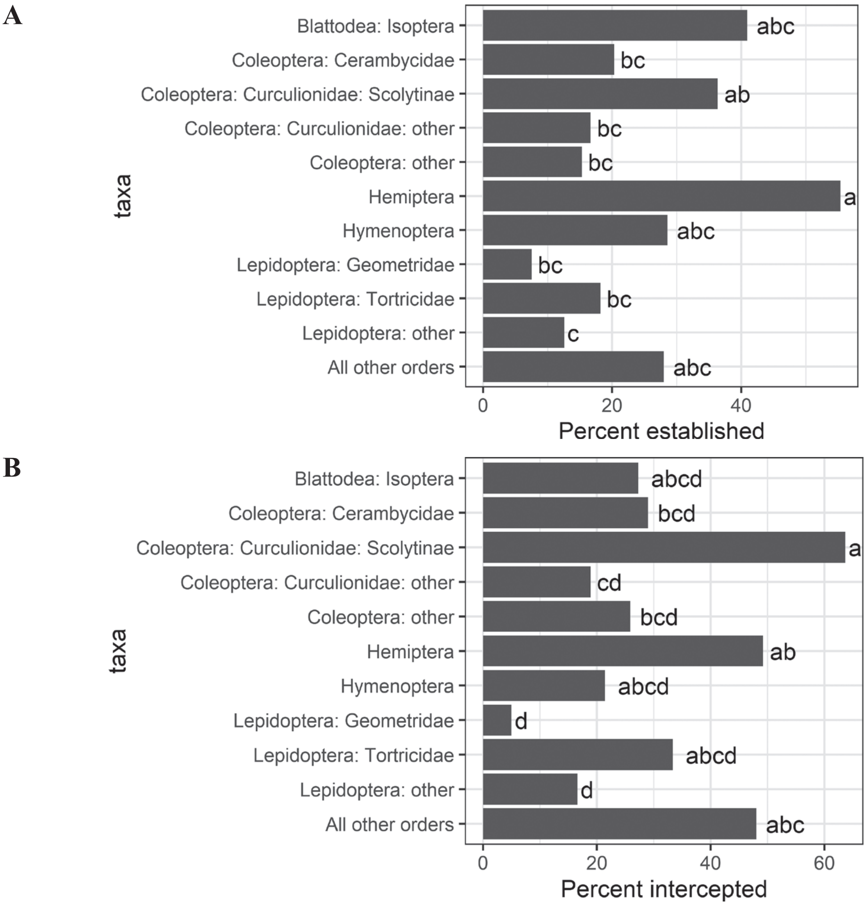

Figure 2. Percentages of each taxon established (**A**) or intercepted (**B**). Bars annotated with the same letter indicate proportions which were not significantly different (i.e. p>0.05) under multiple pairwise comparison of proportions using Fisher's Exact Tests with the Holm (1979) method of P-value adjustment.

and the Neotropic (7%) and West Palearctic regions (7%), whereby the former two have a large proportion of non-native high-impact species. Generally, the proportions of species with high- and low-medium-impact vary considerably among the regions. There was no significant difference in the proportions of species established among the non-negligible compared to among the negligible species (one-sided, 2-sample test for equality of proportions without continuity correction, Chi-squared = 2.622, P=0.053).

High-impact invaders

Twelve of the 28 high-impact species have already become established somewhere in the world, and six of these have become established in more than one biogeographic region (Table 3). The biogeographic regions with the most invasions of high-impact species (6) are the Nearctic or Neotropic (Fig. 1B); all but one of these species are native to Europe (and adjacent parts of the W Palearctic region), with the remainder being a native species from the Nearctic which invaded the Neotropic. Other regions with several establishments of high-impact species are the SW Pacific (four species, two native to the W Palearctic and two native to the Nearctic), and the Afrotropic (four species with three of these being native to Europe), while the Western and the E Palearctic had only one established high-impact species each (Fig. 1B).

Native regions of established non-native species (including within the region)

The SW Pacific is the region with the most native species that became established somewhere outside their native range (both beyond and especially in other countries within their native biogeographic region), followed by the W Palearctic and the E Palearctic (Fig. 1C). However, the W Palearctic contributed by far the most high-impact species that became established somewhere, followed by the E Palearctic and the Nearctic. Although the E Palearctic ranks second in terms of high-impact species that established somewhere outside their native region, these are all species with a native range that extends from Europe across northern Asia, and it is difficult to ascertain the actual part of the region from which the invasion occurred.

Considering the source regions and invaded regions together, a clear picture of invasion routes emerges (Fig. 3). The W Palearctic is the main source region of invaders that colonised mainly the Nearctic, the Neotropic and the SW Pacific regions for all species (Fig. 3A) and species of non-negligible impact (Fig. 3B). Furthermore, the SW Pacific region has by far the most species that invaded other parts of the same region (Figs 3A, B, 4). However, these concern only species of negligible or low-medium impact as there are no high-impact species native to this region which established anywhere.

Border interceptions

Of all the species in the pine pest list, 185 (29%) were intercepted during border import inspections at least once internationally between 1995–2021 (Table 1). Of these, 83 species (13% of the pine pest list) were intercepted specifically at New Zealand's border between 2000–2017. An additional eight species were intercepted earlier (i.e., between 1950 and 2000 and recorded in New Zealand's BUGS database), and a further two species were intercepted and recorded in United States interception records from 1949–2008. Therefore, a total of 195 species were intercepted at least once at a border. More than 60% of species of Scolytinae (bark and ambrosia beetles) on the list were intercepted (at least once, Table 1), a significantly greater percentage than other beetle groups and several other taxa (Fig. 2B). In terms of feeding guilds, the percentage of intercepted species was greatest for sap-feeders and borers, and least for defoliators, while differences from 'other' guild members were not significant (Table 2). The most frequently intercepted species were mainly sap-feeders (including *Thrips tabaci*, Thysanoptera: Thripidae, 42,302 interceptions; *Aonidiella aurantii*, Hemiptera: Diaspididae, 8,782 interceptions; *Pseudococcus longispinus*, Hemiptera: Pseudococcidae, 3,341 interceptions; and sev-

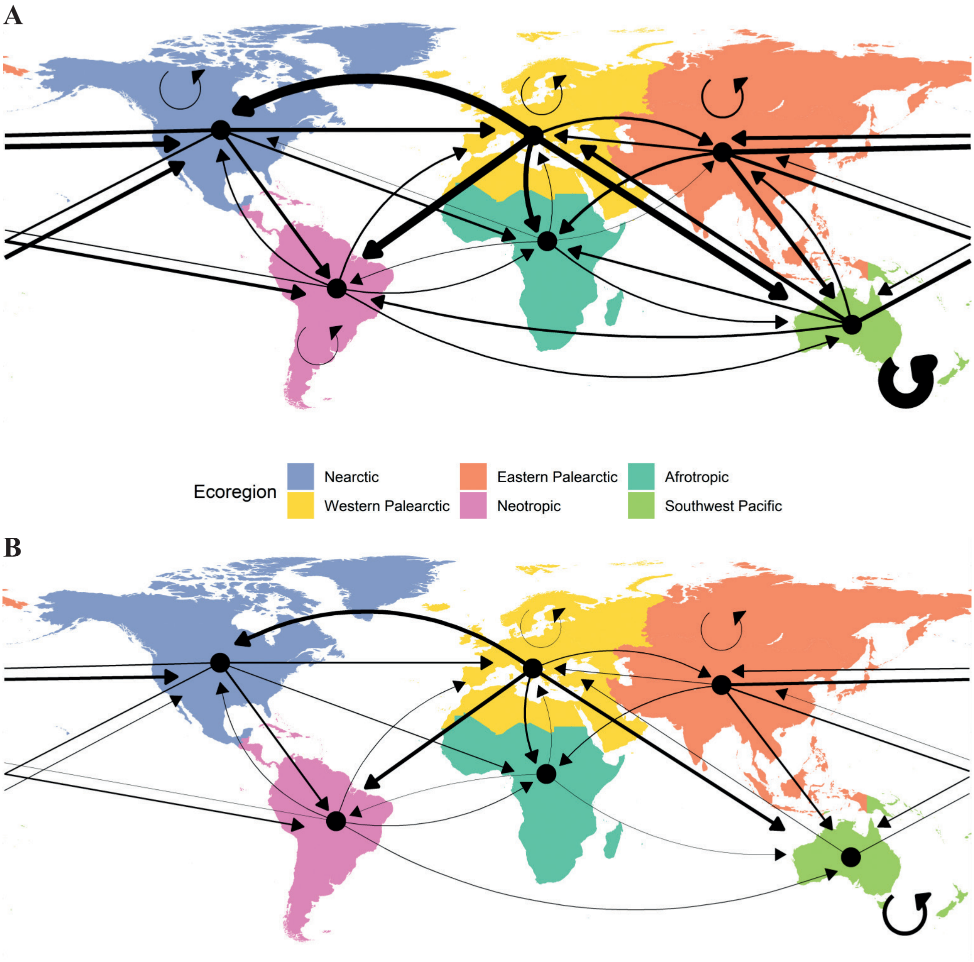

Figure 3. Global movement of all insects feeding on *Pinus radiata* (**A**), and those with non-negligible impact (**B**). The thickness of each arrow is relative to the number of species native to the source biogeographic region established in the destination biogeographic region. Some species had native ranges spanning multiple biogeographic ranges, and in general it is not known if regions were used as bridgeheads, so the arrows represent all possible movements. Note that the East Palearctic includes records from the Indo-Malayan region.

eral other Hemiptera), as well as the defoliators *Helicoverpa armigera* and *Helicoverpa punctigera* (Lepidoptera: Noctuidae, 8,668 and 1,793 interceptions, respectively) and a borer, the bark beetle *Hylurgus ligniperda* (Coleoptera: Curculionidae, 1,766 interceptions) (see Suppl. material 1: table S1 for a complete list of interceptions). The proportion of species that were intercepted was significantly higher among the non-negligible species than for negligible species (One sided, 2-sample test for equality of proportions without continuity correction on log-transformed data, Chi-squared = 8.210, p-value=0.002).

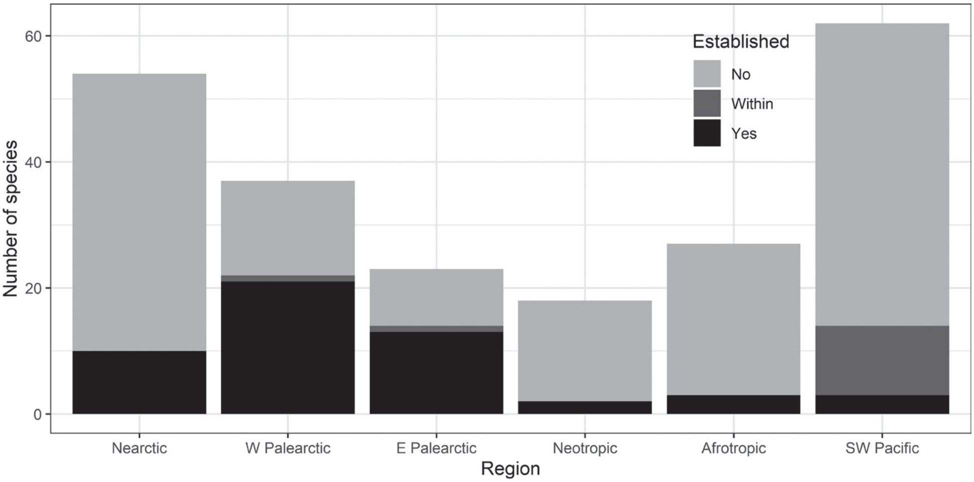

Figure 4. The number of species feeding on *Pinus radiata* that are native to each region and established (or not) outside their native range for non-negligible impact. Cosmopolitan species are excluded. Note, many of the native species from the SW Pacific are native to Australia but established in New Zealand – this is an example of a "Within" region establishment. Also note that some species are native to more than one biogeographic range, e.g., Palearctic species native to Europe and Asia, but this is not shown here. Note that the East Palearctic includes records from the Indo-Malayan region.

Relationships between interceptions and establishments

Among the species feeding on *P. radiata*, the number of intercepted species in a taxonomic group was strongly positively correlated with the number of established species in a family (Pearson's correlation coefficient, using log-transformed data: 0.92, $P<0.001$, Fig. 5). Of the 185 intercepted insect species (considering the international interception dataset from 1995–2021), 104 (56%) have already established somewhere (including cosmopolitan species), and 71% of the 146 species which have already invaded somewhere were intercepted (Fig. 6). Conversely, only 9% of the 464 species that were not intercepted have already invaded somewhere (Fig. 6). This indicates that species that are often intercepted also have a considerably higher likelihood of becoming established. Taxa with particularly high percentages of interceptions include the Scolytinae, Hemiptera and 'other' orders (mainly Orthoptera and Thysanoptera) (Table 1, Fig. 2B). Scolytinae and Hemiptera also have a high percentage of species that became established (Table 1, Fig. 2A).

Relationships between impacts, interceptions and establishments

Of the 28 high-impact species, 15 (54%) have been intercepted internationally (Table 3), and of the 168 species of low-medium impact, 56 (33%) have been intercepted (Suppl. material 1: table S1).

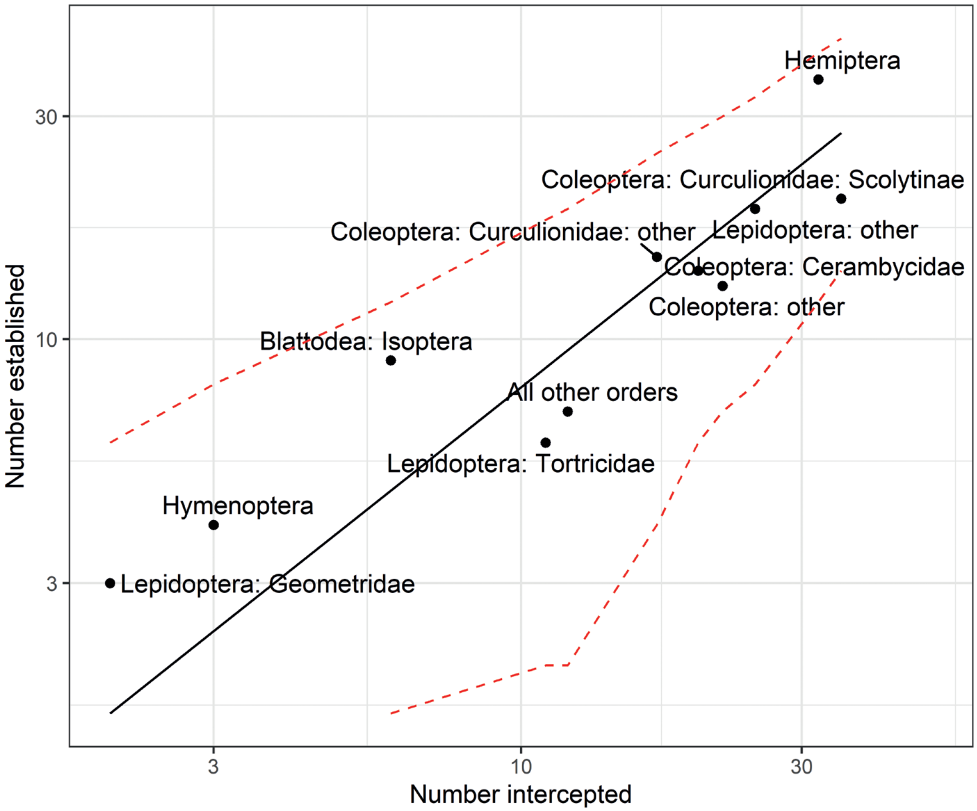

Figure 5. Number of species in the complete all-species pine pest list (649 species total) per taxonomic group that were intercepted and/or established, shown on a log-log scale. The black line represents where the taxa would fall on average if the number of established species was proportional to the number of intercepted species. The dashed lines show the prediction interval within which the taxa groups are expected to fall if establishments occurred at proportionally similar rates to interceptions, based on a Poisson model, alpha = 0.05, with Bonferroni correction accounting for 11 comparisons between taxa.

Of the 196 species with non-negligible impact, 19 species have been intercepted internationally more than 100 times (in decreasing order: *Thrips tabaci*, *Helicoverpa armigera*, *Helicoverpa punctigera*, *Hylurgus ligniperda*, *Hylastes ater*, *Arhopalus ferus*, *Lymantria dispar*, *Ips sexdentatus*, *Heliothrips haemorrhoidalis*, *Epiphyas postvittana*, *Gnathotrichus sulcatus*, *Dendroctonus valens*, *Bradysia impatiens*, *Gnathotrichus retusus*, *Agrotis infusa*, *Nysius vinitor*, *Orthotomicus erosus*, *Arhopalus rusticus* and *Leptoglossus occidentalis*) (Suppl. material 1: table S1). All but four of these 19 species have become established outside their native range (i.e., only *Ips sexdentatus*, *Gnathotrichus sulcatus*, *Gnathotrichus retusus* and *Nysius vinitor* have not yet invaded anywhere, to our knowledge).

A significantly greater percentage (36%) of species with non-negligible impact were intercepted than species with negligible impact (25%¸P=0.002, see details above; Fig. 6), but the difference in impacts between species that had become established (or not) was marginally non-significant (P=0.053, see details above; Fig. 6, Suppl. mate-

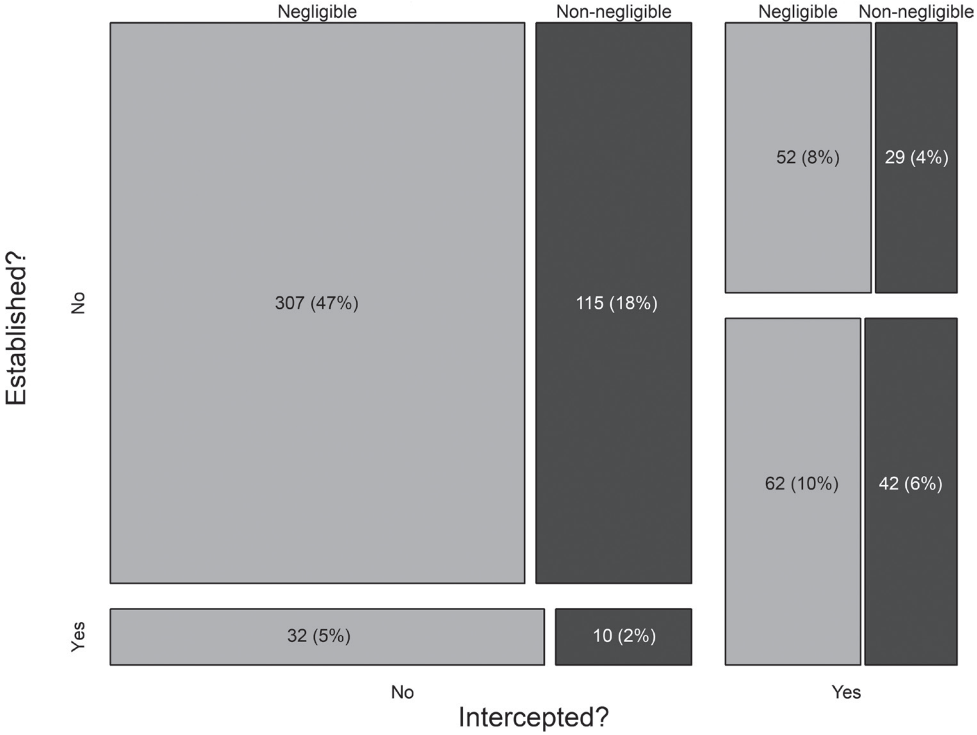

Figure 6. Mosaic plot of the number (and percentages) of species according to their intercepted, establishment, or impact status. Established species are those established in a region outside their native range and are inclusive of cosmopolitan species and species that were subsequently eradicated. Interceptions are based on the international interceptions dataset covering the period 1995–2021. Species with negligible impact on *Pinus radiata* in light grey, those with non-negligible impact (i.e., low-medium and high impact) in dark grey.

rial 3: table S3). For some taxa, differences were observed for both parameters. For example, among Cerambycidae with non-negligible impact, a higher percentage has been intercepted (78%) (compared with only 29% of all Cerambycidae feeding on *Pinus radiata*), and a higher percentage (44%) have established outside their native range (compared with 20% of all Cerambycidae). For Scolytinae feeding on *Pinus radiata*, nearly two thirds (64%) were intercepted, 51% were of non-negligible impact, and 36% are already established (Table 1), significantly higher proportions than for Cerambycidae (Chi-squared tests with Yates' continuity correction, impacts: Chi-squared = 19.19, df = 1, P<0.001; interceptions: Chi-squared = 13.52, df = 1, P< 0.001). This suggests that Scolytinae feeding on *Pinus radiata* are more likely to be intercepted, become established and have negative impacts than Cerambycidae. By contrast, only nine (22%) of the 40 Geometridae on the list have non-negligible impacts (and none fall into the high impact class), and few have been intercepted (5%) or become established (8%) (Table 1).

Results specific to New Zealand and Australia

High-impact species establishments and interceptions

No native high-impact species occur in New Zealand but one such species occurs and is native to Australia (the psychid moth *Hyalarcta huebneri*, Table 3, Suppl. material 1: table S1). Only three and four out of the 28 high-impact insect species are established in New Zealand and Australia, respectively (*Essigella californica*, *Hylastes ater* and *Sirex noctilio* in both, and *Ips grandicollis* in Australia only). Eight other high-impact species (*Hylastes angustatus*, *Lymantria dispar*, *Neodiprion sertifer*, *Orthotomicus erosus*, *Pissodes castaneus*, *Pissodes nemorensis*, *Rhyacionia buoliana* and *Tomicus piniperda*) are established elsewhere outside their native range (but not in New Zealand or Australia) (Suppl. material 1: table S1). Of these established species, all except *Essigella californica*, *Pissodes nemorensis* and *Rhyacionia buoliana* have been recorded in international interceptions (1995–2021) (Suppl. material 1: table S1). Of the internationally established species not yet in New Zealand, *Lymantria dispar* and *Orthotomicus erosus* were the two most frequently intercepted species in the international interceptions (1995–2021 data). In addition, *Ips grandicollis* was also frequently intercepted in New Zealand pre-2000, and is established in Australia and in parts of the E Palearctic. Likewise, *Tomicus piniperda* was also frequently intercepted in New Zealand pre-2000, and has already become established in the Nearctic region. *Ips sexdentatus*, while not yet established outside its native range, has been intercepted internationally (1995–2021) more than 100 times. In addition, ten highly-intercepted species with low-medium impact have already become established in New Zealand and/or Australia (nine and five species, respectively, Suppl. material 1: table S1).

Interceptions and establishment in New Zealand and Australia versus elsewhere

Forty (6%) of the insects on our pine pest list have invaded Australia, and 72 (11%) have invaded New Zealand. Seventy-one percent of the insect species intercepted in New Zealand (irrespective of impacts) have already invaded somewhere, and 58% have already invaded New Zealand. Of the insects intercepted internationally, 32% have invaded New Zealand already. Considering species which have already invaded somewhere, 40% were intercepted in New Zealand between 2000–2017. Of the insects which have already invaded New Zealand, 67% were intercepted in New Zealand between 2000–2017, and 82% were intercepted internationally between 1995–2021.

Feeding guild composition in New Zealand, Australia and elsewhere

The proportions of feeding guilds among native species feeding on *P. radiata* differed significantly between New Zealand and Australia and all other countries and regions (Suppl. material 4: fig. S1) (Pearson's Chi-squared test, Chi-squared = 10.40, df = 6, P = 0.015). In New Zealand, borers represent the largest proportion of native species recorded from *P. radiata* (56%), whereas in Australia there is a high proportion of native defoliators (51%) (Suppl. material 4: fig. S1). In the remaining countries, borers and defoliators are about even.

Discussion

Species recorded on *Pinus radiata* in its native and introduced ranges

With a total of 649 insect species, our compilation of world-wide records of insects feeding on *Pinus radiata* represents a considerable increase over the last such comprehensive effort by Ohmart about 40 years ago (Ohmart 1980, 1981, 1982a, b). Although many of the most damaging insects of *P. radiata* were recognised then, several new threats have emerged. For example, the spongy moth *Lymantria dispar* was known as an occasional defoliator of *P. radiata* but it was considered "of little consequence" (Ohmart 1980). However, major outbreaks of *L. dispar* causing considerable defoliation have been reported recently from Spain (Castedo-Dorado et al. 2016) and we now classify this defoliator as a high-impact species. Another species that has only been recognised in this century as a potentially serious pest of *P. radiata* is the nun moth, *Lymantria monacha* (Withers and Keena 2001). Although other pine species have long been known to suffer sometimes severe defoliation by *L. monacha* in Europe, it was established through laboratory feeding trials that *P. radiata* is a highly suitable host for this defoliator (Withers and Keena 2001). Insects acting as vectors of the pitch canker disease, caused by the fungus *Fusarium circinatum*, are also of particular concern. The severe impacts of this disease on *P. radiata* have been known for some time (Wingfield et al. 2008b), and this is one of the main reasons why *P. radiata* is considered by the IUCN to be 'endangered' in its native range in California (Farjon 2013). However, the important role of insects such as the cone beetle *Conophthorus radiatae* as critical vectors in the transmission of the pathogen has only been appreciated in the last 25 years (Hoover et al. 1996; Brockerhoff et al. 2016). This is the main reason why insects capable of acting as vectors of *F. circinatum* are listed by us as high impact.

High-impact species and their native regions

Altogether, we rated 28 insect species as high impact. Most of these species are native to the Palearctic or Nearctic where pines are native, while only three species originate from parts of the southern hemisphere where *P. radiata* and other pines are planted as non-native species. This is consistent with observations on insects feeding on northern hemisphere plants in southern hemisphere regions such as New Zealand and Australia; these insects originate mainly from the northern hemisphere where their host plants or close relatives are native while comparatively few insects native to the southern hemisphere have colonised these plants which have few or no relatives in the native southern hemisphere flora (Brockerhoff et al. 2010; Harvey et al. 2012). Likewise, few native insects in Europe damage non-native trees without close relatives (i.e., no congeneric species) in the European flora while those with close relatives are colonised by a larger suite of native plant-feeding insects (Branco et al. 2015; Padovani et al. 2020). This applies particularly to insects with a higher degree of host specificity but less so to polyphagous species.

More than half of the high-impact species are from the W Palearctic where they are normally found on European pine species. This means there are more high-impact species that have jumped from other pines to *P. radiata* (with which they have not co-evolved) than high-impact species with long associations with *P. radiata* in its native range. Such new associations between plant-feeding insects and new host plants often cause more severe damage than on their natural hosts. This is well illustrated by the pine processionary moth, *Thaumetopoea pityocampa*, which is considerably more damaging on *P. radiata* planted in Europe than on native European pines (Cobos-Suarez and Ruiz-Urrestarazu 1990), probably because *P. radiata* has not had the opportunity to evolve adaptations against this defoliator, in contrast to southern European pines which have co-evolved with *T. pityocampa*. The European six-toothed bark beetle *Ips sexdentatus* is mainly known as a secondary pest with relatively minor impacts (such as vectoring and facilitating blue-stain fungus infections) but during outbreaks in its native range, it can attack and kill live trees, albeit mainly those that are already weakened by other factors (Cobos-Suarez and Ruiz-Urrestarazu 1990). Other Palearctic species causing high impacts are more problematic in southern hemisphere regions where *P. radiata* has been planted than on *P. radiata* or other pines in Europe. Most notable among these are the Sirex woodwasp, *Sirex noctilio* (Slippers et al. 2015), and the European pine shoot moth, *Rhyacionia buoliana* (Alvarez and Ramirez 1989) which probably benefited from a combination of release from natural enemies (Mitchell and Power 2003; Colautti et al. 2004; Lombardero et al. 2008) and a highly susceptible tree species which has not co-evolved with these insects. High susceptibility in such cases may occur as novel host trees tend to have more limited resistance against non-native insects that are naturally associated with closely related trees, especially when the novel host has no experience with a congeneric native insect (Mech et al. 2019).

Even among the high-impact species native to the Nearctic, several are new associations where *P. radiata* represents a novel host. This includes, most notably, *Ips grandicollis*, the eastern five-spined engraver or five-spined bark beetle, which is native to eastern North America, with its range not sympatric with the natural distribution of *P. radiata*. *Ips grandicollis* invaded Australia where it can be highly damaging in *P. radiata* plantations and sometimes causes tree mortality by itself or in combination with attack by *Sirex noctilio* (Neumann 1987). Another species in this category is *Pissodes nemorensis*, an eastern North American weevil that can damage small trees and also acts as a vector of the pitch canker fungus, both of which have invaded South Africa (Gebeyehu and Wingfield 2003; Brockerhoff et al. 2016).

It is important to note that many of the high-impact species cause more substantial damage on *P. radiata* outside their native range. This applies, for example, to *Sirex noctilio*, *Ips grandicollis*, *Essigella californica* and *Rhyacionia buoliana*. These species probably benefit from freedom of natural enemies compared with the situation in their native regions (Mitchell and Power 2003; Colautti et al. 2004). An equivalent situation may occur in regions where the insect is native but the tree is non-native and probably colonised less by natural enemies as in the case of *T. pityocampa* in *P. radiata* plantations in Europe. In addition, the simplified monoculture environment typical especially of southern hemisphere plantation forests probably has a lower abundance and diversity of natural enemies than more diverse forests which tend to be more common in the native region of *P. radiata* and other pines (Stemmelen et al. 2022).

Native regions of all species (irrespective of impact)

When considering all insects (not only those with high impact), the SW Pacific (i.e., in Australia or New Zealand) was the region with the greatest number of native species feeding on *P. radiata* (42% of all non-cosmopolitan species). This is rather surprising as there are no native pines or other Pinaceae in that region, and consequently, one would not expect a large number of species feeding on *P. radiata*. There are indeed a few native SW Pacific species that have caused noticeable damage in *P. radiata* plantations such as *Pseudocoremia suavis* (Lepidoptera: Geometridae) during outbreaks in New Zealand in the 1950s and 60s (White 1974). However, no outbreaks of this species have been recorded for nearly 50 years, and it is now relatively rare (Berndt et al. 2004), suggesting that these outbreaks were unusual occurrences. Consequently, we have rated *P. suavis* as low-medium impact. The majority of SW Pacific species (about 80%) have no or negligible impacts and the remainder are almost entirely in the low-medium impact category. The reason for the large number of records of species of little relevance is the existence of rigorous forest health surveillance systems in Australia and New Zealand where trees in plantation forests, urban areas, plant nurseries and high-risk sites near ports, airports and transitional facilities (where imports arrive and are cleared by biosecurity officials) are inspected regularly and any insects found are submitted for diagnostic identification (Bulman 2008; Carnegie and Nahrung 2019). These surveillance programmes are designed to detect incursions of non-native insect pests and pathogens as well as damage from known pests and pathogens but they also yield records of native species found on *P. radiata* even though most of these are not damaging. In other regions where *P. radiata* occurs as a native or non-native species, such non-damaging species are not recorded and published to the same extent and publications focus more on species causing more severe damage. Otherwise, the large number of species native to the Nearctic is consistent with this being the region where *P. radiata* and many other pines are native and as a result, there is a large fauna of insects feeding on pines. By contrast, the small number of species native to the E Palearctic may seem somewhat surprising given that there are many native pines and other Pinaceae in that region. However, *P. radiata* is not planted on a large scale in that region, and we are only aware of experimental plantings in China on an area covering hundreds of hectares (Bi et al. 2003, 2008). As the number of species colonising non-native trees is positively correlated with the area planted (Branco et al. 2015), it is plausible that there are comparatively few records of insects feeding on *P. radiata* from China and the E Palearctic (Bi et al. 2008). In addition, our list is probably not entirely complete because sources in languages other than English, especially in the grey literature, may have been missed. This potential bias may have affected especially our records from the E Palearctic and Neotropic with a higher proportion of non-English literature. Furthermore, some regions are under-studied regarding biological invasions, especially in the E Palearctic and Afrotropic regions (Pyšek et al. 2008).

Non-native invasive insects on *P. radiata*

With 146 established non-native insects feeding on *P. radiata*, 22% of all species in our database have already successfully invaded other regions. This large number of invasions is likely to be related to the substantial international trade in pine logs, timber, wood packaging material and propagation material used for the establishment of *P. radiata* plantations in non-native regions. International trade in logs, timber and goods shipped with wood packaging materials such as pallets are important pathways facilitating invasions especially of bark beetles, longhorn beetles and other wood borers (Brockerhoff et al. 2006, 2014; Meurisse et al. 2019; Vilardo et al. 2022), the groups most represented among established non-native species. Trade in live plants used for propagation is another important invasion pathway which is particularly relevant for sap-feeders in the order Hemiptera and defoliating and other Lepidoptera (Liebhold et al. 2012; Meurisse et al. 2019), the second- and third most numerous groups of non-native species feeding on *P. radiata*.

Nearly half of the 28 high-impact species we identified already occur somewhere as established non-native species. However, only six are established in more than one non-native region, indicating a large potential for additional invasions. Also, there are differences between regions in the number of established species. For example, there are only four established high-impact species in the SW Pacific while the remaining 86% are not yet present, which suggests there is considerable benefit in continuing and enhancing biosecurity measures aimed at preventing the arrival and establishment of these species (Sequeira and Griffin 2014; Ormsby and Brenton-Rule 2017).

Border interceptions with imported goods

Nearly a third of the species on our list (29%) were intercepted at least once in the countries for which we could access border interception data. For bark beetles, the percentage of intercepted species was even higher and exceeded 60%. Fifteen of the 28 high-impact species were intercepted, in some cases hundreds of times (e.g., *Ips sexdentatus*, *Lymantria dispar* and *Hylastes ater*). This highlights that pathways exist by which many of these species are transported with international trade and that there is a high potential for additional invasions to occur. Positive relationships between the number of interceptions of species and the probability of invasions have been documented, especially for groups such as bark beetles and longhorn beetles which are often well-identified and are less affected by insufficient identification or omission in interception data (e.g., Brockerhoff et al. 2014; Turner et al. 2020; Nahrung and Carnegie 2021). Our analyses specific to insects feeding on *P. radiata* were consistent with these trends as we found a positive correlation between the number of intercepted species within a taxonomic group and established species in that group (Nahrung and Carnegie 2021). Although some key pathways, such as the use of wood packaging materials, have been mitigated with some effect (Haack et al. 2014), the sheer volume of international trade means that some risk of introduction remains.

Conclusions and outlook

Our compilation and analyses of insects feeding on *P. radiata* has identified numerous species that pose a threat to this tree species in many world regions. Although a large number of damaging native and non-native species have already become associated with *P. radiata* where it is native or has been planted as an introduced tree species, a larger proportion of damaging species could still invade regions where they do not yet occur. Border interceptions of many of these species indicate that pathways exist by which these species move via international trade. Furthermore, there is no sign of saturation of invasions occurring, and additional species continue to be detected as new invaders at a high frequency (Seebens et al. 2018) due to the increasing globalisation of international trade which leads to the expansion of source pools from which potential invaders are being transported. This is partly also noticeable in our analysis where, historically, the W Palearctic has been the dominant source of insects feeding on *Pinus radiata* (particularly of those with non-negligible impact), but this is likely to change with changing global trade relationships.

Although the depth of our global analyses and the large number of species we assessed provide some confidence in our findings and interpretations, there is still considerable uncertainty about the identity of future invaders and damaging species. This stems from the ongoing difficulty of predicting impacts of species that have not yet become established outside their native range. This is illustrated by the cases of species such as *Sirex noctilio*, *Ips grandicollis* and *Essigella californica*, which, based on the low level of damage caused in their native range, would not have been predicted to be so damaging as invaders. Likewise, many insects feeding on other species of pine or Pinaceae probably have the potential to cause damage on *P. radiata* but have not yet crossed paths. For example, in northeast Asia, native species of *Pinus* and other Pinaceae are very common, but there are only few plantings of *P. radiata* and limited research so far on insects feeding on this tree (Bi et al. 2008). It would be very useful to be able to better predict which traits and characteristics predispose such insects to cause substantial damage to *P. radiata*.

New Zealand, Australia and Chile are at a particular risk from such species because of their major reliance on *P. radiata* as a commercial forestry species. Examples of frequently intercepted species that pose a high risk to these and other southern hemisphere countries where *P. radiata* is grown include *Dendroctonus valens* (which has already become established in the E Palearctic), *Hylurgops palliatus* (established in the Nearctic), *Leptoglossus occidentalis* (established in the Afrotropic, W Palearctic, E Palearctic and Neotropic) and *Ips grandicollis* (established in Australia). Although *D. valens* and *L. occidentalis* are only considered low-medium impact on *Pinus radiata*, they are considered highly damaging and have high impact on other *Pinus* species. Furthermore, high-impact species that would probably be highly damaging, but have not yet been intercepted, include *Lymantria monacha*, *Rhyacionia buoliana* and *Thaumetopoea pityocampa*. Finally, there are likely to be many species of 'unknown' risk to *P. radiata* which have not yet come into contact with it yet.

Acknowledgements

The authors are grateful to the late John Bain for his substantial compilation of insects on *Pinus radiata* in New Zealand and internationally which he curated for more than two decades. We would also like to thank Milla Baker for literature searches and curation of the list; Richard Mally, Robert Hoare and Jan Krecek for taxonomic advice; and two anonymous reviewers. This research was funded in part by the New Zealand Ministry of Business Innovation and Employment Strategic Science Investment Funding (CO4X1703, Forest Systems Platform) to Scion and by the HOMED project (http://homed-project.eu/) which received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 771271.

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Supplementary material 1

Pest list of insects feeding on *Pinus radiata* worldwide

Authors: Eckehard G. Brockerhoff, Belinda A. Gresham, Nicolas Meurisse, Helen F. Nahrung, Anouchka Perret-Gentil, Andrew R. Pugh, Stephanie L. Sopow, Rebecca M. Turner

Data type: Occurrences and characteristics of species

Explanation note: Supplementary table providing a detailed list of insects feeding on *Pinus radiata* worldwide, their native range, introduced range (where applicable), impacts, interceptions, and references.

Link: https://doi.org/10.3897/neobiota.84.95864.suppl1

Supplementary material 2

Statistics for Table 2. Statistical tests of proportions out of all species among feeding types for impacts, establishments and interceptions.

Authors: Eckehard G. Brockerhoff, Belinda A. Gresham, Nicolas Meurisse, Helen F. Nahrung, Anouchka Perret-Gentil, Andrew R. Pugh, Stephanie L. Sopow, Rebecca M. Turner

Data type: Statistics

Explanation note: Details on statistical tests of proportions out of all species among feeding types for impacts, establishments and interceptions.

Link: https://doi.org/10.3897/neobiota.84.95864.suppl2

Supplementary material 3

Numbers (and percentages) of species by impact class, and whether or not they have been intercepted (based on the international interceptions dataset covering the period 1995–2021) or established in a region outside their native range.

Authors: Eckehard G. Brockerhoff, Belinda A. Gresham, Nicolas Meurisse, Helen F. Nahrung, Anouchka Perret-Gentil, Andrew R. Pugh, Stephanie L. Sopow, Rebecca M. Turner

Data type: Numbers and percentages of species by impact class

Explanation note: supplementary table providing numbers and percentages of species by impact class, and whether or not they have been intercepted or established in a region outside their native range.

Link: https://doi.org/10.3897/neobiota.84.95864.suppl3

Supplementary material 4

Percentages of species native to a region in each feeding guild, regardless of impact. Those in the "Native country: other" category are species native to other regions but not to Australia or New Zealand.

Authors: Eckehard G. Brockerhoff, Belinda A. Gresham, Nicolas Meurisse, Helen F. Nahrung, Anouchka Perret-Gentil, Andrew R. Pugh, Stephanie L. Sopow, Rebecca M. Turner

Data type: figure on feeding guild percentages

Explanation note: supplementary figure on percentages of species native to a region in each feeding guild (borers, defoliators, sap-feeders and others), regardless of impact.

Link: https://doi.org/10.3897/neobiota.84.95864.suppl4

NeoBiota 84: 169–209 (2023)
doi: 10.3897/neobiota.84.91096
https://neobiota.pensoft.net

RESEARCH ARTICLE

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Worldwide tests of generic attractants, a promising tool for early detection of non-native cerambycid species

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Academic editor: Marc Kenis | Received 31 July 2022 | Accepted 18 November 2022 | Published 18 May 2023

Citation: Roques A, Ren L, Rassati D, Shi J, Akulov E, Audsley N, Auger-Rozenberg M-A, Avtzis D, Battisti A, Bellanger R, Bernard A, Bernadinelli I, Branco M, Cavaletto G, Cocquempot C, Contarini M, Courtial B, Courtin C, Denux O, Dvořák M, Fan J-t, Feddern N, Francese J, Franzen EKL, Garcia A, Georgiev G, Georgieva M, Giarruzzo F, Gossner M, Gross L, Guarneri D, Hoch G, Hölling D, Jonsell M, Kirichenko N, Loomans A, Luo Y-q, McCullough D, Maddox C, Magnoux E, Marchioro M, Martinek P, Mas H, Mériguet B, Pan Y-z, Phélut R, Pineau P, Ray AM, Roques O, Ruiz M-C, Sarto i Monteys V, Speranza S, Sun J-h, Sweeney JD, Touroult J, Valladares L, Veillat L, Yuan Y, Zalucki MP, Zou Y, Žunič-Kosi A, Hanks LM, Millar JG (2023) Worldwide tests of generic attractants, a promising tool for early detection of non-native cerambycid species. In: Jactel H, Orazio C, Robinet C, Douma JC, Santini A, Battisti A, Branco M, Seehausen L, Kenis M (Eds) Conceptual and technical innovations to better manage invasions of alien pests and pathogens in forests. NeoBiota 84: 169–209. https://doi.org/10.3897/neobiota.84.91096

Abstract

A large proportion of the insects which have invaded new regions and countries are emerging species, being found for the first time outside their native range. Being able to detect such species upon arrival at ports of entry before they establish in non-native countries is an urgent challenge. The deployment of traps baited with broad-spectrum semiochemical lures at ports-of-entry and other high-risk sites could be one such early detection tool. Rapid progress in the identification of semiochemicals for cerambycid beetles during the last 15 years has revealed that aggregation-sex pheromones and sex pheromones are often conserved at global levels for genera, tribes or subfamilies of the Cerambycidae. This possibly allows the development of generic attractants which attract multiple species simultaneously, especially when such pheromones are combined into blends. Here, we present the results of a worldwide field trial programme conducted during 2018–2021, using traps baited with a standardised 8-pheromone blend, usually complemented with plant volatiles. A total of 1308 traps were deployed at 302 sites covering simultaneously or sequentially 13 European countries, 10 Chinese provinces and some regions of the USA, Canada, Australia, Russia (Siberia) and the Caribbean (Martinique). We intended to test the following hypotheses: 1) if a species is regularly trapped in significant numbers by the blend on a continent, it increases the probability that it can be detected when it arrives in other countries/continents and 2) if the blend exerts an effective, generic attraction to multiple species, it is likely that previously unknown and unexpected species can be captured due to the high degree of conservation of pheromone structures within related taxa. A total of 78,321 longhorned beetles were trapped, representing 376 species from eight subfamilies, with 84 species captured in numbers greater than 50 individuals. Captures comprised 60 tribes, with 10 tribes including more than nine species trapped on different continents. Some invasive species were captured in both the native and invaded continents. This demonstrates the potential of multipheromone lures as effective tools for the detection of 'unexpected' cerambycid invaders, accidentally translocated outside their native ranges. Adding new pheromones with analogous well-conserved motifs is discussed, as well as the limitations of using such blends, especially for some cerambycid taxa which may be more attracted by the trap colour or other characteristics rather than to the chemical blend.

Keywords
Cerambycidae, early detection, Holarctic, invasion, multi-pheromone blend, pheromone trapping

Introduction

During the last several decades, the unprecedented development of worldwide trade has resulted in increasing translocation and establishment of non-native insects outside their native ranges, with little evidence of saturation (Seebens et al. 2017, 2021). Insect herbivores, accidentally introduced as plant contaminants, appear to be mainly responsible for this sharp increase, at least in Europe (Roques 2010; Pergl et al. 2017). Amongst these non-native herbivores, species associated with woody plants largely dominate, accounting for 76.5% of all herbivore species newly recorded in Europe from 2000 to 2014, while species of importance to agricultural plants and products are a minority (Roques et al. 2016). The increased extent of trade in ornamental plants has been suggested as a major driver of this increase (Liebhold et al. 2012; Eschen et al. 2014; Essl et al. 2015; Roques et al. 2020). Additionally, wood packaging material (e.g. pallets, crating, dunnage) transported with international cargo shipments represents another significant pathway for introduction of non-native phloem- and wood-boring insects (Aukema et al. 2010; Haack et al. 2014; Lovett et al. 2016). An average of 6.1 non-native insect species attacking woody plants became newly established in Europe per year from 2000–2019, compared to 2.4 cases per year from 1950–1970 (Roques et al. 2020). Similar trends were observed in North America (Aukema et al. 2010), New Zealand (Brockerhoff and Liebhold 2017) and at a slower rate in China (Roques et al. 2020).

Another key attribute of this recently-arrived, non-native entomofauna is the increasing presence of “emerging” species, which have not been reported previously as invaders and are not considered to be pests in their native ranges. Arrival of these species probably results from evolving changes in trade routes and imported goods, which leads to accessibility to new pools of species (Seebens et al. 2018). For example, the emerald ash borer, *Agrilus planipennis* Fairmaire, was not considered a significant pest until it invaded North America, where it has caused massive damage (Dang et al. 2022). The same is true for a number of other xylophagous cerambycid beetle species which have recently invaded Europe, such as the Asian mulberry longhorned beetle, *Xylotrechus chinensis* (Chevrolat) (Sarto i Monteys and Torras i Tutusaus 2018), the round-headed apple-tree borer, *Saperda candida* Fabricius (Nolte and Krieger 2008) and the Asian redneck longhorned beetle *Aromia bungii* (Faldermann) (Russo et al. 2020). At first, such species were typically not subject to regulatory measures or strict phytosanitary inspections at borders because their invasive potential had not been recognised. For example, only seven of the 117 non-native insect species that infest woody plants that established in Europe during the period 1995–2012 had been intercepted in such inspections (Eschen et al. 2015). In Australia, 61 of the 135 non-native species established in forests during the period 2003–2016 had never been intercepted, despite relatively intensive border controls (Nahrung and Carnegie 2021). Therefore, the development of new strategies to detect such unanticipated and unregulated species as early as possible is essential to implement rapid and effective eradication or containment measures (Nahrung et al. 2023).

Deployment of traps baited with broad-spectrum semiochemical lures at ports-of-entry (Brockerhoff et al. 2006; Rassati et al. 2014, 2015a; Hoch et al. 2020) or other high-risk sites (e.g. urban wood-waste landfills and industrial sites, Rassati et al. 2015b; Rabaglia et al. 2019) could be one such early detection tool. Given the difficulty of predicting which species may arrive and in what numbers (i.e. propagule pressure), such lures should be efficient even at low population densities and should ideally attract multiple species from different taxa (family, subfamily, tribe). Combining pheromones of several species into blends could be expected to result in such a generic attraction when antagonistic effects amongst blend components are relatively minor, for example, reduced attraction of relatively few species, such that the net effect of blending multiple components is an increase in the number of target taxa detected. The addition of plant volatiles, acting as kairomones, may further enhance the attraction. For instance, a pine specialist, *Monochamus galloprovincialis* (Olivier), was significantly more attracted when its pheromone, monochamol, was combined with volatiles from its pine hosts (Alvarez et al. 2016). Similarly, ethanol had a synergist effect on the capture of species related to broadleaved trees in Eurasia (*Phymatodes testaceus* [L.]; Sweeney et al. 2014; Fan et al. 2019) and in southern USA (Miller et al. 2017). However, the addition of plant volatiles did not affect, either positively or negatively, the captures of several other cerambycid species (Fan et al. 2019). Overall, relationships between host volatiles and cerambycids are probably more dependent on the exploited host and less on insect taxonomy. Potential for using blended lures for detection would be further enhanced if each component of the blend was attractive to multiple related species, i.e. a pheromone or kairomone shared by species within a genus or tribe as occurs in the longhorned beetle family Cerambycidae.

This large family of Coleoptera includes between 34,000 and 38,000 described species (Rossa and Goczał 2021; Tavakilian and Chevillotte 2022). Although recent molecular studies using a multigene approach revealed that the phylogeny at the upper taxonomic levels is not completely resolved and still under debate (Lee and Lee 2020; Nie et al. 2020), Tavakilian and Chevillotte (2022) recognised 13 subfamilies. The subfamily Lamiinae is by far the most diverse with more than 21,000 species, 3,002 genera and 86 tribes, followed by Cerambycinae (> 12,000 species, 1,848 genera, and 119 tribes), Lepturinae (> 1,830 species, 232 genera, 11 tribes), Prioninae (> 1,250 species, 311 genera, 26 tribes) and Spondylidinae (> 150 species, 32 genera, seven tribes); other subfamilies are smaller and much less diverse. Cerambycid larvae of many species develop as endophytic borers concealed beneath the bark of woody plants or, much less frequently, within herbaceous plants. This cryptic lifestyle, coupled with the usual long duration of the hidden larval stages, facilitates the transport of these insects around the world in logs and wooden packing materials (Eyre and Haack 2017), but also via trade in living plants if the plants have a sufficiently large diameter. For example, larvae of the citrus longhorned beetle, *Anoplophora chinensis* (Forster), were detected in Japanese maples, *Acer palmatum* Thunb., shipped to Europe (Eschen et al. 2015). Thus, a steadily increasing number of cerambycid species have become globally important as invasive forest and orchard pests (Venette and Hutchison 2021).

Recent advances in the chemical ecology of cerambycids and, particularly, the identification of volatile pheromones that act as long-range attractants, have provided new tools and opportunities for monitoring invasive woodborers. In total, pheromones or likely pheromones have been identified for more than 400 cerambycid species worldwide (Millar and Hanks 2017). Furthermore, field experiments have shown that these pheromones can be deployed in blends, with a potential generic attraction for both native and non-native species (Hanks et al. 2012; Hanks and Millar 2016; Hanks et al. 2018; Fan et al. 2019; Flaherty et al. 2019; Rassati et al. 2019). Currently, the aggregated data suggest that species in the subfamilies Cerambycinae, Lamiinae and Spondylidinae use male-produced aggregation-sex pheromones to attract both sexes, whereas species in the subfamilies Prioninae and Lepturinae use female-produced pheromones that attract only males (Hanks and Millar 2016). This research has revealed striking patterns in pheromone chemistry. Pheromone components are frequently highly conserved amongst species within genera, tribes and even at the subfamily level (Hanks and Millar 2013, 2016). For example, in the subfamily Lamiinae, hydroxyethers are used as aggregation-sex pheromones by many species native to different continents. Thus, 2-(undecyloxy)ethanol, or monochamol, is a pheromone component shared by European, North American and Asian species in the genus *Monochamus*, all of which vector the pine wood nematode (*Bursaphelenchus xylophilus* [Steiner & Buhrer]) (Pajares et al. 2010; Hanks and Millar 2016; Boone et al. 2018; Lee et al. 2018). In addition, field trials in southern China showed that four lamiine species in genera other than *Monochamus* were attracted to monochamol (Wickham et al. 2014). A number of other compounds are widely shared amongst species within a given subfamily in different world regions. For example, terpenoids such as fuscumol ([*E*]-6,10-dimethyl-5,9-undecadien-2-ol) and its acetate, are aggregation sex-pheromone components for many species in the subfamily Spondylidinae and Laminae (Mitchell et al. 2011; Hanks and Millar 2016). In contrast, many species in the subfamily Cerambycinae from different continents utilise short-chain (6–10 carbon) hydroxyketones, such as 3-hydroxyalkan-2-ones and 2-hydroxyalkan-3-ones and the corresponding *syn*- and *anti*-2,3-alkanediols as aggregation-sex pheromones (Hanks and Millar 2016). Prionic acid ([3*R*,5*S*]-3,5-dimethyldodecanoic acid) similarly appears to be shared as a sex pheromone by several genera of the subfamily Prioninae on different continents (Barbour et al. 2011; Wickham et al. 2016a). This sharing of pheromone components by species in different world regions suggests that traps baited with these compounds have a good chance of detecting non-native, phylogenetically-related invaders that are introduced to another continent. Moreover, combining several of these pheromone components in a single blend has the potential to detect a broader range of species.

During the last 10 years, the generic effectiveness of such multi-component blends has been tested on different continents, but using different pheromone combinations, either alone or in combination with kairomones, such as ethanol and α-pinene (e.g. Miller et al. 2017; Fan et al. 2019). In Illinois, USA, Hanks et al. (2012) first tested a six-component blend, which included racemic 3-hydroxyhexan-2-one, *syn*- and *anti*-2,3-hexanediols, fuscumol, fuscumol acetate, monochamol and racemic 2-methylbutan-1-ol. Ten cerambycid species were caught in significant numbers in these trials, including four species in the subfamily Cerambycinae and six in the subfamily Lamiinae. Hanks et al. (2018) then tested this 6-component blend at a larger scale in several regions of the USA, adding both prionic acid and plant volatiles to the traps. The pheromone blend attracted about twice as many species as any of the individual components and the species attracted by the blend included three subfamilies, whereas individual components attracted species within only one subfamily. The inclusion of prionic acid also resulted in the additional captures of *Prionus* spp. which were not trapped by the previous six-pheromone blend. In a natural reserve in Yunnan (China), Wickham et al. (2021) trapped 71 species with another generic lure comprised of six components, three of which were the same as those used in the USA (*anti*-2,3-hexanediol, racemic 3-hydroxyhexan-2-one and monochamol). In France, using an 8-pheromone blend consisting of the same compounds as Hanks et al. (2018) to which was added geranylacetone targeting Spondylininae (Halloran et al. 2018), Fan et al. (2019) trapped 118 species, of which 114 were native species that represented 48% of the French cerambycid fauna. Trapping more than 50% of the species in 25 of the 41 cerambycid tribes present in the country indicates a considerable generic attraction of this 8-pheromone blend, significantly higher than an earlier trial which tested a blend of four pheromones. By contrast, unbaited control traps deployed in the same French sites caught very few species. Other trials of potentially generic blends, including fewer or different compounds, were carried out in Russia (Sweeney et al. 2014), Australia (Hayes et al. 2016), Brazil (Silva et al. 2017), Poland, Italy and Canada (Flaherty et al. 2019; Rassati et al. 2019, 2021). Results from Australia differed from those reported in other continents because the tested blend attracted no more species than 3-hydroxyhexan-2-one alone (Hayes et al. 2016).

When using multi-pheromone blends, antagonistic effects might occur with either pheromone components or host plant volatiles (e.g. Hanks et al. 2018; Rassati et al. 2021). The North American species *Neoclytus acuminatus acuminatus* (F.), for example, was strongly attracted by *syn*-2,3-hexanediol, but the addition of racemic 3-hydroxyhexan-2-one to the latter pheromone interrupted attraction (Rassati et al. 2021). Addition of host plant volatiles, such as ethanol, significantly enhanced attraction of some cerambycid species (Sweeney et al. 2014; Miller et al. 2017; Hanks et al. 2018), but, with the exception of *P. testaceus*, had little effect on catch of cerambycid species in other studies (Fan et al. 2019). However, as long as inhibition did not completely prevent attraction, one trap with a multi-pheromone lure may still be somewhat more cost-effective than deploying multiple traps baited with individual lures. This can be assessed by a cost-benefit analysis, i.e. estimating the labour and materials costs of deploying and servicing a network of traps baited with single components, versus the costs of deploying and servicing a single trap baited with a blend of the same components.

Results of these different experiments on various continents stimulated us to propose a worldwide trapping programme using a standardised 'generic' 8-pheromone blend in all countries/trapping sites. The blend included the following compounds known to be widely shared amongst cerambycids of related taxa: fuscumol, fuscumol acetate, monochamol, geranylacetone, *anti*-2,3-hexanediol, 3-hydroxyhexan-2-one (C6-ketol), 2-methylbutan-1-ol and prionic acid. The programme relied on the following hypotheses: 1) if a species is attracted in significant numbers by the blend in a region, it increases the probability that it can be detected when it arrives at ports-of-entry in other regions and 2) if the blend exerts an effective, generic attraction to multiple species, it is likely that previously unknown and unexpected species can be captured due to the high degree of conservation of pheromone structures within related taxa, as described above. Our overarching objective was to build a global database of cerambycid species trapped by the 8-pheromone blend. To this end, field trials were conducted during 2018–2021 using operational protocols that were standardised as much as possible at all sites worldwide to cover simultaneously or sequentially 13 European countries, 10 Chinese provinces and some regions of the USA, Canada, Australia, Russia (Siberia) and the Caribbean. Over the course of the study, we also tested the possibility of adding new compounds to enlarge the pool of species trapped. Therefore, in 2020, two additional pheromones, the sex-aggregation pheromones trichoferone (a hydroxyketone pheromone of the velvet longhorned beetle, *Trichoferus campestris* (Faldermann) (Ray et al. 2019) and (*E*)-2-*cis*-6,7-epoxynonenal, the pheromone of the invasive species *A. bungii* (Xu et al. 2017), were added to the original 8-pheromone blend and tested in France and China. In addition, ethanol and α-pinene were included in most trials as synergists for some cerambycids.

Materials and methods

Study sites

The successive or parallel development of three European research projects (HOMED, MULTITRAP, SAMFIX) and two French projects (CANOPEE, PORTRAP) during 2018–2021 allowed us to carry out field trials at 302 sites distributed as follows: 244 in Europe (164 in France, 22 in Italy, 13 in Spain and Switzerland, 6 in Portugal, 5 in Austria and England, 4 in Greece and Slovenia, 3 in the Netherlands, 2 in Bulgaria and the Czech Republic and 1 in Sweden), 38 in Asia (35 in China and three in Siberia, Russia), 11 in North America (10 in the USA and one in Canada), five in the Caribbean (Martinique) and four in Australia (see Table 1 and Suppl. material 1 for details per country, coordinates and the relevant research project). A total of 1308 traps were deployed in stands of broadleaved and/or coniferous trees in natural or managed environments, but also within and nearby potential ports-of-entry (maritime and fluvial ports, airports, national markets). Experiments in these latter sites usually included two traps placed on trees planted within the port and two traps placed in woody areas located within a 1 km-radius from the port, except in 2019 when a larger experiment was carried out (see below).

In 2018, trials were limited to four European countries (Austria, England, France, the Netherlands), including 41 sites with 143 traps. The 2019 trials were much more extensive and involved 12 European countries (the four from 2018, supplemented by Bulgaria, the Czech Republic, Greece, Italy, Portugal, Spain, Sweden and Switzerland), five provinces of China (Beijing, Hebei, Liaoning, Yunnan and Zhejiang), two States of the USA (Michigan and Ohio) and one site in Canada (Nova Scotia), resulting in a total of 79 sites and 626 traps. These 2019 trials included a large trapping programme targeting semi-urban forests located close to ports-of-entry in Europe, USA and Canada where 16 (Czech Republic, Portugal, Sweden) or 32 traps (France, Italy, Nova Scotia, Ohio, Switzerland) were deployed at each target site. The 2020 trials were substantially impacted by the COVID-19 pandemic, but were carried out for at least a part of the spring–summer season in six European countries (France, Greece, Italy, Portugal, Spain and Switzerland), eight provinces of China (those of 2019, except Beijing, to which were added Hunan, Inner Mongolia, Jiangxi and Shandong) and extended to Australia (New South Wales) and the Caribbean (Martinique), resulting in a total of 78 sites and 256 traps. The 2021 trials were deployed in the same countries as in 2020, supplemented by an additional European country (Slovenia), Russia (Siberia) and an additional province of China (Gansu), resulting in a total of 104 sites and 283 traps.

Trapping protocol and 8-pheromone blend

Trials at all sites used either multifunnel or cross-vane panel traps supplied by different companies depending on the country (Econex, Spain; ChemTica Internacional, S.A., Heredia, Costa Rica; Alpha Scents Inc., West Linn, Oregon, USA). Cross-vane traps used in Italy (Colli Euganei area) in 2019 were hand-made (see Cavaletto et al. 2021 for details). Black traps were generally deployed, but other colours were also used in France, Italy, Nova Scotia, Ohio and Switzerland (see Table 1). To improve trapping efficiency, all traps were coated with Fluon (AGC Chemicals Europe Ltd., Thornton Cleveleys, UK) diluted in 1:6 in water (Graham et al. 2010). In forests, traps were usually hung from tree branches or between two trees in the lower canopy, at ~ 3–5 m high. Exceptions are some of the countries involved in the 2019 trapping programme targeting semi-urban forests (i.e. Czech Republic, France, Canada, Italy, Portugal, Sweden, Switzerland and the USA) where traps were placed both in the understory and in the upper canopy (> 20 m), the trial carried out in Italy (Colli Euganei area) in 2019 where traps were placed at 5–7 m above the ground and the trappings carried out in some forests of north-central France during 2019–2021 where traps were placed in the upper canopy (> 20 m). Each trap was separated from the next by 50 m at least and traps were preferentially placed at the forest edge. In ports-of-entry, the traps were attached to branches of available trees, at least 2 m above ground with a minimum distance between traps of 100 m. A similar design was used for the traps placed within the 1 km-radius from the ports-of-entry. Duration of trap deployments were variable amongst sites and years, but in the Northern Hemisphere, experiments were mostly conducted from mid-April at the earliest to mid-October at the latest, except in 2020 when the COVID-19 pandemic delayed the onset of trapping until mid-June or mid-July. In Australia, traps were deployed from December to March, depending on the year. Detailed trap heights and trapping duration by site are provided in the Suppl. material 1.

All lures were prepared by INRAE before being shipped to all study participants. These lures consisted of a blend designed by Fan et al. (2019), which contains eight cerambycid pheromones (fuscumol, fuscumol acetate, monochamol, 3-hydroxyhexan-2-one, *anti*-2,3-hexanediol and 2-methylbutan-1-ol, all at 50 mg/ml; geranylacetone-25 mg/ml; and prionic acid- 0.5 mg/ml; Table 2) dissolved in isopropanol as a carrier to a total volume of 1 ml per lure. The blend composition was expected to attract a large number of cerambycid subfamilies and tribes according to Hanks et al. (2012), (Table 2). Dispensers consisted of a cotton dental pad (to serve as a reservoir and stabilise release rate) placed into a polyethylene sachet (Minigrip, 4 cm × 6 cm × 60 µ; Dutscher, Brumath, France) and dosed with 1 ml of the lure solution. The release rate was estimated by Fan et al. (2019) as 0.0263 ± 0.002 g/d under 20 °C conditions. A dispenser was hung in the centre of each trap.

All primary compounds were obtained from ChemTica Internacional, except prionic acid, which was purchased from Alpha Scents Inc. Commercial high release rate ethanol (100 ml dose, 96% purity, release rate 2 g/day at 20 °C; Econex, Spain) and α-pinene lures (25 ml dose, 98% purity, release rate 0.3 g/day at 20 °C; Econex, Spain) were added to traps in most trials (1076 of the 1308 traps; Table 1). These compounds are known to synergise attraction of some cerambycid species to their pheromones (e.g. Allison et al. 2012; Ryall et al. 2015; Collignon et al. 2016; Miller et al. 2017), but are mildly repellent for others (Collignon et al. 2016). Trap catches were tabulated every 3–4 wk, at which time the pheromone lures were replaced. The ethanol UHR and α-pinene lures, when added, were replaced every 6 wk.

Table 1. Summary of the trapping design per country from 2018 to 2021. Research project: C: CANOPEE; H: HOMED; M: MULTITRAP; P: PORTRAP; S: SAMFIX. Blend: #8: 8-pheromone blend; #8+ET: 8-pheromone blend + ethanol UHR; #8+AP+ ET: 8-pheromone blend + α-pinene + ethanol UHR; #10+ AP+ET: 10- pheromone blend + α-pinene + ethanol UHR. Trap type: MF: multifunnel; CV: crossvane. Trap colour: B: black; G: Green; BG: Black base and green top; P: Purple; Y: Fluorescent yellow; others: brown, blue, red, grey (corresponding to data collected by Cavaletto et al. 2021). Collection type: W: wet; D: dry. Note that the sites could be similar in a country from one year to the next.

Region	Year	Country/ Province	Project	No Sites	No Traps	Blend				Trap type		Trap color						Collection Type	
						#8	#8+ET	#8+AP+ET	#10+AP+ET	MF	CV	B	G	BG	P	Y	Other	W	D
Europe	2018	Austria	M	3	20	10	0	10	0	10	10	20	0	0	0	0	0	0	20
Europe	2018	England	M	3	8	4	0	4	0	6	2	8	0	0	0	0	0	0	8
Europe	2018	France	M,P	32	97	18	0	79	0	41	56	78	7	7	3	0	0	0	97
Europe	2018	Netherlands	M	3	18	6	0	12	0	9	9	9	0	0	0	0	0	18	0
Europe	2019	Austria	H	2	4	0	0	4	0	4	0	4	0	0	0	0	0	0	4
Europe	2019	Bulgaria	H	2	3	0	0	3	0	3	0	3	0	0	0	0	0	0	3
Europe	2019	Czech Rep	H	2	32	0	0	32	0	32	0	32	0	0	0	0	0	32	0
Europe	2019	England	H	2	4	2	0	2	0	4	0	4	0	0	0	0	0	0	4
Europe	2019	France	C, H, P, S	26	170	0	0	170	0	164	6	89	59	13	9	0	0	88	82
Europe	2019	Greece	H	2	3	0	0	3	0	3	0	3	0	0	0	0	0	0	3
Europe	2019	Italy	H, S	19	192	0	128	64	0	64	128	48	48	0	16	16	64	192	0
Europe	2019	Portugal	H	2	32	0	0	32	0	32	0	32	0	0	0	0	0	32	0
Europe	2019	Spain	H	1	2	0	0	2	0	2	0	2	0	0	0	0	0	0	2
Europe	2019	Sweden	H	1	16	0	0	16	0	16	0	16	0	0	0	0	0	16	0
Europe	2019	Switzerland	H	2	64	0	0	64	0	64	0	32	32	0	0	0	0	64	0
Europe	2020	France	C, H, P, S	48	166	2	0	64	100	160	6	77	35	17	17	17	0	18	148
Europe	2020	Greece	H	1	1	0	0	1	0	1	0	1	0	0	0	0	0	0	1
Europe	2020	Italy	H	2	8	0	0	8	0	8	0	2	4	0	2	0	0	0	8
Europe	2020	Portugal	H	2	8	0	0	8	0	8	0	4	4	0	0	0	0	0	8
Europe	2020	Spain	H	6	22	0	0	22	0	22	0	22	0	0	0	0	0	0	22
Europe	2020	Switzerland	H	2	4	0	0	4	0	4	0	4	0	0	0	0	0	4	0
Europe	2021	France	C, H, P, S	58	165	2	0	58	105	162	3	98	42	0	14	11	0	30	135
Europe	2021	Greece	H	1	1	0	0	1	0	1	0	1	0	0	0	0	0	0	1
Europe	2021	Italy	H	1	4	0	0	4	0	4	0	0	2	0	2	0	0	0	4
Europe	2021	Portugal	H	2	8	0	0	8	0	8	0	4	4	0	0	0	0	0	8
Europe	2021	Slovenia	H	4	18	0	0	18	0	18	0	18	0	0	0	0	0	0	18
Europe	2021	Spain	H	6	16	0	0	16	0	16	0	12	4	0	0	0	0	0	16
Europe	2021	Switzerland	H	9	19	8	0	11	0	19	0	8	11	0	0	0	0	11	8
Europe	Total			244	1105	52	128	720	205	885	220	631	252	37	63	44	64	505	600
Asia	2019	China/Beijing	H	1	3	0	0	3	0	3	0	3	0	0	0	0	0	0	3

Region	Year	Country/ Province	Project	No Sites	No Traps	Blend				Trap type		Trap color						Collection Type	
						#8	#8+ET	#8+AP+ET	#10+AP+ET	MF	CV	B	G	BG	P	Y	Other	W	D
Asia	2019	China/Hebei	H	2	6	0	0	6	0	6	0	6	0	0	0	0	0	0	6
Asia	2019	China/InnerMongolia	H	1	3	0	0	3	0	3	0	3	0	0	0	0	0	0	3
Asia	2019	China/Liaoning	H	1	3	0	0	3	0	3	0	3	0	0	0	0	0	0	3
Asia	2019	China/Yunnan	H	1	4	0	0	4	0	4	0	4	0	0	0	0	0	0	4
Asia	2019	China/Zhejiang	H	1	3	0	0	3	0	3	0	3	0	0	0	0	0	0	3
Asia	2020	China/Hebei	H	4	12	0	0	12	0	12	0	12	0	0	0	0	0	0	12
Asia	2020	China/Hunan	H	1	3	0	0	3	0	3	0	3	0	0	0	0	0	0	3
Asia	2020	China/Inner Mongolia	H	2	6	0	0	6	0	6	0	6	0	0	0	0	0	0	6
Asia	2020	China/Jiangxi	H	2	6	0	0	6	0	6	0	6	0	0	0	0	0	0	6
Asia	2020	China/Liaoning	H	1	3	0	0	3	0	3	0	3	0	0	0	0	0	0	3
Asia	2020	China/Shandong	H	1	3	0	0	3	0	3	0	3	0	0	0	0	0	0	3
Asia	2020	China/Yunnan	H	1	4	0	0	4	0	4	0	4	0	0	0	0	0	0	4
Asia	2020	China/Zhejiang	H	1	3	0	0	3	0	3	0	3	0	0	0	0	0	0	3
Asia	2021	China/Beijing	H	1	3	0	0	0	3	3	0	3	0	0	0	0	0	0	3
Asia	2021	China/Gansu	H	1	3	0	0	3	0	3	0	3	0	0	0	0	0	0	3
Asia	2021	China/Hebei	H	2	6	0	0	6	0	6	0	6	0	0	0	0	0	0	6
Asia	2021	China/Hunan	H	1	3	0	0	3	0	3	0	3	0	0	0	0	0	0	3
Asia	2021	China/Inner Mongolia	H	2	6	0	0	3	3	6	0	6	0	0	0	0	0	0	6
Asia	2021	China/Jiangxi	H	2	6	0	0	6	0	6	0	6	0	0	0	0	0	0	6
Asia	2021	China/Lioaning	H	1	3	0	0	3	0	3	0	3	0	0	0	0	0	0	3
Asia	2021	China/Shandong	H	1	3	0	0	3	0	3	0	3	0	0	0	0	0	0	3
Asia	2021	China/Yunnan	H	3	5	0	0	0	5	5	0	5	0	0	0	0	0	0	5
Asia	2021	China/Zhejiang	H	1	3	0	0	3	0	3	0	3	0	0	0	0	0	0	3
Asia	2021	Russia/Siberia	H	3	3	3	0	0	0	3	0	3	0	0	0	0	0	0	3
Asia	Total			38	106	3	0	92	11	106	0	106	0	0	0	0	0	0	106
North America	2019	USA/ Michigan	H	9	18	18	0	0	0	18	0	18	0	0	0	0	0	18	0
North America	2019	USA/ Ohio	H	1	32	0	0	32	0	32	0	16	16	0	0	0	0	32	0
North America	2019	Canada/ Nova Scotia	H	1	32	0	0	32	0	32	0	16	16	0	0	0	0	32	0
North America	Total			11	82	18	0	64	0	82	0	50	32	0	0	0	0	82	0
The Caribbean	2020	France/ Martinique	H	2	3	3	0	0	0	3	0	3	0	0	0	0	0	0	3
The Caribbean	2021	France/ Martinique	H	3	4	4	0	0	0	4	0	4	0	0	0	0	0	0	4
The Caribbean	Total			5	7	7	0	0	0	7	0	7	0	0	0	0	0	0	7
Australia	2020	Australia	H	2	4	0	0	4	0	4	0	4	0	0	0	0	0	0	4
Australia	2021	Australia	H	2	4	0	0	4	0	4	0	4	0	0	0	0	0	0	4
Australia	Total			4	8	0	0	8	0	8	0	8	0	0	0	0	0	0	8
							0												
Grand total				302	1308	80	128	884	216	1088	220	802	284	37	63	44	64	587	721

Table 2. Composition of the 8-pheromone and 10-pheromone blends and targeted sex and cerambycid tribes.

Blend	Compound	Amount/lure (mg/ml)	Target Sex	Target subfamily				Target tribe/ genus	References
				Cerambycinae	Lamiinae	Aseminae	Prioninae		
8-pheromones	Racemic 3-hydroxyhexan-2-one (C6-ketol)	50	M/F	X				Callidiini	Millar et al. (2018)
				X				Clytini	Hanks and Millar (2013), Wickham et al. (2014), Bobadoye et al. (2019)
				X				Hesperophanini	unpub data JGM
				X				Hylotrupini	Reddy et al. (2005)
8-pheromones	Racemic 2-methylbutan-1-ol	50	M/F	X				Callidiini	Hanks et al. (2018)
8-pheromones	2*R**,3*S**-2,3-hexanediol	50	M/F	X				Clytini	Hanks and Millar (2013), Wickham et al. (2014)
8-pheromones	Racemic fuscumol + fuscumol acetate	50+ 50	M/F	X				Obriini	Millar et al. (2018)
					X			Acanthocinini	Millar et al. (2018)
					X			Acanthoderini	Hanks and Millar (2013)
						X		Asemini	Millar et al. (2018)
8-pheromones	Monochamol	50	M/F		X			Monochamini	Hanks et al. (2018)
					X			Lamiini	Wickham et al. (2014)
8-pheromones	Geranylacetone	25	M/F		X			Acanthocinini	Meier et al. (2016, 2019)
						X		Asemini	Halloran et al. (2018)
8-pheromones	Prionic acid (4 stereoisomers)	05	M				X	Prionini	Barbour et al. (2011)
10-pheromones	Racemic trichoferone	25	M/F	X				*Trichoferus*	Ray et al. (2019)
10-pheromones	(*E*)-2-*cis*-6,7-epoxynonenal	50	M/F	X				*Aromia*	Xu et al. (2017)

In most cases, the trapped insects were killed using a section of mesh impregnated with α-cypermethrin insecticide (Storanet, BASF Pflanzenschutz Deutschland, Germany) placed into the trap basins, whose bottoms had been replaced with a wire mesh to allow drainage and to keep specimens dry. However, in the targeted 2019 experiment in forests near ports-of-entry and in the Colli Euganei area (Italy), "wet" trap basins were used, containing water-diluted propylene glycol (50%) to act as a surfactant and preservative. In the trials conducted in Ohio and Michigan, trap collection cups were filled with ~ 200–400 ml of undiluted propylene glycol.

Trapped cerambycids were identified to species by local specialists or sent to INRAE for identification. However, specimens trapped in Australia could not be sent due to restrictions by the customs agency and so most could only be identified to the genus level. Nomenclature used in this article follows the reference checklist of the world database Titan (Tavakilian and Chevillotte 2022).

Preliminary tests of a 10-pheromone blend

In 2020 and 2021, two additional pheromones, trichoferone (the pheromone of *T. campestris*) and (*E*)-2-*cis*-6,7-epoxynonenal (the pheromone of *A. bungii*), were added to the 8-pheromone lures used in France and China, to test for a possible increase in monitoring effectiveness with a 10-pheromone blend (Table 2). Both compounds were synthesised by YFZ and JGM at the University of California, Riverside, using previously-reported syntheses (Ray et al. 2019; Xu et al. 2017, respectively). Lure preparation and insect collection procedures were similar to those described above. Captures were compared with those of the 8-pheromone blend at six sites in France during 2020 by deploying five pairs of traps baited with each blend at each site. The traps were spaced ~ 100 m apart and rotated at each insect collection, which enabled the number of collection dates at each site to be used as replicates. The Student *t*-test for paired samples was then applied to compare the number of species trapped by each lure.

Results

A total of 78,321 longhorned beetles were trapped, representing 376 species, including 373 Cerambycidae, two Vesperidae and one Disteniidae species (Table 3). The cerambycids belonged to eight subfamilies, including 156 species of Cerambycinae, 102 species of Lamiinae, 78 species of Lepturinae, 21 species of Spondylidinae, 12 species of Prioninae, two species of Necydalinae and one species of Parandrinae (Fig. 1). Captures comprised 60 tribes, with 10 tribes including more than nine species trapped on different continents; in decreasing order the tribe Clytini (64 spp.), followed by Lepturini (44 spp.), Rhagiini (32 spp.), Acanthocinini (31 spp.), Callidiini (20 spp.), Monochamini (18 spp.), Saperdini (10 spp.) and Aseminii, Pogonocherini and Prionini (nine spp.

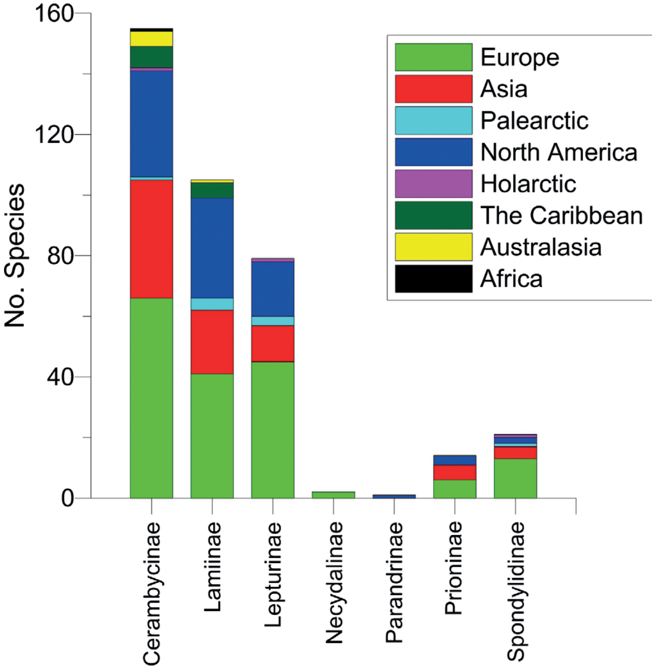

Figure 1. Number of species trapped per subfamily and their region of origin.

each; Fig. 2). Generally, fewer species were trapped in the Caribbean and Australia, where only a limited number of traps had been deployed. Some of the captured species belonged to tribes other than those targeted, such as Callidiopini (*Curtomerus flavus* [F.] in Martinique and *Bethelium* sp. in Australia), Eburiini (*Eburia* spp. in Martinique) and Tillomorphini (*Gourbeyrella madininae* Chalumeau & Touroult in Martinique).

Most tribes included species from the same genera trapped on different continents (Fig. 2). For example, 19 species of the Clytini genus *Xylotrechus* were captured, including 10 in Asia, five in Europe and four in North America. In the same tribe, 12 species of *Chlorophorus* were captured, of which eight were caught in Europe and four in Asia. A total of 12 *Monochamus* species (Lamiinae, Monochamini) were trapped, including five species in Asia, four in North America and three in Europe. The Callidiini genus *Phymatodes* was represented by 11 species, including seven in Europe and four in North America. A number of these species had not been trapped before by any semiochemically-baited trap (e.g. *X. chinensis*, *Chlorophorus glabromaculatus* [Goeze] and *Phymatodes pusillus* [F.]).

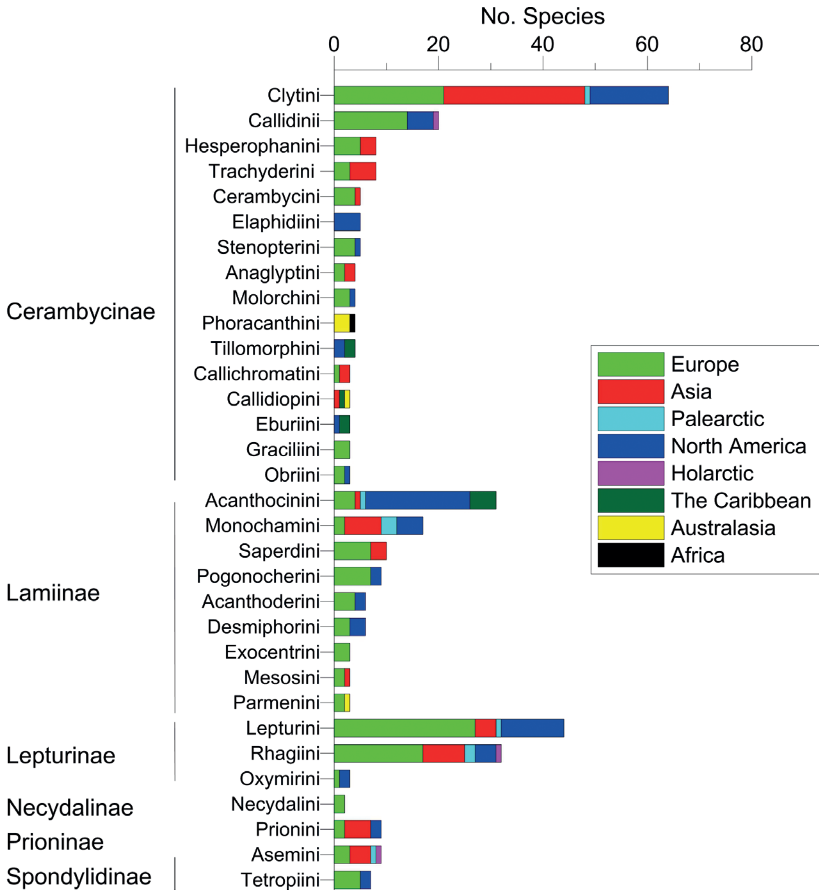

Figure 2. Number of species trapped per tribe in decreasing order and their native region. Only the tribes where > 3 spp were captured are shown.

Table 3. Names of trapped species, origin and specimen numbers captured per continent. Species in bold were trapped in non-native continents.

Subfamily	Tribe	Species	Origin	Europe	Asia	North America	The Caribbean	Australia	Total
Cerambycinae	Anaglyptini	*Anaglyptus gibbosus* (Fabricius, 1787)	Europe	105	0	0	0	0	105
Cerambycinae	Anaglyptini	*Anaglyptus mysticus* (Linnaeus, 1758)	Europe	116	0	0	0	0	116
Cerambycinae	Anaglyptini	*Cyrtophorus verrucosus* (Olivier, 1800)	North America	0	0	197	0	0	197
Cerambycinae	Anaglyptini	*Microclytus compressicollis* (Laporte de Castelnau & Gory, 1841)	North America	0	0	2	0	0	2
Cerambycinae	Bothriospilini	*Chlorida festiva* (Linnaeus, 1758)	Caribbean	0	0	0	0	2	2
Cerambycinae	Callichromatini	*Aromia bungii* Faldermann, 1835	Asia	0	25	0	0	0	25
Cerambycinae	Callichromatini	*Aromia moschata* (Linnaeus, 1758)	Europe	30	0	0	0	0	30
Cerambycinae	Callichromatini	*Aromia moschata orientalis* Plavilstshikov, 1933	Asia	0	3	0	0	0	3
Cerambycinae	Callidiini	*Callidium aeneum* (Degeer, 1775)	Holarctic	120	79	0	0	0	199
Cerambycinae	Callidiini	*Callidium violaceum* (Linnaeus, 1758)	Europe	3	0	0	0	0	3
Cerambycinae	Callidiini	*Lioderina linearis* (Hampe, 1870)	Europe	3	0	0	0	0	3
Cerambycinae	Callidiini	*Phymatodes aereus* (Newman, 1838)	North America	0	0	14	0	0	14
Cerambycinae	Callidiini	*Phymatodes alni* (Linnaeus, 1767)	Europe	2295	0	0	0	0	2295
Cerambycinae	Callidiini	*Phymatodes amoenus* (Say, 1824)	North America	0	0	3100	0	0	3100
Cerambycinae	Callidiini	*Phymatodes dimidiatus* (Kirby, 1837)	North America	0	0	55	0	0	55
Cerambycinae	Callidiini	*Phymatodes fasciatus* (Villers, 1789)	Europe	6	0	0	0	0	6
Cerambycinae	Callidiini	*Phymatodes glabratus* (Charpentier, 1825)	Europe	3	0	0	0	0	3
Cerambycinae	Callidiini	*Phymatodes lividus* (Rossi, 1794)	Europe	7	0	0	0	0	7
Cerambycinae	Callidiini	*Phymatodes pusillus* (Fabricius, 1787)	Europe	37	0	0	0	0	37
Cerambycinae	Callidiini	*Phymatodes rufipes* (Fabricius, 1776)	Europe	3	0	0	0	0	3
Cerambycinae	Callidiini	***Phymatodes testaceus*** **(Linnaeus, 1758)**	Europe	15085	0	41	0	0	15126
Cerambycinae	Callidiini	*Phymatodes varius* (Fabricius, 1776)	North America	0	0	29	0	0	29
Cerambycinae	Callidiini	*Physocnemum brevilineum* (Say, 1824)	North America	0	0	4	0	0	4
Cerambycinae	Callidiini	*Pyrrhidium sanguineum* (Linnaeus, 1758)	Europe	4388	0	0	0	0	4388
Cerambycinae	Callidiini	*Ropalopus clavipes* (Fabricius, 1775)	Europe	69	0	0	0	0	69
Cerambycinae	Callidiini	*Ropalopus femoratus* (Linnaeus, 1758)	Europe	35	0	0	0	0	35
Cerambycinae	Callidiini	*Ropalopus macropus* (Germar, 1823)	Europe	21	0	0	0	0	21
Cerambycinae	Callidiini	*Ropalopus varini* (Bedel, 1870)	Europe	1	0	0	0	0	1
Cerambycinae	Callidiopini	*Bethelium* sp.	Australasia	0	0	0	5	0	5
Cerambycinae	Callidiopini	*Curtomerus flavus* (Fabricius, 1775)	Caribbean	0	0	0	0	7	7
Cerambycinae	Callidiopini	*Stenodryas clavigera* Bates, 1873	Asia	0	1	0	0	0	1
Cerambycinae	Cerambycini	*Cerambyx cerdo* Linnaeus, 1758	Europe	20	0	0	0	0	20
Cerambycinae	Cerambycini	*Cerambyx miles* Bonelli, 1812	Europe	3	0	0	0	0	3
Cerambycinae	Cerambycini	*Cerambyx scopolii* Fueßlins, 1775	Europe	141	0	0	0	0	141
Cerambycinae	Cerambycini	*Cerambyx welensii* (Küster, 1845)	Europe	22	0	0	0	0	22
Cerambycinae	Cerambycini	*Nadezhdiella cantori* (Hope, 1842)	Asia	0	1	0	0	0	1
Cerambycinae	Clytini	*Chlorophorus figuratus* (Scopoli, 1763)	Europe	42	0	0	0	0	42
Cerambycinae	Clytini	*Chlorophorus glabromaculatus* (Goeze, 1777)	Europe	1391	0	0	0	0	1391
Cerambycinae	Clytini	*Chlorophorus glaucus* (Fabricius, 1781)	Europe	36	0	0	0	0	36
Cerambycinae	Clytini	*Chlorophorus herbstii* (Brahm, 1790)	Europe	6	0	0	0	0	6
Cerambycinae	Clytini	*Chlorophorus miwai* Gressitt, 1936	Asia	0	9	0	0	0	9
Cerambycinae	Clytini	*Chlorophorus motschulskyi* (Ganglbauer, 1887)	Asia	0	7	0	0	0	7
Cerambycinae	Clytini	*Chlorophorus ruficornis* (Olivier, 1790)	Europe	41	0	0	0	0	41
Cerambycinae	Clytini	*Chlorophorus sartor* (Müller, 1766)	Europe	482	0	0	0	0	482
Cerambycinae	Clytini	*Chlorophorus signaticollis* (Laporte de Castelnau & Gory, 1836)	Asia	0	1	0	0	0	1
Cerambycinae	Clytini	*Chlorophorus tredecimmaculatus* (Chevrolat, 1863)	Asia	0	2	0	0	0	2
Cerambycinae	Clytini	*Chlorophorus trifasciatus* (Fabricius, 1781)	Europe	33	0	0	0	0	33

Subfamily	Tribe	Species	Origin	Europe	Asia	North America	The Caribbean	Australia	Total
Cerambycinae	Clytini	*Chlorophorus varius* (Müller, 1766)	Europe	36	0	0	0	0	36
Cerambycinae	Clytini	*Clytoleptus albofasciatus* (Laporte de Castelnau & Gory, 1841)	North America	0	0	6	0	0	6
Cerambycinae	Clytini	*Clytus arietis* (Linnaeus, 1758)	Europe	52	0	0	0	0	52
Cerambycinae	Clytini	*Clytus lama* Mulsant, 1850	Europe	123	0	0	0	0	123
Cerambycinae	Clytini	*Clytus rhamni* Germar, 1817	Europe	85	0	0	0	0	85
Cerambycinae	Clytini	*Clytus ruricola* (Olivier, 1800)	North America	0	0	25	0	0	25
Cerambycinae	Clytini	*Clytus tropicus* (Panzer, 1795)	Europe	73	0	0	0	0	73
Cerambycinae	Clytini	*Cyrtoclytus capra* (Germar, 1823)	Asia	0	24	0	0	0	24
Cerambycinae	Clytini	*Cyrtoclytus caproides* (Bates, 1873)	Asia	0	5	0	0	0	5
Cerambycinae	Clytini	*Demonax diversefasciatus* Pic, 1920	Asia	0	1	0	0	0	1
Cerambycinae	Clytini	*Demonax nansenensis* Pic 1903	Asia	0	1	0	0	0	1
Cerambycinae	Clytini	*Demonax sp. 1*	Asia	0	1	0	0	0	1
Cerambycinae	Clytini	*Demonax sp. 2*	Asia	0	1	0	0	0	1
Cerambycinae	Clytini	*Glycobius speciosus* (Say, 1824)	North America	0	0	1	0	0	1
Cerambycinae	Clytini	*Isotomus speciosus* (Schneider, 1787)	Europe	1	0	0	0	0	1
Cerambycinae	Clytini	*Megacyllene caryae* (Gahan, 1908)	North America	0	0	22	0	0	22
Cerambycinae	Clytini	***Neoclytus acuminatus acuminatus* (Fabricius, 1775)**	North America	37	0	28	0	0	65
Cerambycinae	Clytini	*Neoclytus caprea* (Say, 1824)	North America	0	0	3	0	0	3
Cerambycinae	Clytini	*Neoclytus leucozonus* (Laporte de Castelnau & Gory, 1841)	North America	0	0	15	0	0	15
Cerambycinae	Clytini	*Neoclytus mucronatus mucronatus* (Fabricius, 1775)	North America	0	0	323	0	0	323
Cerambycinae	Clytini	*Neoclytus muricatulus* (Kirby, 1837)	North America	0	0	1	0	0	1
Cerambycinae	Clytini	*Neoclytus scutellaris* (Olivier, 1790)	North America	0	0	2	0	0	2
Cerambycinae	Clytini	*Perissus paulonotatus* (Pic, 1902)	Asia	0	21	0	0	0	21
Cerambycinae	Clytini	*Plagionotus arcuatus* (Linnaeus, 1758)	Europe	95	0	0	0	0	95
Cerambycinae	Clytini	*Plagionotus christophi* (Kraatz, 1879)	Asia	0	1	0	0	0	1
Cerambycinae	Clytini	*Plagionotus detritus* (Linnaeus, 1758)	Europe	299	0	0	0	0	299
Cerambycinae	Clytini	*Pseudosphegesthes cinerea* (Laporte de Castelnau & Gory, 1841)	Europe	27	0	0	0	0	27
Cerambycinae	Clytini	*Raphuma anongi* Gressitt & Rondon, 1970	Asia	0	96	0	0	0	96
Cerambycinae	Clytini	*Raphuma gracilipes* (Faldermann, 1835)	Asia	0	24	0	0	0	24
Cerambycinae	Clytini	*Raphuma laosica* Gressitt & Rondon, 1970	Asia	0	22	0	0	0	22
Cerambycinae	Clytini	*Raphuma* sp.	Asia	0	1	0	0	0	1
Cerambycinae	Clytini	*Rhabdoclytus acutivittis* (Kraatz, 1879)	Asia	0	1	0	0	0	1
Cerambycinae	Clytini	*Sarosesthes fulminans* (Fabricius, 1775)	North America	0	0	39	0	0	39
Cerambycinae	Clytini	*Xylotrechus antilope* (Schönherr, 1817)	Europe	1303	0	0	0	0	1303
Cerambycinae	Clytini	*Xylotrechus antilope* var *sekerai* Podaný, 1970	Europe	16	0	0	0	0	16
Cerambycinae	Clytini	*Xylotrechus arvicola* (Olivier, 1800)	Europe	379	0	0	0	0	379
Cerambycinae	Clytini	*Xylotrechus atronotatus* Pic, 1917	Asia	0	1	0	0	0	1
Cerambycinae	Clytini	*Xylotrechus buqueti* (Laporte de Castelnau & Gory, 1841)	Asia	0	38	0	0	0	38
Cerambycinae	Clytini	***Xylotrechus chinensis* (Chevrolat, 1852)**	Asia	41	3	0	0	0	44
Cerambycinae	Clytini	*Xylotrechus clarinus* Bates, 1884	Asia	0	1	0	0	0	1
Cerambycinae	Clytini	*Xylotrechus colonus* (Fabricius, 1775)	North America	0	0	484	0	0	484
Cerambycinae	Clytini	*Xylotrechus gratus* Viktora, 2020	Asia	0	1	0	0	0	1
Cerambycinae	Clytini	*Xylotrechus integer* (Haldeman, 1847)	North America	0	0	1	0	0	1
Cerambycinae	Clytini	*Xylotrechus latefasciatus ochroceps* Gressitt, 1951	Asia	0	1	0	0	0	1
Cerambycinae	Clytini	*Xylotrechus magnicollis* (Fairmaire, 1888)	Asia	0	1	0	0	0	1
Cerambycinae	Clytini	*Xylotrechus pantherinus* (Savenius, 1825)	Europe	1	0	0	0	0	1
Cerambycinae	Clytini	*Xylotrechus pekingensis* Pic, 1939	Asia	0	1	0	0	0	1

Subfamily	Tribe	Species	Origin	Europe	Asia	North America	The Caribbean	Australia	Total
Cerambycinae	Clytini	*Xylotrechus rufilius* Bates, 1884	Asia	0	27	0	0	0	27
Cerambycinae	Clytini	*Xylotrechus rusticus* (Linnaeus, 1758)	Europe/Asia	161	1	0	0	0	162
Cerambycinae	Clytini	*Xylotrechus sagittatus* (Germar, 1821)	North America	0	0	34	0	0	34
Cerambycinae	Clytini	**_Xylotrechus stebbingi_ Gahan, 1906**	Asia	6089	0	0	0	0	6054
Cerambycinae	Clytini	*Xylotrechus undulatus* (Say, 1824)	North America	0	0	26	0	0	26
Cerambycinae	Deilini	*Deilus fugax* (Olivier, 1790)	Europe	87	0	0	0	0	87
Cerambycinae	Dryobiini	*Dryobius sexnotatus* Linsley, 1957	North America	0	0	2	0	0	2
Cerambycinae	Eburiini	*Eburia dejeani* Gahan, 1895	Caribbean	0	0	0	0	2	2
Cerambycinae	Eburiini	*Eburia octomaculata* Chevrolat, 1862	Caribbean	0	0	0	0	1	1
Cerambycinae	Eburiini	*Eburia quadrigeminata* (Say, 1827)	North America	0	0	3	0	0	3
Cerambycinae	Elaphidiini	*Anelaphus pumilus* (Newman, 1840)	North America	0	0	531	0	0	531
Cerambycinae	Elaphidiini	*Anelaphus villosus* (Fabricius, 1793)	North America	0	0	8	0	0	8
Cerambycinae	Elaphidiini	*Elaphidion mucronatum* (Say, 1824)	North America	0	0	110	0	0	110
Cerambycinae	Elaphidiini	*Parelaphidion aspersum* (Haldeman, 1847)	North America	0	0	3	0	0	3
Cerambycinae	Elaphidiini	*Parelaphidion incertum* (Newman, 1840)	North America	0	0	4	0	0	4
Cerambycinae	Graciliini	*Axinopalpis gracilis* (Krynicki, 1832)	Europe	8	0	0	0	0	8
Cerambycinae	Graciliini	*Gracilia minuta* (Fabricius, 1781)	Europe	12	0	0	0	0	12
Cerambycinae	Graciliini	*Penichroa fasciata* (Stephens, 1831)	Europe	41	0	0	0	0	41
Cerambycinae	Hesperophanini	*Gnatholea eburifera* Thomson, 1861	Asia	0	10	0	0	0	10
Cerambycinae	Hesperophanini	*Hesperophanes sericeus* (Fabricius, 1787)	Europe	8	0	0	0	0	8
Cerambycinae	Hesperophanini	*Stromatium auratum* (Böber, 1793)	Europe	7	0	0	0	0	7
Cerambycinae	Hesperophanini	**_Trichoferus campestris_ (Faldermann, 1835)**	Asia	45	12	0	0	0	57
Cerambycinae	Hesperophanini	*Trichoferus fasciculatus* (Faldermann, 1837)	Europe	135	0	0	0	0	135
Cerambycinae	Hesperophanini	*Trichoferus guerryi* (Pic, 1915)	Asia	0	1	0	0	0	1
Cerambycinae	Hesperophanini	*Trichoferus holosericeus* (Rossi, 1790)	Europe	187	0	0	0	0	187
Cerambycinae	Hesperophanini	*Trichoferus pallidus* (Olivier, 1790)	Europe	145	0	0	0	0	145
Cerambycinae	Hylotrupini	*Hylotrupes bajulus* (Linnaeus, 1758)	Europe	79	0	0	0	0	79
Cerambycinae	Molorchini	*Dolocerus reichii* Mulsant, 1862	Europe	1	0	0	0	0	1
Cerambycinae	Molorchini	*Molorchus bimaculatus* Say, 1824	North America	0	0	122	0	0	122
Cerambycinae	Molorchini	*Molorchus minor* (Linnaeus, 1758)	Europe	15	0	0	0	0	15
Cerambycinae	Molorchini	*Molorchus umbellatarum* (Schreber, 1759)	Europe	55	0	0	0	0	55
Cerambycinae	Neoibidionini	*Neocompsa cylindricollis* (Fabricius, 1798)	Caribbean	0	0	0	0	1	1
Cerambycinae	Obriini	*Obrium brunneum* (Fabricius, 1793)	Europe	3	0	0	0	0	3
Cerambycinae	Obriini	*Obrium cantharinum* (Linnaeus, 1767)	Europe	44	0	0	0	0	44
Cerambycinae	Obriini	*Obrium maculatum* (Olivier, 1800)	North America	0	0	1	0	0	1
Cerambycinae	Phoracanthini	**_Cordylomera spinicornis_ (Fabricius, 1775)**	Africa	4	0	0	0	0	4
Cerambycinae	Phoracanthini	**_Phoracantha recurva_ Newman, 1840**	Australasia	8	0	0	0	0	8
Cerambycinae	Phoracanthini	**_Phoracantha semipunctata_ (Fabricius, 1775)**	Australasia	11	0	0	0	0	11
Cerambycinae	Phoracanthini	*Thoris* sp.	Australasia	0	0	0	2	0	2
Cerambycinae	Psebiini	*Nathrius brevipennis* (Mulsant, 1839)	Europe	649	0	0	0	0	649
Cerambycinae	Pytheini	*Certallum ebulinum* (Linnaeus, 1767)	Europe	1	0	0	0	0	1
Cerambycinae	Rhopalophorini	*Rhopalophora longipes* (Say, 1824)	North America	0	0	1	0	0	1
Cerambycinae	Stenhomalini	*Stenhomalus fenestratus* White,1855	Asia	0	3	0	0	0	3
Cerambycinae	Stenoderini	*Syllitus* sp.	Australasia	0	0	0	2	0	2
Cerambycinae	Stenopterini	*Callimoxys sanguinicollis* (Olivier, 1800)	North America	0	0	1	0	0	1
Cerambycinae	Stenopterini	*Callimus abdominalis* (Olivier, 1800)	Europe	11	0	0	0	0	11
Cerambycinae	Stenopterini	*Callimus angulatus* (Schrank, 1789)	Europe	1	0	0	0	0	1
Cerambycinae	Stenopterini	*Stenopterus ater* (Linnaeus, 1767)	Europe	20	0	0	0	0	20
Cerambycinae	Stenopterini	*Stenopterus rufus* (Linnaeus, 1767)	Europe	83	0	0	0	0	83
Cerambycinae	Tillomorphini	*Bonfilsia pejoti* Chalumeau & Touroult, 2004	Caribbean	0	0	0	0	1	1

Subfamily	Tribe	Species	Origin	Europe	Asia	North America	The Caribbean	Australia	Total
Cerambycinae	Tillomorphini	*Euderces picipes* (Fabricius, 1787)	North America	0	0	9	0	0	9
Cerambycinae	Tillomorphini	*Euderces pini* (Olivier, 1800)	North America	0	0	93	0	0	93
Cerambycinae	Tillomorphini	*Gourbeyrella madininae* Chalumeau & Touroult, 2004	Caribbean	0	0	0	0	3	3
Cerambycinae	Trachyderini	*Anoplistes halodendri* (Pallas, 1773)	Asia	0	2	0	0	0	2
Cerambycinae	Trachyderini	*Dicelosternus corallinus* Gahan, 1900	Asia	0	3	0	0	0	3
Cerambycinae	Trachyderini	*Purpuricenus budensis* (Götz, 1783)	Europe	18	0	0	0	0	18
Cerambycinae	Trachyderini	*Purpuricenus globulicollis* Dejean, 1839	Europe	2	0	0	0	0	2
Cerambycinae	Trachyderini	*Purpuricenus kaehleri* (Linnaeus, 1758)	Europe	261	0	0	0	0	261
Cerambycinae	Trachyderini	*Purpuricenus lituratus* Ganglbauer, 1887	Asia	0	1	0	0	0	1
Cerambycinae	Trachyderini	*Purpuricenus temminckii* (Guérin-Méneville, 1844)	Asia	0	10	0	0	0	10
Cerambycinae	Trachyderini	*Amarysius altajensis* (Laxmann, 1770)	Asia	0	20	0	0	0	20
Lamiinae	Acanthocinini	*Acanthocinus aedilis* (Linnaeus, 1758)	Europe/Asia	6	24	0	0	0	30
Lamiinae	Acanthocinini	*Acanthocinus griseus* (Fabricius, 1793)	Europe/Asia	114	106	0	0	0	220
Lamiinae	Acanthocinini	*Acanthocinus pusillus* (Kirby, 1837)	North America	0	0	21	0	0	21
Lamiinae	Acanthocinini	*Amniscus similis* (Gahan, 1895)	Caribbean	0	0	0	0	5	5
Lamiinae	Acanthocinini	*Astyleiopus variegatus* (Haldeman, 1847)	North America	0	0	11	0	0	11
Lamiinae	Acanthocinini	*Astylidius parvus* (LeConte, 1873)	North America	0	0	17	0	0	17
Lamiinae	Acanthocinini	*Astylopsis macula* (Say, 1827)	North America	0	0	47	0	0	47
Lamiinae	Acanthocinini	*Astylopsis sexguttata* (Say, 1827)	North America	0	0	19	0	0	19
Lamiinae	Acanthocinini	*Astylopsis* sp.	North America	0	0	2	0	0	2
Lamiinae	Acanthocinini	*Graphisurus despectus* (LeConte, 1850)	North America	0	0	3	0	0	3
Lamiinae	Acanthocinini	*Graphisurus fasciatus* (Degeer, 1775)	North America	0	0	86	0	0	86
Lamiinae	Acanthocinini	*Graphisurus triangulifer* (Haldeman, 1847)	North America	0	0	3	0	0	3
Lamiinae	Acanthocinini	*Hyperplatys maculatus* Haldeman, 1847	North America	0	0	4	0	0	4
Lamiinae	Acanthocinini	*Lagocheirus araneiformis insulorum* Dillon, 1957	Caribbean	0	0	0	0	4	4
Lamiinae	Acanthocinini	*Leiopus fallaciosus* Holzschuh, 1993	Asia	0	5	0	0	0	5
Lamiinae	Acanthocinini	*Leiopus femoratus* Fairmaire, 1859	Europe	3461	0	0	0	0	3461
Lamiinae	Acanthocinini	*Leiopus linnei* Wallin, Nýlander & Kvamme, 2009	Europe	548	0	0	0	0	548
Lamiinae	Acanthocinini	*Leiopus nebulosus* (Linneus, 1758)	Europe	1473	0	0	0	0	1473
Lamiinae	Acanthocinini	*Leptostylus transversus* (Gyllenhal, 1817)	North America	0	0	101	0	0	101
Lamiinae	Acanthocinini	*Lepturges angulatus* (LeConte, 1852)	North America	0	0	20	0	0	20
Lamiinae	Acanthocinini	*Lepturges confluens* (Haldeman, 1847)	North America	0	0	26	0	0	26
Lamiinae	Acanthocinini	*Lepturges* sp.	North America	0	0	2	0	0	2
Lamiinae	Acanthocinini	*Sternidius alpha* (Say, 1827)	North America	0	0	3	0	0	3
Lamiinae	Acanthocinini	*Sternidius punctatus* (Haldeman, 1847)	North America	0	0	1	0	0	1
Lamiinae	Acanthocinini	*Sternidius rusticus* (LeConte, 1852)	North America	0	0	19	0	0	19
Lamiinae	Acanthocinini	*Styloleptus posticalis* (Gahan, 1895)	Caribbean	0	0	0	0	1	1
Lamiinae	Acanthocinini	*Trypanidius spilmani* Villiers, 1980	Caribbean	0	0	0	0	1	1
Lamiinae	Acanthocinini	*Urgleptes cobbeni* Gilmour, 1963	Caribbean	0	0	0	0	1	1
Lamiinae	Acanthocinini	*Urgleptes querci* (Fitch, 1859)	North America	0	0	2	0	0	2
Lamiinae	Acanthocinini	*Urgleptes signatus* (LeConte, 1852)	North America	0	0	1	0	0	1
Lamiinae	Acanthoderini	*Aegomorphus clavipes* (Schrank von Paula, 1781)	Europe	1412	0	0	0	0	1412
Lamiinae	Acanthoderini	*Aegomorphus francottei* Sama, 1994	Europe	181	0	0	0	0	181
Lamiinae	Acanthoderini	*Aegomorphus krueperi* (Kraatz, 1859)	Europe	7	0	0	0	0	7
Lamiinae	Acanthoderini	*Aegomorphus modestus* (Blais, 1817)	North America	0	0	58	0	0	58
Lamiinae	Acanthoderini	*Aegomorphus quadrigibbus* (Say, 1831)	North America	0	0	3	0	0	3
Lamiinae	Acanthoderini	*Oplosia cinerea* (Mulsant, 1839)	Europe	63	0	0	0	0	63
Lamiinae	Acanthoderini	*Oplosia nubila* (LeConte, 1862)	North America	0	0	4	0	0	4

Subfamily	Tribe	Species	Origin	Europe	Asia	North America	The Caribbean	Australia	Total
Lamiinae	Agapanthiini	*Agapanthia cardui* (Linnaeus, 1767)	Europe	1	0	0	0	0	1
Lamiinae	Agapanthiini	*Agapanthia villosoviridescens* (Degeer, 1775)	Europe	1	0	0	0	0	1
Lamiinae	Apomecynini	*Apomecyna saltator* (Fabricius, 1787)	Asia	0	1	0	0	0	1
Lamiinae	Ceroplesini	*Moechotypa diphysis* (Pascoe, 1871)	Asia	0	2	0	0	0	2
Lamiinae	Ceroplesini	*Thysia wallichii tonkinensis* (Kreische, 1924)	Asia	0	1	0	0	0	1
Lamiinae	Desmiphorini	*Anaesthetis testacea* (Fabricius, 1781)	Europe	17	0	0	0	0	17
Lamiinae	Desmiphorini	*Deroplia genei* (Aragona, 1830)	Europe	1	0	0	0	0	1
Lamiinae	Desmiphorini	*Deroplia troberti* (Mulsant, 1843)	Europe	3	0	0	0	0	3
Lamiinae	Desmiphorini	*Eupogonius pauper* LeConte, 1852	North America	0	0	1	0	0	1
Lamiinae	Desmiphorini	*Eupogonius tomentosus* (Haldeman, 1847)	North America	0	0	3	0	0	3
Lamiinae	Desmiphorini	*Psenocerus supernotatus* (Say, 1824)	North America	0	0	9	0	0	9
Lamiinae	Dorcaschematini	*Dorcaschema cinereum* (Olivier, 1800)	North America	0	0	1	0	0	1
Lamiinae	Dorcaschematini	*Olenecamptus bilobus* (Fabricius, 1801)	Asia	0	3	0	0	0	3
Lamiinae	Exocentrini	*Exocentrus adspersus* Mulsant, 1846	Europe	5	0	0	0	0	5
Lamiinae	Exocentrini	*Exocentrus lusitanus* (Linnaeus, 1767)	Europe	29	0	0	0	0	29
Lamiinae	Exocentrini	*Exocentrus punctipennis* Mulsant & Guillebeau, 1856	Europe	28	0	0	0	0	28
Lamiinae	Lamiini	*Lamiomimus gottschei* Kolbe, 1886	Asia	0	1	0	0	0	1
Lamiinae	Lamiini	*Pharsalia subgemmata* (Thomson, 1857)	Asia	0	375	0	0	0	375
Lamiinae	Mesosini	*Mesosa curculionoides* (Linnaeus 1761)	Europe	37	0	0	0	0	37
Lamiinae	Mesosini	*Mesosa myops* (Dalman, 1817)	Asia	0	29	0	0	0	29
Lamiinae	Mesosini	*Mesosa nebulosa* (Fabricius, 1781)	Europe	132	0	0	0	0	132
Lamiinae	Monochamini	*Anoplophora beryllina* (Hope, 1840)	Asia	0	1	0	0	0	1
Lamiinae	Monochamini	*Anoplophora chinensis* (Forster, 1771)	Asia	0	1	0	0	0	1
Lamiinae	Monochamini	*Anoplophora glabripennis* (Motschulsky, 1854)	Asia	0	9	0	0	0	9
Lamiinae	Monochamini	*Microgoes oculatus* (LeConte, 1862)	North America	0	0	3	0	0	3
Lamiinae	Monochamini	*Monochamus alternatus* Hope, 1842	Asia	0	1246	0	0	0	1246
Lamiinae	Monochamini	*Monochamus bimaculatus* Gahan, 1888	Asia	0	1	0	0	0	1
Lamiinae	Monochamini	*Monochamus carolinensis* (Olivier, 1797)	North America	0	0	77	0	0	77
Lamiinae	Monochamini	*Monochamus galloprovincialis* (Olivier, 1800)	Europe/Asia	6209	87	0	0	0	6296
Lamiinae	Monochamini	*Monochamus maculosus* Haldeman, 1847	North America	0	0	3	0	0	3
Lamiinae	Monochamini	*Monochamus notatus* (Drury, 1773)	North America	0	0	256	0	0	256
Lamiinae	Monochamini	*Monochamus saltuarius* Gebler, 1830	Asia/Europe	13	985	0	0	0	998
Lamiinae	Monochamini	*Monochamus sartor* (Fabricius, 1787)	Europe	20	0	0	0	0	20
Lamiinae	Monochamini	*Monochamus sartor urussovii* (Fischer von Waldheim, 1806)	Asia/Europe	1	41	0	0	0	42
Lamiinae	Monochamini	*Monochamus scutellatus* (Say, 1824)	North America	0	0	216	0	0	216
Lamiinae	Monochamini	*Monochamus sutor* (Linnaeus, 1758)	Europe/Asia	30	22	0	0	0	52
Lamiinae	Monochamini	*Monochamus sutor longulus* Pic, 1898	Asia	0	22	0	0	0	22
Lamiinae	Monochamini	*Uraecha angusta* (Pascoe, 1857)	Asia	0	15	0	0	0	15
Lamiinae	Obereini	*Oberea linearis* (Linnaeus, 1761)	Europe	8	0	0	0	0	8
Lamiinae	Parmenini	*Mesolita* sp.	Australasia	0	0	0	3	0	3
Lamiinae	Parmenini	*Parmena balteus* (Linnaeus, 1767)	Europe	7	0	0	0	0	7
Lamiinae	Parmenini	*Parmena unifasciata* (Rossi, 1790)	Europe	3	0	0	0	0	3
Lamiinae	Phytoeciini	*Phytoecia pustulata* (Schrank von Paula, 1776)	Europe	1	0	0	0	0	1
Lamiinae	Phytoeciini	*Phytoecia nigricornis* (Fabricius, 1782)	Europe	2	0	0	0	0	2
Lamiinae	Pogonocherini	*Pogonocherus caroli* Mulsant, 1862	Europe	5	0	0	0	0	5
Lamiinae	Pogonocherini	*Pogonocherus decoratus* Fairmaire, 1855	Europe	139	0	0	0	0	139
Lamiinae	Pogonocherini	*Pogonocherus fasciculatus* (Degeer, 1775)	Europe	16	1	0	0	0	17
Lamiinae	Pogonocherini	*Pogonocherus hispidulus* (Piller & Mitterpacher, 1783)	Europe	6	0	0	0	0	6

Subfamily	Tribe	Species	Origin	Europe	Asia	North America	The Caribbean	Australia	Total
Lamiinae	Pogonocherini	*Pogonocherus hispidus* (Linnaeus, 1758)	Europe	55	0	0	0	0	55
Lamiinae	Pogonocherini	*Pogonocherus mixtus* Haldeman, 1847	North America	0	0	8	0	0	8
Lamiinae	Pogonocherini	*Pogonocherus ovatus* (Goeze, 1777)	Europe	19	0	0	0	0	19
Lamiinae	Pogonocherini	*Pogonocherus penicillatus* LeConte, 1850	North America	0	0	11	0	0	11
Lamiinae	Pogonocherini	*Pogonocherus perroudi* Mulsant, 1839	Europe	127	0	0	0	0	127
Lamiinae	Pteropliini	*Niphona picticornis* Mulsant, 1839	Europe	127	0	0	0	0	127
Lamiinae	Pteropliini	*Sthenias gracilicornis* Gressitt, 1937	Europe	0	3	0	0	0	3
Lamiinae	Saperdini	*Menesia bipunctata* (Zoubkoff, 1829)	Europe	2	0	0	0	0	2
Lamiinae	Saperdini	*Paraglenea fortunei* (Saunders, 1853)	Asia	0	1	0	0	0	1
Lamiinae	Saperdini	*Saperda alberti* Plavilstshikov, 1915	Asia	0	1	0	0	0	1
Lamiinae	Saperdini	*Saperda hosokawai* Hasegawa, 2017	Asia	0	1	0	0	0	1
Lamiinae	Saperdini	*Saperda octopunctata* (Scopoli, 1772)	Europe	1	0	0	0	0	1
Lamiinae	Saperdini	*Saperda perforata* (Pallas, 1773)	Europe	21	0	0	0	0	21
Lamiinae	Saperdini	*Saperda populnea* (Linnaeus, 1758)	Europe	4	0	0	0	0	4
Lamiinae	Saperdini	*Saperda scalaris* (Linnaeus, 1758)	Europe	24	0	0	0	0	24
Lamiinae	Saperdini	*Stenostola dubia* (Laicharting, 1784)	Europe	2	0	0	0	0	2
Lamiinae	Saperdini	*Stenostola ferrea* (Schrank von Paula, 1776)	Europe	28	0	0	0	0	28
Lepturinae	Lepturini	*Alosterna tabacicolor* (Degeer, 1775)	Europe	9	0	0	0	0	9
Lepturinae	Lepturini	*Anastrangalia dubia* (Scopoli, 1763)	Europe	6	0	0	0	0	6
Lepturinae	Lepturini	*Anastrangalia reyi* (Heyden, 1889)	Europe	1	0	0	0	0	1
Lepturinae	Lepturini	*Anastrangalia sanguinolenta* (Linnaeus 1761)	Europe	13	0	0	0	0	13
Lepturinae	Lepturini	*Anastrangalia scotodes continentalis* (Plavilstshikov, 1936)	Asia	0	1	0	0	0	1
Lepturinae	Lepturini	*Anoplodera rufipes* (Schaller, 1783)	Europe	5	0	0	0	0	5
Lepturinae	Lepturini	*Anoplodera sexguttata* (Fabricius, 1775)	Europe	9	0	0	0	0	9
Lepturinae	Lepturini	*Brachyleptura brevis* (Kirby, 1837)	North America	0	0	1	0	0	1
Lepturinae	Lepturini	*Brachyleptura circumdata* (Olivier, 1800)	North America	0	0	1	0	0	1
Lepturinae	Lepturini	*Brachyleptura rubrica* (Say, 1824)	North America	0	0	1	0	0	1
Lepturinae	Lepturini	*Leptura thoracica* Creutzer, 1799	Asia	0	1	0	0	0	1
Lepturinae	Lepturini	*Leptura aethiops* Poda von Neuhaus, 1761	Europe	2	0	0	0	0	2
Lepturinae	Lepturini	*Leptura aurulenta* Fabricius, 1793	Europe	7	0	0	0	0	7
Lepturinae	Lepturini	*Leptura quadrifasciata* Linnaeus, 1758	Europe	9	0	0	0	0	9
Lepturinae	Lepturini	*Neoalosterna capitata* (Newman, 1841)	North America	0	0	2	0	0	2
Lepturinae	Lepturini	*Pachytodes erraticus* (Dalman, 1817)	Europe	232	0	0	0	0	232
Lepturinae	Lepturini	*Paracorymbia fulva* (Degeer, 1775)	Europe	8	0	0	0	0	8
Lepturinae	Lepturini	*Paracorymbia hybrida* (Rey, 1885)	Europe	1	0	0	0	0	1
Lepturinae	Lepturini	*Pedostrangalia revestita* (Linnaeus, 1767)	Europe	12	0	0	0	0	12
Lepturinae	Lepturini	*Pseudovadonia livida* (Fabricius, 1776)	Europe	5	0	0	0	0	5
Lepturinae	Lepturini	*Rutpela maculata* (Poda von Neuhaus, 1761)	Europe	74	0	0	0	0	74
Lepturinae	Lepturini	*Stenurella nigra* (Linnaeus 1758)	Europe	2	0	0	0	0	2
Lepturinae	Lepturini	*Stenurella bifasciata* (Müller, 1776)	Europe	16	0	0	0	0	16
Lepturinae	Lepturini	*Stenurella septempunctata* (Fabricius, 1793)	Europe	5	0	0	0	0	5
Lepturinae	Lepturini	*Stenurella melanura* (Linnaeus 1758)	Europe	33	0	0	0	0	33
Lepturinae	Lepturini	*Stictoleptura canadensis* (Olivier, 1800)	North America	0	0	8	0	0	8
Lepturinae	Lepturini	*Stictoleptura cordigera* (Fueßlins, 1775)	Europe	203	0	0	0	0	203
Lepturinae	Lepturini	*Stictoleptura erythroptera* (Hagenbach, 1822)	Europe	1	0	0	0	0	1
Lepturinae	Lepturini	*Stictoleptura fontenayi* (Mulsant, 1839)	Europe	1	0	0	0	0	1
Lepturinae	Lepturini	*Stictoleptura maculicornis* (Degeer, 1775)	Europe	2	0	0	0	0	2
Lepturinae	Lepturini	*Stictoleptura rubra* (Linnaeus, 1758)	Europe/asia	11	1	0	0	0	12
Lepturinae	Lepturini	*Stictoleptura scutellata* (Fabricius, 1781)	Europe	29	0	0	0	0	29
Lepturinae	Lepturini	*Stictoleptura succedanea* (Lewis, 1879)	Asia	0	1	0	0	0	1
Lepturinae	Lepturini	*Stictoleptura trisignata* (Fairmaire, 1852)	Europe	7	0	0	0	0	7
Lepturinae	Lepturini	*Strangalepta abbreviata* (Germar, 1823)	North America	0	0	1	0	0	1

Subfamily	**Tribe**	**Species**	**Origin**	**Europe**	**Asia**	**North America**	**The Caribbean**	**Australia**	**Total**
Lepturinae	Lepturini	*Strangalia attenuata* (Linnaeus 1758)	Europe	1	0	0	0	0	1
Lepturinae	Lepturini	*Strangalia luteicornis* (Fabricius, 1775)	North America	0	0	4	0	0	4
Lepturinae	Lepturini	*Strophiona nitens* (Forster, 1771)	North America	0	0	3	0	0	3
Lepturinae	Lepturini	*Trachysida mutabilis* (Newman, 1841)	North America	0	0	4	0	0	4
Lepturinae	Lepturini	*Trigonarthris proxima* (Say, 1824)	North America	0	0	1	0	0	1
Lepturinae	Lepturini	*Trigonarthris subpubescens* (Kirby, 1837)	North America	0	0	3	0	0	3
Lepturinae	Lepturini	*Typocerus lunulatus* (Swederus, 1787)	Asia	0	1	0	0	0	1
Lepturinae	Lepturini	*Typocerus velutinus* (Olivier, 1800)	North America	0	0	1	0	0	1
Lepturinae	Lepturini	*Vadonia unipunctata* (Fabricius, 1787)	Europe	2	0	0	0	0	2
Lepturinae	Oxymirini	*Anthophylax cyaneus* (Haldeman, 1848)	North America	0	0	2	0	0	2
Lepturinae	Oxymirini	*Anthophylax viridis* LeConte, 1850	North America	0	0	6	0	0	6
Lepturinae	Oxymirini	*Oxymirus cursor* (Linnaeus, 1758)	Europe	4	0	0	0	0	4
Lepturinae	Rhagiini	*Anisorus quercus* (Götz, 1783)	Europe	3	0	0	0	0	3
Lepturinae	Rhagiini	*Brachyta interrogationis* (Linnaeus, 1758)	Europe	2	0	0	0	0	2
Lepturinae	Rhagiini	*Carilia virginea* (Linnaeus, 1758)	Europe	2	0	0	0	0	2
Lepturinae	Rhagiini	*Carilia virginea thalassina* (Schrank von Paula, 1781)	Asia	0	14	0	0	0	14
Lepturinae	Rhagiini	*Centrodera decolorata* (Harris, 1838)	North America	0	0	3	0	0	3
Lepturinae	Rhagiini	*Cortodera femorata* (Fabricius, 1787)	Europe	11	0	0	0	0	11
Lepturinae	Rhagiini	*Cortodera flavimana* (Waltl, 1838)	Europe	8	0	0	0	0	8
Lepturinae	Rhagiini	*Cortodera humeralis* (Schaller, 1783)	Europe	99	0	0	0	0	99
Lepturinae	Rhagiini	*Dinoptera collaris* (Linnaeus, 1758)	Europe	3	0	0	0	0	3
Lepturinae	Rhagiini	*Acmaeops marginatus* (Fabricius, 1781)	Europe/asia	3	11	0	0	0	14
Lepturinae	Rhagiini	*Acmaeops pratensis* (Laicharting, 1784)	Europe	10	0	0	0	0	10
Lepturinae	Rhagiini	*Acmaeops proteus* (Kirby, 1837)	North America	0	0	14	0	0	14
Lepturinae	Rhagiini	*Acmaeops septentrionis* (C G Thomson, 1866)	Europe/asia	24	28	0	0	0	52
Lepturinae	Rhagiini	*Acmaeops smaragdulus* (Fabricius, 1793)	Europe	6	0	0	0	0	6
Lepturinae	Rhagiini	*Evodinellus borealis* (Gyllenhal, 1827)	Asia	0	2	0	0	0	2
Lepturinae	Rhagiini	*Gaurotes cyanipennis* (Say, 1824)	North America	0	0	2	0	0	2
Lepturinae	Rhagiini	*Grammoptera abdominalis* (Stephens, 1831)	Europe	31	0	0	0	0	31
Lepturinae	Rhagiini	*Grammoptera ruficornis* (Fabricius, 1781)	Europe	266	0	0	0	0	266
Lepturinae	Rhagiini	*Grammoptera ustulata* (Schaller, 1783)	Europe	56	0	0	0	0	56
Lepturinae	Rhagiini	*Pachyta mediofasciata* Pic 1936	Asia	0	3	0	0	0	3
Lepturinae	Rhagiini	*Pachyta quadrimaculata* (Linnaeus, 1758)	Asia	0	1	0	0	0	1
Lepturinae	Rhagiini	*Paragaurotes ussuriensis* (Blessig, 1873)	Asia	0	1	0	0	0	1
Lepturinae	Rhagiini	*Pidonia lurida* (Fabricius, 1792)	Europe	3	0	0	0	0	3
Lepturinae	Rhagiini	*Pseudosieversia japonica* (Ohbayashi, 1937)	Asia	0	1	0	0	0	1
Lepturinae	Rhagiini	*Rhagium bifasciatum* Fabricius, 1775	Europe	20	0	0	0	0	20
Lepturinae	Rhagiini	*Rhagium inquisitor* (Linnaeus, 1758)	Holarctic	524	5	110	0	0	639
Lepturinae	Rhagiini	*Rhagium japonicum* Bates, 1884	Asia	0	21	0	0	0	21
Lepturinae	Rhagiini	*Rhagium mordax* (Degeer, 1775)	Europe	41	0	0	0	0	41
Lepturinae	Rhagiini	*Rhagium rugipenne* Reitter, 1898	Asia	0	4	0	0	0	4
Lepturinae	Rhagiini	*Rhagium sycophanta* (Schrank von Paula, 1781)	Europe	32	0	0	0	0	32
Lepturinae	Rhagiini	*Stenocorus cinnamopterus* (Randall, 1838)	North America	0	0	1	0	0	1
Lepturinae	Rhagiini	*Stenocorus meridianus* (Linnaeus, 1758)	Europe	71	0	0	0	0	71
Necydalinae	Necydalini	*Necydalis major* Linnaeus 1758	Europe	3	0	0	0	0	3
Necydalinae	Necydalini	*Necydalis ulmi* (Chevrolat, 1838)	Europe	1	0	0	0	0	1
Parandrinae	Parandrini	*Neandra brunnea* (Fabricius, 1798)	North America	0	0	1	0	0	1
Prioninae	Aegosomatini	*Aegosoma scabricorne* (Scopoli, 1763)	Europe	33	0	0	0	0	33
Prioninae	Macrotomini	*Prinobius myardi* Mulsant, 1842	Europe	3	0	0	0	0	3
Prioninae	Meroscelisini	*Tragosoma harrisii* LeConte, 1851	North America	0	0	236	0	0	236
Prioninae	Prionini	*Dorysthenes sternalis* (Fairmaire, 1902)	Asia	0	25	0	0	0	25

Subfamily	Tribe	Species	Origin	Europe	Asia	North America	The Caribbean	Australia	Total
Prioninae	Prionini	*Dorysthenes paradoxus* (Faldermann, 1833)	Asia	0	22	0	0	0	22
Prioninae	Prionini	*Dorysthenes* sp.	Asia	0	2	0	0	0	2
Prioninae	Prionini	*Mesoprionus besikanus* (Fairmaire, 1855)	Europe	46	0	0	0	0	46
Prioninae	Prionini	*Orthosoma brunneum* (Forster, 1771)	North America	0	0	1	0	0	1
Prioninae	Prionini	*Prionus coriarius* (Linnaeus, 1758)	Europe	4112	0	0	0	0	4112
Prioninae	Prionini	*Prionus insularis* Motschulsky, 1857	Asia	0	241	0	0	0	241
Prioninae	Prionini	*Prionus laticollis* (Drury, 1773)	North America	0	0	3	0	0	3
Prioninae	Prionini	*Prionus* sp.	Asia	0	1	0	0	0	1
Spondylidinae	Anisarthrini	*Alocerus moesiacus* (Frivaldszky, 1837)	Europe	4	0	0	0	0	4
Spondylidinae	Anisarthrini	*Anisarthron barbipes* (Schrank von Paula, 1781)	Europe	19	0	0	0	0	19
Spondylidinae	Asemini	*Arhopalus ferus* (Mulsant, 1839)	Europe	338	0	0	0	0	338
Spondylidinae	Asemini	***Arhopalus rusticus* (Linnaeus, 1758)**	Europe/Asia	4264	702	5	0	0	4971
Spondylidinae	Asemini	*Asemum amurense* Kraatz, 1879	Asia	0	5	0	0	0	5
Spondylidinae	Asemini	*Asemum striatum* (Linnaeus, 1758)	Holarctic	21	181	289	0	0	491
Spondylidinae	Asemini	*Asemum tenuicorne* Kraatz, 1879	Europe	1	0	0	0	0	1
Spondylidinae	Asemini	*Cephalallus oberthueri* Sharp, 1905	Asia	0	14	0	0	0	14
Spondylidinae	Asemini	*Cephalallus* sp.	Asia	0	3	0	0	0	3
Spondylidinae	Asemini	*Cephalallus unicolor* (Gahan, 1906)	Asia	0	15	0	0	0	15
Spondylidinae	Asemini	*Cephalocrius syriacus* (Reitter, 1895)	Europe	2024	0	0	0	0	2024
Spondylidinae	Nothorhinini	*Nothorhina punctata* (Fabricius, 1798)	Europe	2	0	0	0	0	2
Spondylidinae	Saphanini	*Oxypleurus nodieri* Mulsant, 1839	Europe	25	0	0	0	0	25
Spondylidinae	Spondylidini	*Spondylis buprestoides* (Linnaeus, 1758)	Europe	2149	8	0	0	0	2157
Spondylidinae	Tetropiini	*Tetropium castaneum* (Linnaeus, 1758)	Europe	53	8	0	0	0	61
Spondylidinae	Tetropiini	*Tetropium cinnamopterum* Kirby, 1837	North America	0	0	2	0	0	2
Spondylidinae	Tetropiini	*Tetropium fuscum* (Fabricius, 1787)	Europe	100	0	0	0	0	100
Spondylidinae	Tetropiini	*Tetropium gabrieli* Weise, 1905	Europe	166	0	0	0	0	166
Spondylidinae	Tetropiini	*Tetropium schwarzianum* Casey, 1891	North America	0	0	2	0	0	2
Spondylidinae	Tetropiini	*Tetrops praeustus* (Linnaeus, 1758)	Europe	7	0	0	0	0	7
Spondylidinae	Tetropiini	*Tetrops starkii* Chevrolat, 1859	Europe	23	0	0	0	0	23
Disteniidae	Disteniini	*Elytrimitatrix undata* (Fabricius, 1775)	North America	0	0	6	0	0	6
Vesperidae	Vesperini	*Vesperus conicicollis* Fairmaire & Coquerel, 1866	Europe	1	0	0	0	0	1
Vesperidae	Vesperini	*Vesperus strepens* (Fabricius, 1793)	Europe	6	0	0	0	0	6

In Europe, a total of 192 cerambycid species were trapped, of which seven were non-natives (three Clytini: the North American *N. a. acuminatus* and the Asian *X. chinensis* and *Xylotrechus stebbingi* Gahan; three Phoracanthini: the African *Cordylomera spinicornis* (F.) and the Australasian *Phoracantha recurva* Newman and *P. semipunctata* (F.); and one Hesperophanini: the Asian *T. campestris*). The captures amounted to about 20% of the total European cerambycid fauna (955 species, including apterous species, according to Vitali and Schmitt 2017). In North America, a total of 97 species were captured, including two non-native species originating from Europe: the Aseminii *Arhopalus rusticus* (L.) and the Callidiini *P. testaceus*. In contrast, no non-native species were trapped in Asia (95 total species), the Caribbean (12 total species; i.e. 18% of the 65 species known in Martinique; Touroult and Poirier 2021) and Australia. Three species with Holarctic distribution (the Aseminii *Asemum striatum* [L.], the Callidiini *Callidium aeneum* [Degeer] and the Rhagiini *Rhagium inquisitor* [L.]) were trapped in Europe, Asia and North America, whereas nine species with northern Palaeartic distribution were captured in both Europe and Asia (the Clytini *Xylotrechus rusticus* [L.], the Acanthocinini *Acanthocinus griseus* [F.], the Monochamini *M. galloprovincialis* and congeners *M. saltuarius* [Say] and *M. sartor urussovii* [Fischer von Waldheim], the Lepturini *Stictoleptura rubra* [L.], the Rhagiini congeners *Acmaeops marginatus* [F.] and *A. septentrionis* [C. G. Thomson] and the Asemini *A. rusticus*).

Three species were notably abundant with captures exceeding > 5,000, including the European native *P. testaceus* (which was also trapped in the USA as a non-native species), the Palaearctic *M. galloprovincialis* (trapped in Europe and Northern China) and the Asian *X. stebbingi* which has invaded Europe. Sixteen species were represented by more than 1,000 specimens, 58 species by more than 100 specimens (Fig. 3) and 84 species by more than 50 individuals. In contrast, 109 cerambycid species from the total of 374 species were represented by only one or two specimens. More than 1,000 individuals were caught for four of the Callidiini species, of which three were native European species (*P. testaceus* – 15,126 individuals, 41 of which were trapped in North America where they have been introduced; *Pyrrhidium sanguineum* [L.]- 4,388 individuals and *Phymatodes alni* [L.]- 2,295 individuals), along with the North American *Phymatodes amoenus* (Say) (3,100 individuals).

Some invasive species were trapped in both their native range and in invaded regions (Fig. 4). The Chinese Clytini *X. chinensis* was captured in its native range around Beijing as well as in the invaded European areas in Spain, Greece (Crete) and southern France. The European species *P. testaceus* (Callidiini) and *A. rusticus* (Asemini) were

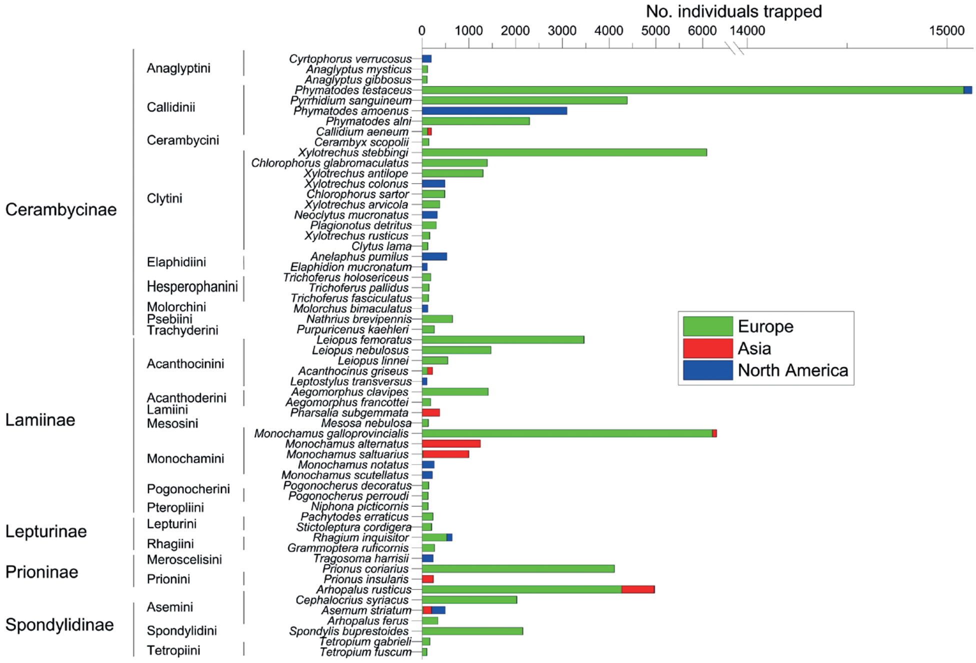

Figure 3. Cerambycid species with more than 100 inviduals captured, ranked by decreasing order and showing the continent of capture.

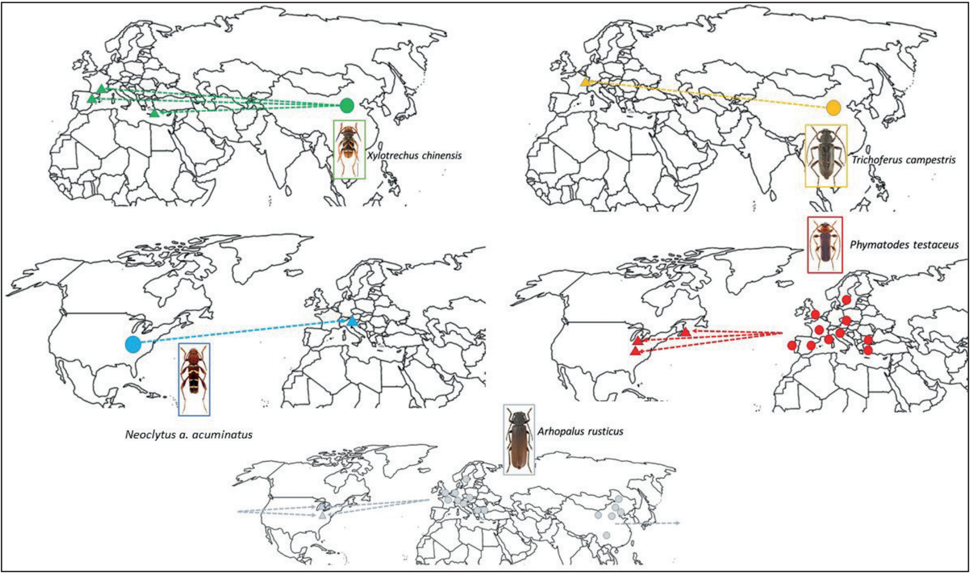

Figure 4. Cerambycid species trapped in both their native range (circles) and invaded range (triangles). Each species is shown by a different colour.

trapped in large numbers in their native Europe, but also as non-native species in North America. Conversely, the North American Clytini *N. a. acuminatus* was captured in its native range in the USA, but also in the invaded areas of Italy. A noticeable anomaly was the Himalayan Clytini *X. stebbingi*, which was caught in large numbers in southern Europe (6,089 specimens) where it is invasive, but not at all in the traps deployed in Asia. The African Phoracanthini *C. spinicornis* was regularly trapped within European ports-of-entry, but not in nearby woody areas.

Simultaneous captures of non-target Coleopteran species were mostly bark and ambrosia beetles (> 100,000 individuals), which are not yet identified to species, but also predators in the family Cleridae, essentially *Clerus mutillarius* Fabricius, 1775 (> 5,000 individuals) and *Thanasimus* spp. (> 2,000 individuals) and Trogossitidae (*Temnoscheila* spp.; > 500 individuals).

Additional captures resulting from the 10-pheromone blend

The addition of trichoferone and (*E*)-2-*cis*-6,7-epoxynonenal to the 8-pheromone blend in France and China in 2019 onwards, did not significantly change the previous trapping spectrum of the 8-pheromone blend (paired *t*-test; $P = 0.750$). However, the 10-pheromone blend resulted in trapping large numbers of four *Trichoferus* species, including the Asian *T. campestris* in its invasive range in Europe and native range in China (Table 3). In addition, large numbers of *Aromia bungii* were trapped in their native Chinese range. Only a few specimens of these five species had been previously trapped using the 8-pheromone blend.

Discussion

Capturing 376 species of cerambycid beetles from eight different subfamilies and 60 tribes on different continents, with 84 species captured in numbers greater than 50 individuals, clearly demonstrates the potential of the multi-pheromone lure to constitute an effective tool for the detection of 'unexpected' cerambycid invaders that are accidentally translocated outside their native ranges. Our hypothesis regarding the generic effectiveness of the blend was based on the evolutionary conservatism observed in many cerambycid pheromone structures. Pheromone constituents of the blend composition are shared by phylogenetically-related species on different continents (cf. references in Table 2). Therefore, their combination was expected to simultaneously attract multiple species of different tribes and subfamilies. These expected generic effects were largely supported for the targeted subfamilies, namely the Cerambycinae, Lamiinae, Spondylidinae and Prioninae.

The best represented tribe was Clytini (Cerambycinae). A total of 64 species were trapped overall, including catches in Asia (27 spp.), Europe (22 spp.) and North America (15 spp.). Two of these species were captured in both the native and invaded continents (*X. chinensis*- Asia/Europe, *N. a. acuminatus*- North America/Europe). This richness probably resulted from the presence in the blend of C6-ketol (3-hydroxyhexan-2-one) and *anti*-2,3-hexanediol. Both are known to be male-emitted attractants for a number of species in this tribe (Millar and Hanks 2017; Imrei et al. 2021). Furthermore, using these two compounds in a similar multipheromone blend in tropical China, Wickham et al. (2021) captured 26 Clytini species of which only four were in common with the present study (*Rhaphuma anongi* Gressit & Rondon, *Rhaphuma laosica* Gressit & Rondon, *Xylotrechus buqueti* [Laporte de Castelnau & Gory] and *X. chinensis*), thus suggesting an even larger potential of the blend. The Asian Clytini *X. stebbingi*, a native of the Himalayas (India, Pakistan), provides an illustrative example of the potential of detection of 'unexpected' cerambycid invaders, with a total of 6,089 specimens captured in the invaded countries of southern Europe. The species was trapped each year at more than 50 sites in southern France, Greece, Italy, Spain and Switzerland, but also in nurseries near Paris far from the invaded areas. However, it has apparently not invaded China. Somewhat surprisingly, the pheromone blend of this abundant invasive species has not yet been identified, although racemic 3-hydroxyhexan-2-one was suggested to be a key component of its pheromone (Rassati et al. 2021) and a number of other *Xylotrechus* species have pheromones comprised of 3-hydroxyalkan-2-ones, 2-hydroxyalkan-3-ones and/or 2,3-alkanediols (Millar and Hanks 2017). The richness in the trapped *Xylotrechus* species (19 spp.), a genus known to include a number of invasive species, is especially important for the early detection of new invaders.

In the same subfamily Cerambycinae, the tribe Callidiini was represented by 15 species trapped in Europe and five in North America, including a total of 11 species in the genus *Phymatodes* on the two continents. The very large number of captures (from ~ 2,000 to more than 15,000 individuals) of three *Phymatodes* species, two native to

Europe (*P. testaceus* and *P. alni*) and one from North America (*P. amoeneus*) and those of the closely-related European *P. sanguineum*, probably reflects the inclusion in the blend of both C6-ketol and 2-methylbutan-1-ol, known to be attractants for a number of *Phymatodes* spp. (Millar and Hanks 2017). Hanks et al. (2019) had previously trapped *P. testaceus* and *P. amoenus* in large numbers using these compounds, but also confirmed that the attraction to C6-ketol is antagonised by 2-methylbutan-1-ol for another species of *Phymatodes*, *P. aereus* (Newman) (Mitchell et al. 2011). Probably for the same reason, few *P. aereus* (14 individuals) were trapped during our study. A species of the Callidiini, *Callidium aeneum*, was represented by more than 200 individuals, but is another example of a species for which pheromones have not yet been identified. However, the congeners *C. antennatum hesperum* Casey and *C. pseudotsugae* Fisher are known to use C6-ketol along with semanopyrrole as their aggregation-sex pheromone blend (Millar et al. 2019). The C6-ketol compound is also likely to be a pheromone component for *Bethelium tillides* (Pascoe), a representative of another Cerambycinae tribe, Callidiopini, in Australia (Hayes et al. 2016), as indicated by our captures of *Bethelium* sp. in Australia and another Callidiopini, *C. flavus*, in Martinique.

In the subfamily Lamiinae, large numbers of individuals of 12 species of Monochamini in the genus *Monochamus* were trapped in Europe, Russia (Siberia), China and North America. This likely resulted from the inclusion in the blend of monochamol (2-[undecyloxy]-ethanol), known as a sex-aggregation pheromone for at least 14 *Monochamus* species in Europe (*M. galloprovincialis*; Pajares et al. 2010), North America (e.g. *M. carolinensis* [Olivier] and *M. scutellatus* [Say]; Millar and Hanks 2017) and Asia (*M. alternatus* Hope; Lee et al. 2018). The captures included species such as *M. sartor* (F.) and *M. sutor longulus* Pic for which no attractant had previously been recorded (but known for *M. sutor* [L.], Pajares et al. 2013). These results confirmed those obtained by Boone et al. (2018), who trapped six *Monochamus* species in North America and *M. alternatus* in Asia using monochamol. Given the importance of *Monochamus* beetles in vectoring the lethal pinewood nematode, the multilure blend would be useful for early detection of such invading species in ports and other high-risk sites. Additionally, another Monochamini in a different genus, *Uraecha angusta* (Pascoe), was trapped in China in high numbers, further indicating that monochamol is not restricted to the genus *Monochamus* Dejean.

In the subfamily Spondylidinae, Žunič-Kosi et al. (2019) recently showed that (*S*)-fuscumol, with geranylacetone as a minor component, is a sex-aggregation pheromone for a European representative of the Asemini tribe, *A. rusticus*. They also suggested that the fuscumol motif is probably shared more broadly in this subfamily. Our trapping of 15 spondylidine species supports this assumption, as the blend included fuscumol, fuscumol acetate and geranylacetone. Besides the ~ 5,000 trapped *A. rusticus* individuals, other European Asemini were captured in substantial numbers as well, including *Cephalocrius syriacus* Sharp (> 2,000 individuals), *Arhopalus ferus* (Mulsant) and *A. striatum* in Europe, but also other *Asemum* and *Cephalallus* species in China (Table 3). High numbers of captures were also noted for several species in other spondylidine tribes, such as *Spondylis buprestoides* (L.) (Spondylidini, > 2,000 individuals) in both Europe and China, for which no attractants are yet known and the alpine *Tetropium gabrieli* Weise (Tetropiini), for which Schroeder et al. (2021) recently showed an attraction to (*E*)-fuscumol.

In the subfamily Prioninae, the inclusion of prionic acid, originally identified as a female-produced sex pheromone of the North American species *Prionus californicus* Motschulsky (Rodstein et al. 2009), but also as an attractant for most, if not all, other North American species of *Prionus* (Barbour et al. 2011; Millar and Hanks 2017) and for the Asian Prionini *Dorysthenes granulosus* (Thomson) (Wickham et al. 2016a), resulted in substantial catches (> 4,000 specimens) of the European *Prionus coriarius* (L.). Additional captures of other Prionini, such as the Balkanic *Mesoprionus besikanus* (Fairmaire), the Asian *Prionus insularis* Motschulsky, and three Chinese species of *Dorysthenes* (*D. sternalis* [Fairmaire], *D. paradoxus* [Faldermann] and an, as yet, unidentified species), suggest a broad attractive spectrum for prionic acid in this tribe.

Despite the general efficiency of our blend, a number of species, especially those trapped with less than 50 individuals, are likely either random catches or were attracted by physical characteristics (e.g. trap shape and/or colour). Based on the previous results of Fan et al. (2019), who showed highly significant differences in the number of species trapped by multipheromone blends and unbaited traps, we did not deploy any unbaited control traps in this study which could help in clarifying this point. However, it is noteworthy that, very unexpectedly, we trapped a total of 79 lepturine species, including 49 in Europe, 12 in Asia and 18 in North America, although few pheromones are known in the subfamily Lepturinae and none of them, such as *cis*-vaccenyl acetate (Ray et al. 2011) or (*R*)-desmolactone (Ray et al. 2014), was included in the blend. Most of these species were caught in small numbers, consistent with random captures of individuals. Only three species (*Pachytodes erraticus* [Dalman], *Stictoleptura cordigera* [Fueßlins] and *Rutpela maculata* [Poda von Neuhaus]) out of the 44 captured in the tribe Lepturini, were represented by more than 50 individuals (Table 3). For these species, we can only speculate that the beetles were attracted to the trap colour or silhouette, rather than to the lure (see details in Cavaletto et al. 2021). Analogous results were obtained for another Lepturine tribe, the Rhaginii, where more than 600 specimens of a species considered as Holarctic, *R. inquisitor*, were caught in Europe, Asia and North America, together with the closely-related *R. japonicum* Bates trapped in China.

Attraction of these lepturines may also have been a result of the addition of high release rate ethanol and α-pinene lures to traps, rather than attraction to the blend of synthetic pheromones. Plant volatiles can, in some cases, effectively enhance the attraction of cerambycids to pheromone lures (e.g. for *Monochamus* species; Pajares et al. 2010). Indeed, plant volatiles alone, such as turpentine, have long been used as generic attractants for wood-boring insects, including some species of cerambycids, but are not as effective as pheromone-baited traps for target species. For example, Rassati et al. (2019) showed that traps deployed in Italy with a multi-lure including most of the compounds of our blend (C6-ketol, racemic 3-hydroxyoctan-2-one, *syn*-2,3-hexanediols, [*E*/*Z*]-fuscumol and [*E*/*Z*]-fuscumol acetate) caught more than twice the number of cerambycid species as ethanol-baited traps.

Our results also provide leads to possible pheromone structures in new species (see also above), building on the previously-articulated concept of "pheromone identification by proxy", in which identification of pheromones for one species may provide leads for the identification of pheromones of related taxa (Millar et al. 2019). For the 16 species caught in numbers > 1,000 individuals, it is likely that the major component(s) of their pheromones were present in the blend. Pheromones or possible pheromones had been identified from only about half of these species (*M. alternatus*, *M. galloprovincialis* and *M. saltuarius*, *A. rusticus*, *P. amoenus* and *P. testaceus*, *P. sanguineum*, *P. coriarius*, *Xylotrechus antilope* [Schönherr]; Millar and Hanks 2017). Thus, our data represent the first possible leads to the pheromone structures for a number of cerambycine species in the tribe Clytini (*C. glabromaculatus*, > 1,000 individuals), three Lamiinae in the tribes Acanthocinini (*Leiopus femoratus* Fairmaire, > 3,000 individuals; *Leiopus nebulosus* [L.], > 1,000 individuals) and Acanthoderini (*Aegomorphus clavipes* [Schrank von Paula], > 1,000 individuals) and two Spondylidinae in the tribes Asemini (*C. syriacus*, > 2,000 individuals) and Spondylidini (*S. buprestoides*, > 2,000 individuals). It is likely that the same could be true for at least some of the remaining 46 species caught in numbers > 100 individuals (and maybe even > 50), but for which pheromones have not been formally identified. For instance, Wickham et al. (2021) considered that their substantial captures of the Clytini *R. anongi* and *R. laosica* in tropical China suggested the presence of pheromone components in the blend that they tested, which was similar to ours. Our results further support this assumption because we trapped ~ 100 *R. anongi* and > 25 *R. laosica* with traps deployed at a single site of southern China.

Trapping of some invasive species in both the native and invaded ranges revealed the potential of the multilure blend for detecting invaders. Some of these non-native species have been present for a long time in the invaded areas (e.g. the European *P. testaceus* and *A. rusticus* in North America and the North American *N. a. acuminatus* in Europe). However, the trapping of very recent invaders within and near ports-of-entry is noteworthy and is indicative of the sensitivity of the blend for early detection at low population levels. For example, the Chinese Clytini *X. chinensis* was captured in its native range around Beijing, as well as in all the scattered European areas it has invaded and established in relatively recently (2013 in Spain-Catalonia; 2017 in Greece-Crete island and 2018 in southern France-Port of Sète; https://gd.eppo.int/taxon/XYLOCH/distribution/ES). Similarly, when the 10-pheromone blend including trichoferone was deployed, the Chinese Hesperophanini *T. campestris* was trapped in both its native range in China and in the river port of Huningue (France), where this invasive species had not yet been recorded. Interestingly, despite its presumably low abundance, our trapping studies allowed us to follow the dispersal of this invading species from the port. For example, in 2019 and 2020, specimens were only detected in traps placed within the Huningue Port but, in 2021, the species was captured in traps placed within a 1 km-radius from the Port. Numerous catches of *X. stebbingi* in ports-of-entry and nurseries of northern France, far from the known invaded southern area of France, also highlighted the sensitivity of the blend for its detection.

What possible improvements can be expected?

Is it possible and useful to continue increasing the number of pheromones included in the blend? The addition of trichoferone and the pheromone of *Aromia bungii* to the 8-pheromone blend in some field trials in France and China since 2020 resulted in relatively high numbers of captures of several *Trichoferus* species (three native European species and one native Chinese species invasive in Europe), as well as individuals of *A. bungii* in China, without reducing the trapping scope observed in nearby traps baited with the primary blend, especially the cerambycine *P. testaceus*. Millar et al. (2021) obtained analogous results in Pennsylvania, USA, where the responses of the major cerambycid species were not affected, except for the lamiine species *Sternidius alpha* (Say) whose catches were shut down by the addition of the pheromones of the two non-native species. Preliminary experiments (not detailed here) carried out in 2020 and 2021 in south-central France consisting of adding the sex-aggregation pheromone of *Rosalia alpina* (Linnaeus), an alkylated pyrone (Žunič-Kosi et al. 2017), to the 8-pheromone blend also resulted in captures of *R. alpina* without altering the cerambycid species richness. Tests of addition of the semanopyrrole structure (1-[1*H*-pyrrol-2-yl]-1,2-propanedione) could also be of interest, given that it occurs in pheromones of species from several continents. Wickham et al. (2016b) suggested that semanopyrrole may correspond to another well-conserved sex-aggregation pheromone motif within the subfamily Cerambycinae, being highly attractive in combination with C6-ketol for the Callidiini *Callidiellum villosulum* (Fairmaire) and as a single component for the Phoracanthini *Allotraeus asiaticus* (Schwarzer) in China. Silva et al. (2017) also identified semanopyrrole as a component of the sex-aggregation pheromone of two South American Cerambycinae in the tribe Elaphidiini, *Ambonus distinctus* (Newman) and *Ambonus electus* (Gahan). Recent work has also shown that 10-methyldodecanol, 11-methyltridecanol and their corresponding aldehydes may form another conserved structural motif, with examples of pheromones from South American (Silva et al. 2020) and North American cerambycid species (JGM and LMH, work in progress). As all these additional compounds exhibit chemical structures substantially different from the ones used in the 8-pheromone blend, it may be hypothesised that their addition will be unlikely to interfere with the attraction of species to the other eight pheromones.

For a more general approach of early detection of xylophagous invaders, targeting not only cerambycids, but also other groups, such as bark and ambrosia beetles (Curculionidae, Scolytinae), woodwasps (Siricidae) and jewel beetles (Buprestidae), represents a valuable opportunity. In fact, traps baited with some (e.g. Marchioro et al. 2020; Miller et al. 2022) or all (Cavaletto et al. 2020) the eight longhorn beetle pheromones used in this study, allowed us to catch a high number of other wood-boring beetle species and associated predators, especially when synergised by generic attractants, such as ethanol or α-pinene. The possibility of augmenting the blend with complementary attractants specific and/or generic to each of these groups is also of interest. Miller et al. (2016) already tested the combination of monochamol and α-pinene with ipsenol, a pheromone component of *Ips* bark beetles (Curculionidae: Scolytinae) and did not observe any negative effect nor synergy on catches of Cerambycidae or on any associated species of bark beetles, weevils or bark beetle predators. However, some of the cerambycid pheromones used in the 8-pheromone blend have been shown to have either positive or negative effects on catches of bark and ambrosia beetles, depending on the species (Marchioro et al. 2020).

The position of the trap also has rather to be carefully managed. In our study, standardisation of trap position was not possible due to the different trapping locations (ports-of-entry, urban parks, forests) and the variety of environments amongst the countries included in the study. However, several recent studies have confirmed that trap position can have a considerable influence on the captures of cerambycid beetles, on a vertical gradient from the forest understorey up to the canopy (Wermelinger et al. 2007; Graham et al. 2012; Flaherty et al. 2019; Rassati et al. 2019; Miller et al. 2020; Wickham et al. 2021), as well as on a horizontal gradient between the forest edge and forest interior (Allison et al. 2019; Sweeney et al. 2020). For instance, *Monochamus maculosus* Haldeman and *M. scutellatus* were more abundant in the clearing adjacent to the forest than in the forest, whereas the contrary was observed for *M. carolinensis* and *M. titillator* (F.). As most of our field trials did not consider the upper canopy and were not deployed along forest edge-interior gradients, except for the specific 2019 experiment, it is possible that a number of species with specific types of behaviour related to these micro-environments were not trapped at all. Moreover, weather conditions during trapping periods, as well as the size of the local populations, were also likely to influence the captures.

Another important point is the colour of the trap. Most traps used in the study were black multifunnel traps (1069 out of 1289; 83%). However, Cavaletto et al. (2021), using the same 8-pheromone blend, showed that trap colour had a considerable influence on cerambycid captures in both species' richness and abundance for several subfamilies, but in different ways. For example, black traps caught significantly fewer species of Cerambycinae than yellow ones, whereas for Lamiinae, both brown and red traps caught significantly more species than black traps and colours in the portion of the visible electromagnetic spectrum (yellow, green, blue) attracted higher numbers of lepturine species than did black traps. A relationship with adult beetle behaviour could be hypothesised. Cavaletto et al. (2021) observed that the number of species of flower-visiting cerambycids was significantly lower in black traps than in yellow, blue and green ones, whereas yellow and green traps were significantly less efficient than black traps for non-flower-visiting species. Therefore, systematic tests of traps of different colours in different world regions could significantly enhance the efficiency of trapping species which respond to the multipheromone blend. Data obtained during the present study will be later merged with those of specifically-designed experiments to test for the influence of trap colour.

The impact of such trappings on local insect biodiversity could be questioned. As all specimens from non-target Coleopteran groups have not been identified yet, we cannot exclude that a few species other than cerambycids, bark and ambrosia beetles and beetle predators (clerids, trogossitids) have also been trapped in significant numbers (> 500 ind.). However, any trapping study, like our one, is necessarily limited in scope by cost and logistical factors. Thus, unless trappings are intensively conducted over a whole region or country, which is very unlikely, they are likely to affect local biodiversity in a very limited way.

In conclusion, we are delivering a database of nearly 400 species which were trapped during the course of our multiyear field trials with the multipheromone blend, and the two hypotheses of our study are strongly supported. First, the trapping of a species in significant numbers on a continent effectively increased the probability that it can be detected upon arrival in other countries/continents, as shown by the species trapped in large numbers in both native and invaded ranges, supporting hypothesis 1. Second, the multipheromone blend was shown to be an effective generic attractant for multiple species from several cerambycid subfamilies, including numerous species for which pheromones have not yet been identified, supporting hypothesis 2. In addition, some species, such as the lepturine species caught in large numbers, were probably trapped because of trap colour or the host plant lure, rather than as a result of the blend composition. However, regardless of cues used by beetles, trapping of non-native species when they arrive at ports-of-entry has the same value for phytosanitary officials. Antagonistic effects between compounds exist, but appear to be fairly limited and so should not compromise the overall detection potential. Finally, further advances in the effectiveness of detection of cerambycids by multipheromone lures can be expected as parameters, such as trap colour and height, are optimised and as the number of pheromone components which are found to be conserved within and across related taxa and continents expands.

Acknowledgements

We thank Filippo Giannone, Riccardo Poloni, Kate Van Rooyen, Chantelle Kostanowicz, Vincent Webster, Andrej Kapla, Matic Gabor, Mischa Giasson and Cory Hughes for technical assistance in the lab and field and for species identification. Paige Payter, Michigan State University (MSU), installed and monitored traps in Michigan and Page Payter and Gary Parsons (MSU) identified the captured cerambycids. We are indebted to Fréderic Delport, François-Xavier Saintonge, Jean-Baptiste Daubrée and all colleagues of the "Santé des Forêts" Department (DSF) and local offices (SRAL) of the French Ministry of Agriculture for the management of the traps in France. Marie-Pierre Dufresne from Fredon Centre - Val de Loire and Sylvain Amiot from the Direction Patrimoine végétal et Biodiversité of Tours-Métropole helped to settle traps in the Val de Loire area, France. We are also grateful to Eddy Poirier and Nicolas Moulin for the management of the traps in Martinique and to the forest health team of Vaersa and the forest management service (SOGF) of the Generalitat Valenciana (Spain). We thank very much Xing Zhong-Ping for his help in the trappings in Yunnan and Anastasia Knorre for helping us with field research in the State Nature Reserve "Stolby" (Krasnoyarsk, Russia). We also want to thank Prof. Ana Paula Ramos for enabling a connection with the administration of the municipalities of Lisbon and

Setúbal for the trappings in Portugal. We are indebted to Eng. Rui Simão and Eng. Ana Júlia Francisco in CM- Lisboa, Dr. António Nobre from the administration board of Lisbon harbour, Eng. Sérgio Gaspar from CM-Setúbal for allowing us to conduct this work in their municipalities. The municipality of L'Argentière la Bessée provided invaluable assistance for the management of the traps in the southern French Alps. We thank Robert Haack, Nicolas Meurisse and a third anonymous reviewer for their very helpful comments and suggestions on the manuscript.

This work was essentially supported by the HOMED project (HOlistic Management of Emerging Forest Pests and Diseases) which received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 771271 (https://homed-projecteu/). We also acknowledge funding from the European project LIFE SAMFIX (SAving Mediterranean Forests from Invasions of *Xylosandrus* Beetles and associated Pathogenic Fungi, LIFE17 NAT/IT/000609, https://wwwlifesamfixeu/), from the EUPHRESCO project MULTITRAP ("Multi-lure and multi-trap surveillance for invasive tree pests"). Trappings in France were supported from funding by the French Ministry of Agriculture under the PORTRAP project "Test de l'efficacité de pièges génériques multicomposés pour la détection précoce d'insectes exotiques xylophages dans les sites potentiels d'entrée sur le territoire national" and by the Région Centre- Val de Loire under the CANOPEE project ("Biodiversité des insectes des canopées de chênes dépérissants"- Project No. 2018-00124136). Trapping in Austria was supported by the Austrian Federal Ministry for Agriculture, Forestry, Regions and Water Management (Research Project 101183). Trapping in China was partially supported by a grant from the French Embassy in Beijing under the programme CaiYuanPei. LR, JS, YQL and YY are grateful for funding by the National Key Research and Development Program of China (Grant No 2021YFC2600400) and the National Natural Science Foundation of China (31770687). Research conducted in Nova Scotia, Canada, was funded by the Pest Risk Management Program of Natural Resources Canada, Canadian Forest Service. Trapping in Michigan was supported by a grant from the Michigan Department of Agriculture and Rural Development. Research in Siberia (Russia) was supported by Sukachev Institute of Forest SB RAS (the basic project, grant No 0287-2021-0011) [field collection] and the Russian Science Foundation (grant No 22-16-00075) [species identification]. AMR and EKLF are grateful for the Robert Borcer Endowment and the Undergraduate Research Fund of Xavier University and USDA-APHIS cooperative agreement numbers AP19PPQS and T00C082 and AP20PPQS and T00C173. MZ and CM acknowledge funding by the New South Wales government and Hort Innovation project 16004 NSW DPI component of the Macadamia Integrated Pest Management. Trials in Martinique were part of a natural site inventory funded by the DEAL Martinique (French Ministry of Environment). The work in Slovenia was financially supported by the Slovenian Research Agency (Research Core Funding P1-0255). Trapping in England was supported by the Department for Environment, Food and Rural Affairs (Defra). JGM and LMH gratefully acknowledge support from United States Department of Agriculture, Animal and Plant Health Inspection Service (APHIS) grants 19- to 22-8130-1422-CA.

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Supplementary material 1

Total trapping network

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Data type: site description (excel document)

Link: https://doi.org/10.3897/neobiota.84.91096.suppl1

NeoBiota 84: 211–229 (2023)
doi: 10.3897/neobiota.84.95177
https://neobiota.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
NeoBiota
Advancing research on alien species and biological invasions

Citizen science at school increases awareness of biological invasions and contributes to the detection of exotic ambrosia beetles

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Academic editor: C. Orazio | Received 21 September 2022 | Accepted 6 March 2023 | Published 18 May 2023

Citation: Colombari F, Battisti A (2023) Citizen science at school increases awareness of biological invasions and contributes to the detection of exotic ambrosia beetles. In: Jactel H, Orazio C, Robinet C, Douma JC, Santini A, Battisti A, Branco M, Seehausen L, Kenis M (Eds) Conceptual and technical innovations to better manage invasions of alien pests and pathogens in forests. NeoBiota 84: 211–229. https://doi.org/10.3897/neobiota.84.95177

Abstract

The serious and growing threat posed by biological invasions to biodiversity and livelihoods means that public engagement in dealing with problems of invasive alien species is ever more urgent and necessary hence a citizen science experiment was carried out in north-eastern Italy. The study aimed *i*) to raise awareness of invasive alien species threatening trees and forests, and *ii*) to perform monitoring activities of a group of wood borers as an example, involving teachers and students (aged 11 to 18) of high schools. Students and teachers were given background knowledge and trained about the protocol for collecting data in schoolyards/grounds. Native (*Anisandrus dispar* (Fabricius, 1792), *Xyleborinus saxesenii* (Ratzeburg, 1837)) and exotic ambrosia beetles (*Anisandrus maiche* Kurentsov, *Cnestus mutilatus* (Blandford), *Xylosandrus crassiusculus* (Motschulsky, 1866), *Xylosandrus germanus* (Blandford, 1894)) were used as the target species, as they could be easily detected through accessible and low-cost traps requiring limited effort. The traps were exposed for 24 hours weekly between March and June 2021. The experiment also aimed to strengthen public involvement, connecting environmental education and experiential outdoor learning. The mutual collaboration resulted in a wider knowledge of the potential impact of exotic species. It also led to new geographical citizen-science records of two alien ambrosia beetles considered to be quarantine pests by the European Union: *C. mutilatus*, new for the European part of the EPPO (European and Mediterranean Plant Protection Organization) region, and *A. maiche*, which was previously found only in Eastern European EPPO member countries.

Keywords
Anisandrus maiche, *Cnestus mutilatus*, environmental education, first record, invasive alien species, knowledge, students, surveillance

Introduction

Humans have traded and transported alien species for decades, intentionally or unintentionally, but, over the last 50 years, the rates of Invasive Alien Species' (IAS) introductions have significantly grown and currently do not show, at a global scale, any sign of saturation (Hulme 2009). Instead, the trend is increasing, especially for insect species mainly introduced accidentally as stowaways or contaminants (Hulme et al. 2008; Seebens et al. 2017; Kenis et al. 2019; Turbelin et al. 2022). The introduction of IAS as contaminants may be predicted from their pathways, e.g., by plants, fruits, or wood (Kenis et al. 2007; Hulme et al. 2008; Pergl et al. 2020). Or stowaways can arrive independently of a specific commodity, as hitchhikers on ships, in containers, vehicles (car, train, airplane), packing material, and in the luggage of tourists (Hulme et al. 2008; Pergl et al. 2020; Turbelin et al. 2022). Identifying and managing the pathways through which IAS arrive and spread is an important component of any strategy aimed at reducing the threat of biological invasions but difficult to carry out (Roques 2015; Seebens et al. 2017; Pergl et al. 2020; Turbelin et al. 2022). Consequently, given sets of gaps that regulations cannot cover, the direct involvement of the wider public in the surveillance and management of IAS is crucial (Hulme et al. 2008; Faulkner et al. 2020; Turbelin et al. 2022).

Citizen science, which Pocock et al. (2019) defined as "the involvement of people in the scientific process, including participating in environmental recording and monitoring", represents an opportunity to raise awareness of common problems of biological invasions and to expand better monitoring efforts conducted by researchers and phytosanitary personnel in detecting IAS (Sagy et al. 2019). IAS exemplify an excellent case of citizen science application as they threaten the environment and society (García-Llorente et al. 2008; Novoa et al. 2017). Increasing the public's perception of IAS is essential (Jubase et al. 2021) and it has been shown that after educational and informative efforts the public was much more aware of IAS (García-Llorente et al. 2008). Actions aimed at increasing public knowledge and awareness, such as workshops, training programs, or media involvement, must be supported in policy decisions and by funding (García-Llorente et al. 2008; Novoa et al. 2017).

In particular, educating and training school students in IAS has become a commonly recognised innovative and holistic approach because students bring home the message and share it with relatives, in this way supporting intergenerational learning (Verbrugge et al. 2021). Among IAS affecting trees and forests, bark and ambrosia beetles (Coleoptera Curculionidae Scolytinae) represent an important group and have already been used in a citizen science initiative, called 'Backyard Bark Beetles' (Steininger et al. 2015). The purposes of our study were to implement a large-scale surveillance and increase high school students' awareness about two recently reported ambrosia beetle species (*Anisandrus maiche* Kurentsov, 1941 and *Cnestus mutilatus* (Blandford, 1894)). This paper aims to disseminate how environmental education can strengthen public engagement and contribute to IAS detection.

Materials and methods

Study organisms

Ambrosia beetles were used as target species in this project, as they can be easily detected through accessible traps activated with a freely available attractant and following a simple trapping protocol. Moreover, the phenology of species allowed their monitoring during an ideal season for outdoor activities compatible with school time. Ambrosia beetles include thousands of species worldwide and are currently one of the main issues of phytosanitary measures and controls. The specimens are usually identified using morphological features and diagnostic keys, as in the current study (Rabaglia et al. 2009; Gomez et al. 2018; Smith et al. 2020), although DNA barcoding is generally required to confirm the taxonomic identity of new records and/or cryptic species. For these purposes, DNA barcoding using primers LCO- 1490/HCO- 2198 as in Folmer et al. (1994) was used, whenever necessary, by comparing the sequences obtained with those already available for the target species.

Study area

The monitoring sites made available by participants (see Increase public awareness on IAS) numbered 15 in total, all located in the Veneto Region (NE part of Italy; Fig. 1). All the schools were in urban areas in temperate and warm-temperate climate, with mainly various species of broadleaf trees and shrubs, both native and exotic, and a lower percentage of conifers in their immediate surroundings. Landscape composition around schools (i.e., the percentages of the different land-cover classes) was highly heterogeneous with values spread out over a wide range (Table 1). The categories "areas devoid of vegetation" and "agricultural areas' were dominant, while "green urban areas" and "forest and semi-natural areas" occupied only a small percentage of the buffers despite a slight increase of their sum (from 10 to 20%) with increasing distance.

Increase public awareness on IAS

In 2019 and 2020, a total of 66 headmasters of high schools were asked to collaborate on the project. Sixteen schools accepted the proposal for a total of 30 classes, and about 500 students aged 11 to 18. The project workflow is shown in Fig. 2. Before and after the background lecture, students were asked to complete anonymous questionnaires to test their knowledge and awareness on IAS.

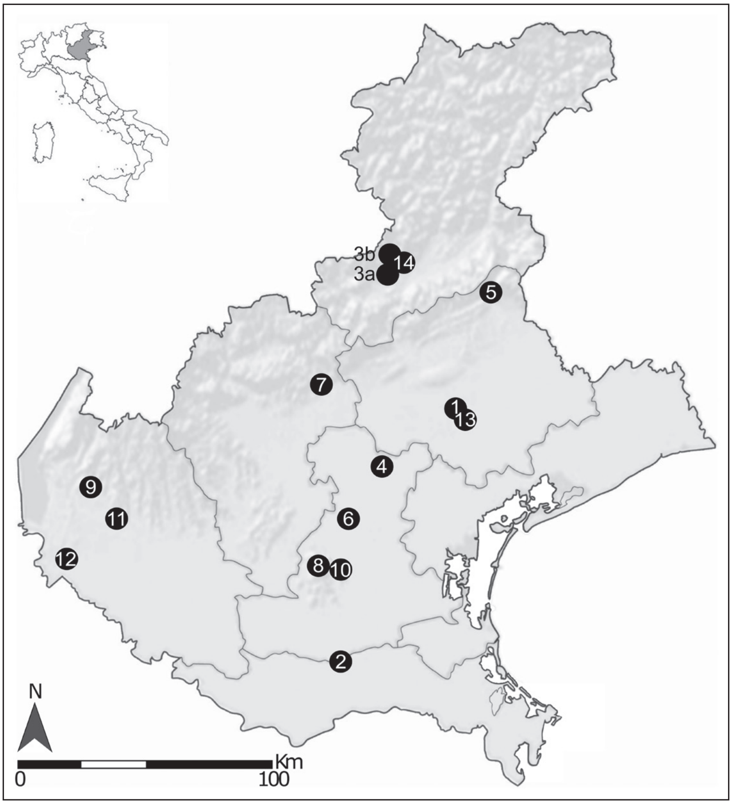

Figure 1. Map of the study area (the Veneto Region) indicating the high school locations.

Table 1. Landscape composition (mean % ± SE) within three different radii (0.5, 1 and 2 km) around schools.

	Areas devoid of vegetation		Agricultural areas		Green urban areas		Forest and semi-natural areas		Discontinuous urban fabric		Water bodies	
Radius	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
0.5 km	60.47	5.56	26.93	5.41	7.23	2.15	3.56	2.02	0.97	0.33	0.84	0.49
1 km	48.23	5.47	35.73	5.75	4.86	1.21	7.71	3.10	1.25	0.24	2.23	1.03
2 km	36.03	5.30	42.49	5.80	3.67	0.80	14.82	5.61	1.64	0.23	1.35	0.45

A simple pre-lecture questionnaire had to be filled out based only on the IAS definition (FAO 2007), provided at the beginning of the test. It contained 10 questions (Suppl. material 1) relating to knowledge of IAS definition and IAS species, perception of the possible impact of an introduced alien species in the country, and propensity to get information about IAS. A one-hour lecture entitled ‘Monitoring of insect species harmful to trees and forests’ (Suppl. material 2) was delivered in the classes, both face-to-face and online. This activity was launched in Spring 2020, interrupted due the COVID-19 pandemic, and resumed in March and April 2021. In addition, a short video lecture (length of about 17 minutes) was provided (Suppl. material 2).

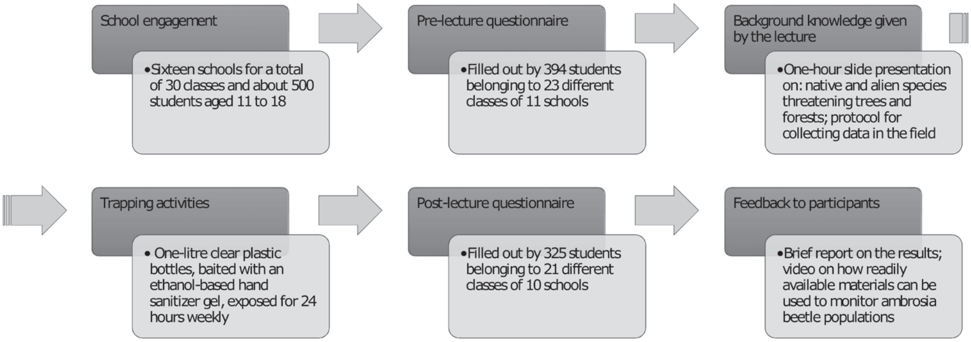

Figure 2. Flowchart of the citizen science project on ambrosia beetles.

A further simple post-lecture questionnaire aimed at testing students on the new knowledge they had acquired about the subject. It contained 10 questions, all closed-ended, with 4 of them having the possibility to provide a brief opinion or explanation (Suppl. material 1). The questionnaire was addressing the increase of awareness and knowledge of IAS, the understanding of their impacts, and the role of information campaigns, such as the one developed with the schools. A brief report on the main results of the experiment and a video on how readily available materials can be used to monitor ambrosia beetle populations (Suppl. material 2) were sent in 2022 to all headmasters and teachers to be shared with the students who had actively participated in the project.

IAS monitoring activity

The traps were prepared using one-litre clear plastic bottles baited with Septaman Gel (Nuova Farmec® S.r.l., Settimo di Pescantina, Italy), a 70% ethanol unscented hand sanitizer gel (Steininger et al. 2015). Traps were tested at the university campus and shown to be efficient in trapping the target ambrosia beetles (see Study organisms). Starting from late March 2021, 3 traps were deployed at each school (Fig. 3), placed within the understory and hung at a height of about 1.5 m above the ground on a branch of trees or shrubs, depending on the vegetation available around each school. The distance from one trap to another varied with the area available, but usually was not less than 8 m. Traps were filled with gel on a day of the week chosen by the school and checked after 24 hours. The short duration of trap exposure prevented complete ethanol evaporation, which would lead to the formation of a crust on the gel surface. Trapped insects were collected by a spoon, then put in a small, labelled plastic bag that was stored in a fridge until delivery to the laboratory for identification (see Study organisms). In one school (school n. 1; Fig. 1) the survey was repeated from the second half of July 2021 until October, to get one more confirmation of the occurrence of new IAS species (for Italy and the EPPO region) detected during the spring trapping (Colombari et al. 2022).

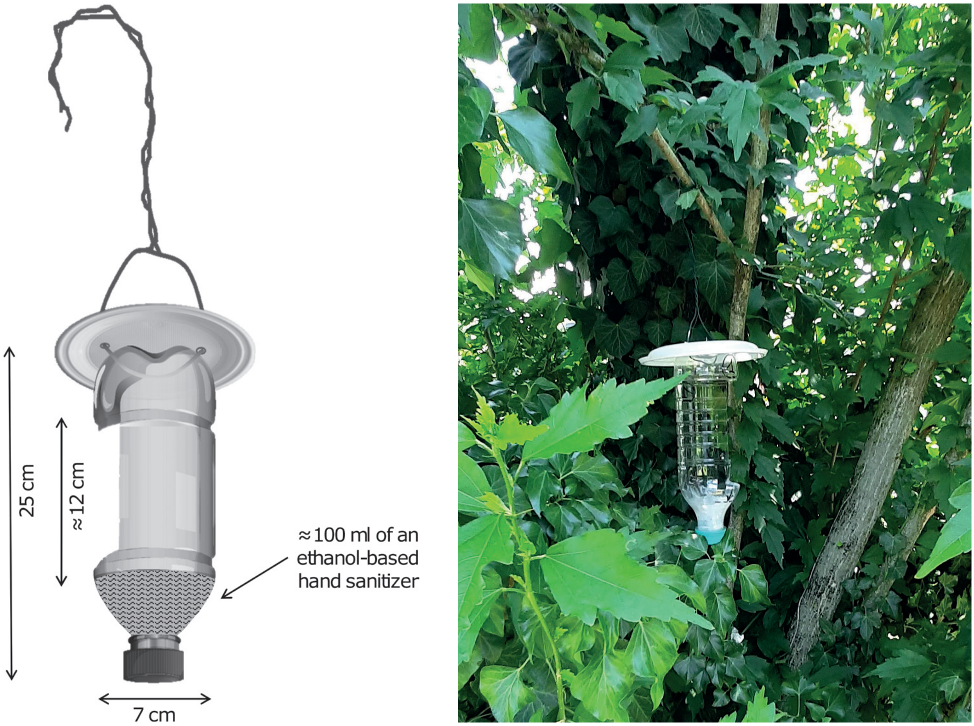

Figure 3. Schematic and photographic representation of a plastic bottle trap filled with hand-sanitizer as attractant.

Data analyses

To compare the trapping data collected by the schools that differed in the duration of the monitoring period and in the number of trapping rounds, the number of captured individuals was standardised by dividing it by the number of trapping rounds. The standardised number of native, alien species, and individuals was considered as dependent variable; while elevation, land cover class, and the number of samples were independent variables.

Landscape composition within three different circles (radius of 0.5, 1 and 2 km) around each school was assessed to determine the impact of habitat variability on data. In QGIS 3.22 (QGIS.org 2022), from the regional land cover map (Regione del Veneto, Sezione Pianificazione Territoriale Strategica e Cartografia – https://idt2.regione.veneto.it/) the percentage (in hectares) of six land cover class of interest was quantified. CORINE land cover nomenclature was followed: (1) discontinuous urban fabric, (2) green urban areas, (3) forest and semi-natural areas, (4) agricultural areas, (5) areas devoid of vegetation, and (6) water bodies. Percentages obtained in QGIS were eventually adjusted after examining high-resolution satellite images in Google Earth Pro (version 7.3.4; © 2022, Google LLC, Mountain View, CA).

A correlation matrix and the Pearson correlation coefficient were used through the 'Tidyverse' and 'Reshape2' packages in the software R (R Core Team 2022) before testing with linear regression the possible relationships. Trap catch data were log-transformed for the analysis.

Results

Increasing public awareness on IAS: Questionnaires

A total of 394 students, belonging to 23 different classes of 11 schools, participated in the pre-lecture questionnaire (Fig. 2). Thirty percent of respondents did not know what an IAS was, and 42% were unsure. Among the "aware" students (28%) the most mentioned insect species were the brown marmorated stink bug, *Halyomorpha halys* (Stål, 1855), and the Asian tiger mosquito (*Aedes albopictus* (Skuse, 1894)). Coypu, *Myocastor coypus* Molina, 1782, was the species cited most among mammals, whereas, among fishes, *Silurus glanis* Linnaeus, 1758 commonly named wels catfish, was very popular. When answered about the main source of information, "aware" respondents indicated "School" followed by "TV" (28% and 21%, respectively), whereas "Social media'"and "Family/Friends'" achieved almost the same percentages (12% and 11%, respectively). "Radio", instead, was sorely neglected (1%). Only 23% of the students perceived alien species to be invasive, i.e., causing damage.

Awareness of the possible occurrence of alien and invasive alien species in Italy was quite high, 87% and 81%, respectively, but the real number of invasive alien species was underestimated by 70% of total respondents. However, 60% considered native Italian species as a possible threat for ecosystems of other countries when introduced. Regarding their motivation for having interest in IAS, on a scale of 1 to 5 respondents rated almost a five (4.8) "if it causes harm to human or animal health", followed by "if it is present in the area where I live" (3.8), "if it causes harm to the environment" (2.9), and "if it can limit my hobbies or activities" (2.4). The motivation "if the species causes harm to the economy" had the lowest score (1.4). When answering the question "How would you prefer to receive information on invasive alien species"? students rated as most favourite source of information "School" (4.8), followed by "TV", "Family/Friends" and "Social media "(3.3, 3.2 and 3, respectively), whereas "Radio" had the lowest score (1.2).

Subsequently, the post-lecture questionnaire was completed (except by two entire classes of two schools and by some absentees from the other classes). Thus, in total, the survey involved 325 students belonging to 21 different classes of 10 schools (Fig. 2). The percentage of correct answers to the two questions present in the pre-lecture questionnaire and repeated in this questionnaire increased from 23% to 89% ("is an alien species always invasive"?) and from 60% to 75% ("could a species native to Italy be defined as invasive when introduced into ecosystems/areas of other countries"?). The five questions related to the new pieces of information given during the lecture got 87%

of correct answers. Many students demonstrated an understanding of environmental issues, with 45% of them providing an adequate explanation of the risks associated with the introduction of an alien species, although beneficial such as an organism that can be used in a classical biological control programme. Ninety percent of respondents believed the role of information is crucial for the proper management of IAS, and 86% that citizen science can be a very useful tool for the management of these harmful species. For almost all students (94%) the lecture was helpful to raise awareness and better understand the threat caused by IAS and the difficulties in managing them. Eighty percent commented that the lecture was fully comprehensive (20% did not comment) and more than half of them (55%) were really intrigued and wanted to gain a better insight into different points (e.g., pathways of introduction, degree of damage, ID, impact on environment and economies, control methods, etc.).

Large-scale surveillance of ambrosia beetles

Fifteen schools out of 16 provided insects caught in traps. The length of the monitoring period varied from one month to two-and-a-half months, whereas the interval between two consecutive samplings was one week with only one exception (2 weeks). Overall, 621 bark and ambrosia beetles, belonging to 9 genera and 11 species, were obtained across the whole monitoring period (Suppl. material 3).

Ambrosia beetles were the large majority (606 individuals) and four out of six species detected were non-native: *A. maiche*, *C. mutilatus*, *Xylosandrus crassiusculus* (Motschulsky, 1866), *Xylosandrus germanus* (Blandford, 1894) (Fig. 4). Native species were *Anisandrus dispar* (Fabricius, 1792) and *Xyleborinus saxesenii* (Ratzeburg, 1837). When assessing the respective percentage of captured individuals, the proportion of non-native was 34.8%, while the native *X. saxesenii* accounted for 62.2% of total insects caught.

Among alien ambrosia beetles, the two congeneric *Xylosandrus germanus* and *X. crassiusculus* constituted 33.0% and 1.3% of the overall catches, respectively. The remaining 0.5% was represented by three females of two new introduced alien species, *C. mutilatus* and *A. maiche*, as already reported by Colombari et al. (2022). Trapping conducted from July to October at the same school of the first records (school n. 1; Fig. 1), revealed the non-occasional occurrence of both the new species, as another 8 *C. mutilatus* (7.3%) and 5 *A. maiche* (4.6%) were captured (Fig. 5). Moreover, in this second monitoring period, total alien ambrosia species accounted for 54.1% given the large percentage of *X. germanus* (42.2%) that was the most common species found in the traps (Fig. 5).

There were strong differences in trap catch among schools (Fig. 6). The number of species and individuals showed a quite strong positive relationship with the sampling frequency, with correlation coefficients' (*r*) values ranging from 0.65 to 0.82. In particular, using regression analyses, it was found that sampling frequency significantly predicted the total numbers of both species and individuals ($R^2 = 0.52$, $F_{(1, 12)} = 13.28$, $P = 0.0034$ and $R^2 = 0.45$, $F_{(1, 12)} = 9.73$, $P = 0.0089$, respectively). That is, the higher

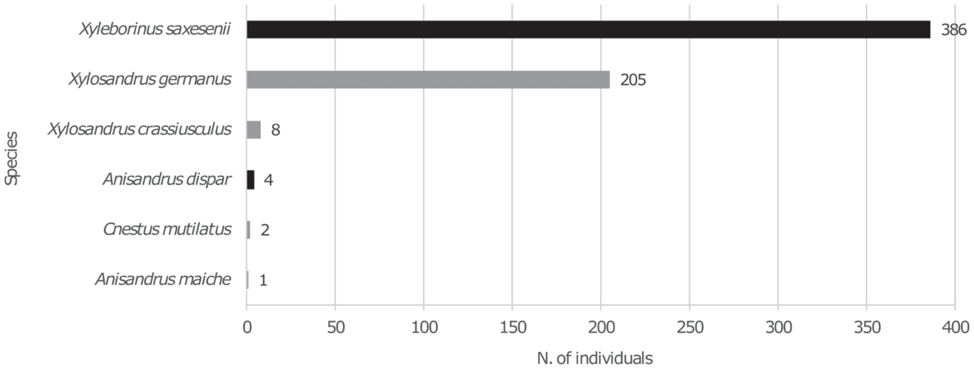

Figure 4. Overall species and abundance of ambrosia beetles (606 specimens). Black and grey bars indicate native and alien species, respectively).

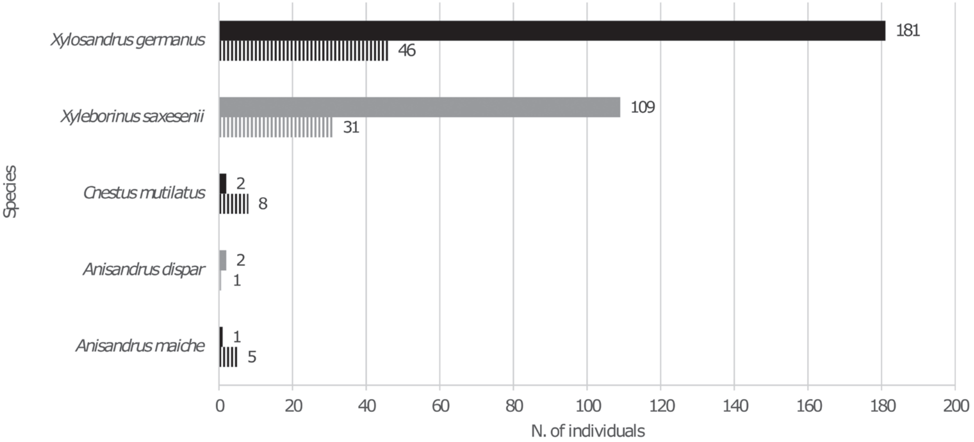

Figure 5. Overall species and abundance of ambrosia beetles at the site of first records of the two new alien species (school n. 1; see Fig. 1 – Map indicating the high school locations). Black and grey bars indicate native and alien species, respectively. Plain colour bars and striped bars indicate the first (from March to June) and the second (from July to October) 2021 monitoring periods, respectively.

the number of samples, the larger the number of species and individuals caught. The same was observed when the number of species and individuals of alien ambrosia beetles were considered alone ($R^2 = 0.43$, $F_{(1, 12)} = 9.11$, $P = 0.0107$ and $R^2 = 0.52$, $F_{(1, 12)} = 12.96$, $P = 0.0036$, respectively). The trap catch was not explained by any of the explanatory variables considered.

Bark beetles accounted for a very small percentage of the total (2.4%), as only fifteen specimens were found in traps, and were represented by five species: *Hypothenemus eruditus* (Westwood, 1834), *Phloeotribus scarabaeoides* (Bernard, 1788), *Hypoborus ficus* Erichson, 1836, *Trypophloeus asperatus* (Gyllenhal, 1813), and *Phloeosinus* spp.

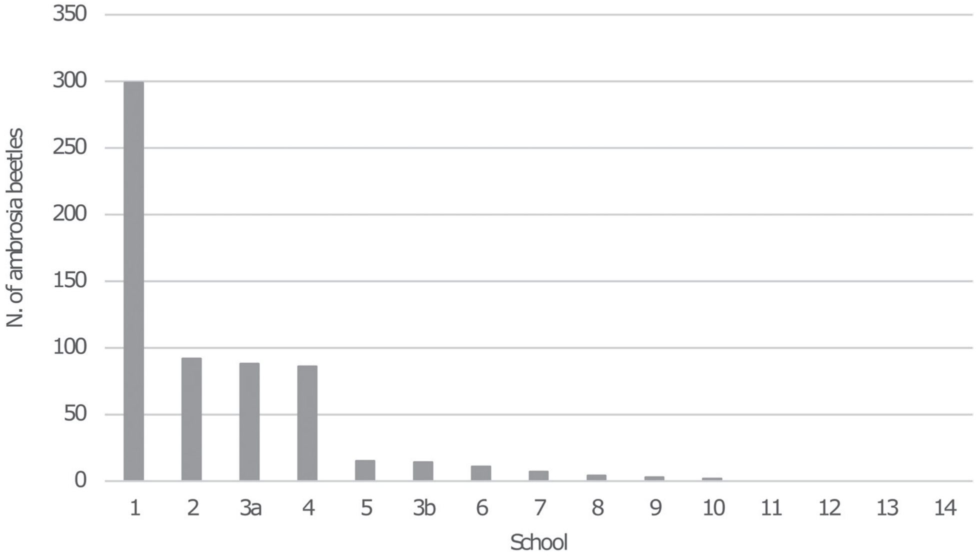

Figure 6. Total number of ambrosia beetles caught at different schools identified by number along the × axis (see Fig. 1 – Map indicating the high school locations).

Discussion

Our study aimed to both educate students and collect scientific data at sites such as schools where surveillance for potentially invasive ambrosia beetles is not usually conducted, or where it is sometimes misunderstood. Student involvement and curiosity were aroused during a pre-survey lecture where we stressed the importance of their role in assessing the unknown species and abundance of ambrosia beetles in their school yards/grounds, and the potential occurrence of undetected invasive species that could pose a serious threat to trees and forests in the surrounding environments. The results obtained provide strong evidence covering all prior expectations.

The citizen science approach contributed important goals for monitoring and alien species detection. Six different species of ambrosia beetles were recorded during the present study, two native species, *A. dispar* and *X. saxesenii*, and four alien species (35% of the total catch). *Xylosandrus germanus* and *X. crassiusculus* were accidentally introduced into Italy in 1992 and 2003, respectively, and are present in some deciduous temperate forests of the Veneto region (EPPO 2010; Rassati et al. 2016), whereas *C. mutilatus* and *A. maiche* were both first records in Italy and first and third records, respectively, in the European part of the EPPO region (Colombari et al. 2022; EPPO 2022).

Among ambrosia beetles, *X. saxesenii*, *X. germanus*, and *X. crassiusculus* were the most represented and our results are consistent with previous research using bottles and ethanol-based lures (Miller and Rabaglia 2009; Reding et al. 2011; Werle et al. 2012; Steininger at al. 2015; Tarno et al. 2021). In particular, ethanol has been demonstrated to have a significant effect on *X. saxesenii*, which usually accounted for the greater percentage of the overall catches (Oliver and Mannion 2001; Miller and Rabaglia 2009;

Galko et al. 2014; Steininger at al. 2015; Chen et al. 2021; Cavaletto et al. 2022) as our data confirm (62.2% of the total number of individuals collected). By contrast, the only other native species, *A. dispar*, was scarce even though it is lured to ethanol-baited traps, and it often represents alongside *X. saxesenii* one of the most trapped ambrosia beetles (Galko et al. 2014; Holuša et al. 2021). The two new species found and confirmed as successfully established, *C. mutilatus* and *A. maiche*, are known instead to respond almost exclusively to baits containing ethanol alone (Sweeney et al. 2016; EPPO 2020) and are significantly more attracted to bottle than to funnel traps (Klingeman et al. 2017; Miller et al. 2018; Tobin and Ginzel 2022).

Different catches indeed can be interpreted as the complex result of many variable factors that can affect trapping rates such as, among others, the aggressiveness of the species and their preferred host condition for attack and/or breeding (Oliver and Mannion 2001; Chen et al. 2021). Anyway, none of the overall species recorded were aggressive, able to attack healthy trees, but were instead associated with stressed (*X. saxesenii* and *X. germanus*), weakened (*X. crassiusculus* and *Hypothenemus* spp.) or dying hosts (*A. dispar*) (Chen et al. 2021; Holuša et al. 2021). Although *C. mutilatus* and *A. maiche* are classified as non-aggressive because of their preference for unhealthy (stressed and weakened) or recently dead small diameter materials, their low host specificity may raise concerns for forest ecosystems and particularly for nurseries, plantations, ornamentals, and fruit trees (Klingeman et al. 2017; EPPO 2020; Chen et al. 2021; Tobin and Ginzel 2022).

Diversity in species richness and abundance among sites was best predicted by sampling effects as more individual, and thus more species were caught where the monitoring effort was more prolonged (McCabe 2011; Brown et al. 2016). Thus, we cannot exclude that more species could be present at those sites where a low number of individuals was sampled and well aware of a bias in our analyses due to method we used, i.e., the ethanol-based lure known to be less attractive to host-specific bark and ambrosia beetles (Miller and Rabaglia 2009; Steininger at al. 2015). The time dimension in a sampling program is of great importance, as it considers temporal dynamics and phenological maturation of populations (Binns and Nyrop 1992). At the site where the monitoring period lasted more than two months, species richness and abundance were the highest, but considering the sites that collected no beetles at all, only in one case out of three a likely explanation was the methodological limitation. In the other two cases, sampling effort was not so different from that of other schools.

Undoubtedly, at the small scale, abundance and species richness are sensitive to sample size, but they also vary naturally, being affected by many geographic, abiotic and biotic factors such as resource availability, environmental heterogeneity, and ability to disperse (McIntyre et al. 2001; Brown et al. 2016). This is particularly true for dynamic and vulnerable urban ecosystems, where free movements of goods linked to population density can act as another driver influencing the occurrence of beetles (McIntyre et al. 2001; Branco et al. 2019; Meurisse et al. 2019; EPPO 2020). The complex interactions among all these local and landscape factors must be studied further for a better explanation of our results.

Questionnaire results showed that students acquired a greater knowledge and increased their awareness and interest by on average +57%, +40% and +55%, respectively, changing their attitude about IAS. At the beginning of the project, less than one third of the students were "aware" of biological invasions and their impact and were able to list some IAS, whereas a large majority of respondents were "unsure", a result in line with the research of Waliczek et al. (2017). It must be considered, however, that there is a great deal of confusion surrounding some terms and concepts relevant to biological invasion as they are open to subjective interpretation, such as the term 'invasive' on which a consensus has not been reached (Colautti and Mc Isaac 2004). If ambiguous, or very technical terminology is used by scientists, for example, invasive and alien species erroneously generate synonyms, which can be expected to create confusion for a lay audience that require a basic understanding of the terms (Verbrugge et al. 2021).

To improve clarity and avoid such unwanted discrepancies that emerged from the results of the pre-lecture questionnaire, during the lecture we devoted time to state key complex terms and concepts in a concise and simple manner. A better understanding of IAS, and a greater awareness of the serious threats they pose, does not always translate into a change in behaviour regarding invasive species (Jordan et al. 2011). Nonetheless, in our case, those students with more positive attitudes towards control and eradication were those "aware" of IAS, as found by Waliczek et al. (2017). These students, being children or grandchildren of farmers, experienced in some way negative/economic impact of IAS and were very knowledgeable about names and number of IAS occurring regionally, in Italy or neighbours' countries. Family was indeed selected as a reliable source of information, and this is a very important fact on which recent literature focused (Halmatov and Ekin 2017; Masykuroh et al. 2022). Quoting van Noordwijk et al. (2021) "people are more often inspired to change their behaviour if they are influenced by their own social contacts, including friends, family, colleagues, and neighbours". But the majority of the "aware" students said they learned about IAS mainly from school, as part of biology curricula. However, they amounted to half of total respondents showing that education on IAS at the high school level must improve to gain support for control and management (Waliczek et al. 2017).

After the lecture and once monitoring activities began, almost all students (87%) gave correct answers to questions related to the new topics explained. If we consider that in the pre-test half of respondents answered correctly, the increase in knowledge is 34%, but if we exclude students "aware" of alien species who had probably a greater basic knowledge, then the increase rises to 57%. Waliczek et al. (2018) has demonstrated that delivering comprehensive lectures and laboratory work on invasive species to students led them to perform better in a post-test than students who only attended lectures. The higher content knowledge was likely attributable to the active learning activity, which is known to have a positive influence on further outcomes more than a simple learning method (Verbrugge et al. 2021), and a combination of the two experiences could achieve even better results.

Another very satisfying feedback was that almost all the students enjoyed the lecture and found it an appropriate awareness-raising initiative for gaining new knowledge and greater awareness of the topic. Remarkably, more than half of them would like to learn more, especially about the multiple negative effects related to the introduction of IAS and practices to manage them (limit their spread).

Citizen science empowers school interest when students had to choose their preferred sources of information. Here school was the most favourite, whereas social media were placed only second, which was unexpected given the age of the respondents. Cerri et al. (2022) analysed whether European Union blacklists of IAS with media coverage increased the curiosity of laypersons seeking further information online, and concluded that there were not more visits than expected after the lists were posted. Considering that Wikipedia is the most famous online encyclopaedia largely used by students as a first (and often unique) approach to various issues, this is a noteworthy result highlighting the need for specific education programmes or public awareness campaigns from school level onwards (Hulme et al. 2010; Butkevičienė et al. 2021). Furthermore, the author's analysis took into consideration invasive alien mammals (such as the raccoon, *Procyon lotor* (Linnaeus, 1758) and the Eastern gray squirrel, *Sciurus carolinensis* Gmelin, 1788) that generally arouse interest and are held in higher affection by people, so making eradication and control difficult (Novoa et al. 2017), unlike insects that rarely pose ethical problems. But sensational news or viral videos may greatly affect interest (Cerri et al. 2022) and indeed, after school, TV and social media were selected as second preferred sources of info.

Our results confirm the primary role of education, which has been recognized as a major driver of change in dealing with sustainability challenges (Leicht et al. 2018). For this reason, teachers and environmental educators are expected to reach the knowledge and skills' objectives required to promote sustainable development (Leicht et al. 2018; Sosa et al. 2021). However, a study of a representative sample of teachers/educators found that half of them had never heard about IAS, or were unsure of the issues, stressing the need for well-trained teachers able to bridge the gap between scientists and students (Sosa et al. 2021; Verbrugge et al. 2021).

Concluding thoughts on citizen science

Despite many global measures implemented to limit the risk of IAS introduction, current tools are ineffective at slowing down the ever-increasing arrivals into new regions at unprecedented rates. An effective early detection of invasive forest pests should involve citizens, as most first records occur in cities or suburban areas. People are often unaware of the role they have in the entire invasive process. Therefore, promoting interest and receiving public collaboration and support through educational activities and information campaigns should be seen as a good long-term investment to counter biological invasions. We show here that citizen science can successfully involve school students, giving them an opportunity to participate and contribute in detection of ambrosia beetle species, a group associated with a number of pathways in international trade. Citizens can significantly help with the collection of scientific data to improve the management of natural and cultivated ecosystems.

Acknowledgements

The authors would like to thank all the headmasters, teachers and students who accepted the collaboration and collected the data. Special thanks go to headmaster Mario Dalle Carbonare, Professor Michele Zanata and their students (III H and IV H classes, SY 2020–2021; high secondary school "Leonardo da Vinci" – Treviso, Italy) for their enthusiastic participation in the project. Finally, the authors thank the reviewers and editors for all their useful and helpful comments on the manuscript. The monitoring programme was conducted in the context of the European project HOMED (Holistic management of emerging forest pests and diseases), which receives funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 771271.

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Supplementary material 1

Lecture questionnaires

Authors: Fernanda Colombari, Andrea Battisti

Data type: PDF file

Link: https://doi.org/10.3897/neobiota.84.95177.suppl1

Supplementary material 2

Slides of the lecture on 'Monitoring of insect species harmful to trees and forests' and link to educational videos

Authors: Fernanda Colombari, Andrea Battisti

Data type: PDF file

Link: https://doi.org/10.3897/neobiota.84.95177.suppl2

Supplementary material 3

Number of individuals of each species captured at each school

Authors: Fernanda Colombari, Andrea Battisti

Data type: table (PDF file)

Link: https://doi.org/10.3897/neobiota.84.95177.suppl3

NeoBiota 84: 231–246 (2023)
doi: 10.3897/neobiota.84.90981
https://neobiota.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
NeoBiota
Advancing research on alien species and biological invasions

Vertical spread of *Hymenoscyphus fraxineus* propagules

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Academic editor: Andrea Battisti | Received 28 July 2022 | Accepted 25 October 2022 | Published 18 May 2023

Citation: Dvořák M, Štoidl P, Rost M (2023) Vertical spread of *Hymenoscyphus fraxineus* propagules. In: Jactel H, Orazio C, Robinet C, Douma JC, Santini A, Battisti A, Branco M, Seehausen L, Kenis M (Eds) Conceptual and technical innovations to better manage invasions of alien pests and pathogens in forests. NeoBiota 84: 231–246. https://doi.org/10.3897/neobiota.84.90981

Abstract

Currently, the ash dieback causal agent *Hymenoscyphus fraxineus* is an established invasive pathogen in most European countries. Its potential to spread quickly among invaded forests is based on its propagules: airborne inoculum composed mainly of ascospores originated in apothecia growing on leaf litter infected during the previous vegetation season. The spread of the inoculum by air masses to distant areas is probable and depends on the availability of the ascospores in higher levels of air. Our study aimed to detect the inoculum in an infected area at heights of more than 20 meters. Our study was conducted in a municipal locality (Boršov nad Vltavou) with tens of infected ash trees (*Fraxinus excelsior*) in South Bohemia (SW Czechia). The infected trees surround an agricultural silo where five rotating arm spore traps (rotorods) were mounted for ten consequent 48h samplings during the peak of the sporulating season (17th July to 6th August 2020). The spore traps were mounted 48, 37, 25, 14 and 0,3 meters above ground. Samples were quantified by qPCR. Results clearly proved the ability of the spores to reach a height of 48 meters. Furthermore, *H. fraxineus* DNA was detected from all five spore traps during all ten samplings. Mostly, the amount of detected spores showed a decreasing trend with height, and varied a lot. During some of the samplings, higher spore concetrations were achieved at the top than at the lower traps, which can be explained by horizontal air transfer of the inoculum from other infected areas. Based on GLM analyses, higher spore concentrations were achieved during days without rain, lower air temperatures, after cloudy, humid and rainy weather without strong winds. A combination of rotorod ROTTRAP 52 with qPCR quantification proved to be an efficient technology for a study focused on the vertical spread of *H. fraxineus* propagules.

Keywords

airborne inoculum, ash dieback, riseability, rotorod, ROTTRAP, spore trap

Introduction

Hymenoscyphus fraxineus (T. Kowalski) Baral, Queloz, and Hosoya is the causal agent of the ash dieback (Kowalski 2006). It is an invasive pathogen introduced to Europe from Eastern Asia (Zhao et al. 2012) probably during the 1990's (Przybył 2002), first reported in Poland (Kowalski 2006). Its current distribution apart from Eastern Asia covers the whole of Europe apart from Portugal, Greece, Albania, Macedonia, Bulgaria and Moldova (EPPO 2022). *H. fraxineus* severely attacks native European ash species *Fraxinus excelsior* and *F. angustifolia* (Kowalski and Holdenrieder 2009) causing a serious threat to mixed deciduous forest habitats, especially riparian forests where other deciduous tree species are endangered by invasive pathogens (Jankovský and Holdenrieder 2009).

The symptoms of the ash dieback and mechanism of infection of the host trees have been well described in numerous publications (Kowalski 2006; Kowalski and Holdenrieder 2009; Gross et al. 2012, 2014; Husson et al. 2012; Cleary et al. 2013; Kräutler et al. 2015; Chandelier et al. 2016; Fones et al. 2016; Haňáčková et al. 2017)

The predominant way to spread this pathogen is via the airborne ascospores (Gross et al. 2012), although the conidia of the imperfect stage *Chalara fraxinea* play an important role (Fones et al. 2016). The ascospores are typically released from apothecia growing on shredded infected leaf rachises during the following growing season, mainly from June to September (Timmermann et al. 2011; Gross et al. 2012; Hietala et al. 2013; Chandelier et al. 2014; Dvořák et al. 2016). The ascospores have been shown to be able to span long distances and in France they have been observed to even reach 50 to 100 km (Grosdidier et al. 2018b).

The probability of the long distance spread of the ascospores depends on the height they can reach to be blown with the air masses (Chandelier et al. 2014; Oteros et al. 2015; Aguayo et al. 2021). Existing studies proved that the inoculum of *H. fraxineus* rises from the ground where it is actively ejected from the apothecia (Timmermann et al. 2011) and it is reliably detectable in aerobiological samples collected by the RNSA (French Network of Aerobiology) at heights of between 10 to 20 meters (Aguayo et al. 2021). According to these authors, ascospores present at this height prove the presence of the pathogen in the landscape scale in the range of tens of kilometres.

The aim of our study was i) to test a methodological approach for describing the vertical spore dispersal pattern of *H. fraxineus*, and ii) to prove the presence of the inoculum as high as possible, at such a height where the spread of the aerosols is more likely affected by horizontal movement of air masses rather than by convection from the ground surface.

Methods

Sampling in ADB infected locality

The sampling point was located in an agricultural silo in Boršov nad Vltavou (South Bohemia; SW Czech Republic); GPS: 48.9244°N, 14.4414°E; 412 m a. s. l. It is an industrial area of a village adjacent to Moldava River. It is located on the SE edge of a large plain called České Budějovice Basin (Českobudějovická pánev), where foothills of Šumava mountains called Blanský les start to rise. Due to the surrounding landscape being characterised by plains (especially in NW, N and E direction), the 52 m high silo of Boršov is probably exposed to wind currents blowing from areas that are at least tens of kilometres away. Trees in this area are mostly represented by mixtures of deciduous middle-European species formed at the riverbanks, gardens and parks, or alleys along railways and roads. Most of the groups of trees comprise a significant portion of ashes (*Fraxinus excelsior*) with typical symptoms of ash dieback (Fig. 1A); in a few cases they are even completely dead trees. The closest source of inoculum = rachises with apothecia was found 60 m from the silo, where a lowest spore trap (R1) was installed. This closest point is an edge of mostly untreated vegetation with many infected ashes following adjacent railway and roads, partly visible in Fig. 1B at the right side of the horizon. This forest-like suburban vegetation is in the northern direction altered with recently recovered park with heavily infested trees; one of them, 170 m distanced, is well visible in the foreground of the Fig. 1A. The sampling was carried out during the peak of the ascospore production season in Czechia (Dvořák et al. 2016) continuously from 17th July to 6th August, 2020. The presence of apothecia was checked in the litter of ash trees adjacent to the sampling point.

Air samplers

To sample the air inoculum rotating arm spore traps (rotorods) ROTTRAP 52 (Miloň Dvořák, Boršov nad Vltavou, Czech Republic) were employed (Fig. 1C). Our air-sampler was a further developed rotorod of Chandelier et al. (2014), based on the description of Perkins and Leighton (1957) and improved by McCartney et al. (1997). A 10 cm-long aluminium arm whirled 2067 rotations per minute with vertical squared brass rods mounted on both ends. The impaction side of the rod (0.8 × 50 mm) was covered by double-sided non-woven tape (Tesa SE, Norderstadt, Germany), which was renewed every 48 hours; exposed stored in sterile 2-ml microtubes at -20 °C before further processing. The rotorods were powered from an electric network via adaptor 220V AC/9V DC. Such an arrangement of rotorod samples 52 litres of air per minute, with a theoretical sampling efficiency close to 100% for particles with a diameter bigger than 10.94 µm (Noll 1970; Dhingra and Sinclair 2017). Further methodological details are described in Dvořák (2022).

Sampling points

The rotorods were installed at five different heights (Fig. 1B): 0.3 m (further mentioned as „*R1*“), 13.84 m („*R2*“), 25.09 m („*R3*“), 36.57 m („*R4*“) and 48.06 m („*R5*“) above ground. The *R1* was positioned close to the ground of the nearest group (60 m apart from the silo) of infected ash trees to monitor the source of inoculum. The *R2* – *R5* were mounted on windows in regular heights on the NNW side of the silo. This

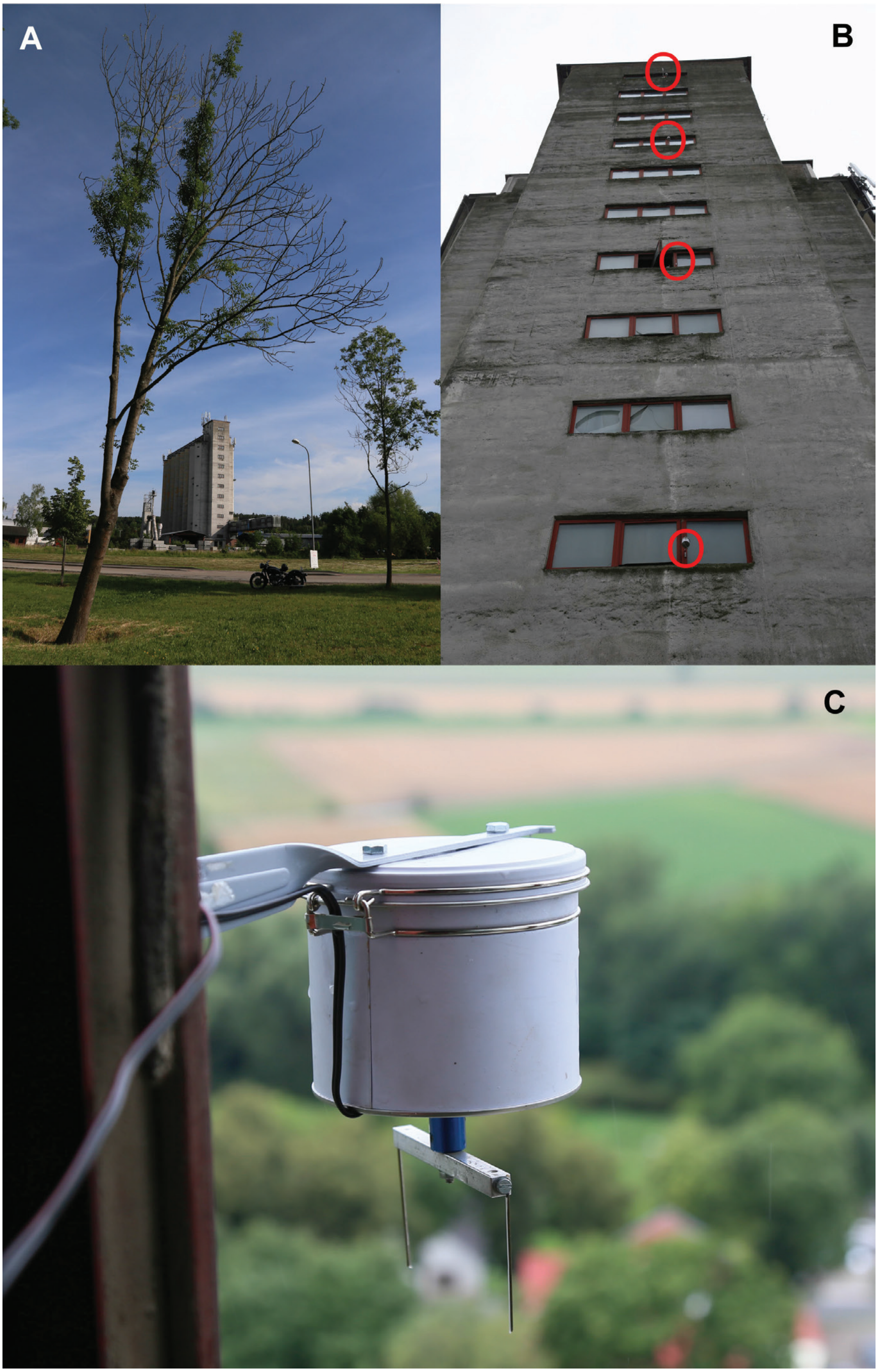

Figure 1. Sampling locality and sampling tools **A** the sampling point in the focus of ash dieback. Agricultural silo is surrounded by ashes infected with *H. fraxineus* **B** sampling spots in the windows of the silo. ROTTRAPs 52 are installed in different heights *R2 - R5* in the windows (red circles) of the silo on its NNW side **C** rotating arm spore trap ROTTRAP 52 installed at *R5*.

side was chosen for its non-exposure to sun (to avoid additional thermic air currents), and due to the prevailing wind direction. It is exposed to the most common wind (NW), which is supposedly bringing the airborne inoculum from the infection sources present in that direction, including the closest inoculum source where *R1* was installed.

Meteorological data

Meteorological data were partly measured by automatic meteorological station Signalizátor (AMET, Velké Bílovice, Czech Republic) and partly received from the archive of the Czech Hydrometeorological Institute (CHMI), station České Budějovice – Rožnov. From the sampling point the CHMI meteorological station is positioned 3.6 km to NE. Data taken from Signalizátor were daily means of relative air humidity (further in the text only air humidity). Data received from CHMI were: i) daily mean of air temperature (air temperature); ii) daily duration of sunshine (sunshine); iii) daily mean of air pressure (air pressure); iv) daily amount of precipitation (precipitation) and v) daily mean of wind speed (wind speed).

DNA extraction

The genomic DNA from samples was extracted with a DNeasy plant minikit (Düsseldorf, Germany). Each microtube with exposed tape was supplemented with one 3-mm sterile tungsten bead and 20 pcs 2-mm glass beads, 400 µl of AP1 buffer and 4 µl of RNase. This mix was ground twice for 60 seconds using a high speed homogenizer Millmix 20 (Domel, d.o.o., Železniki, Slovenia) set at 30 Hz and incubated for 10 minutes at 65 °C. The microtubes were inverted three times during the incubation. Further steps were following the manufacturer's instructions; however, the last step (elution) was not repeated to obtain higher concentration of DNA. DNA samples were eluted in 100 µl and stored at -20 °C before further processing.

Real-time quantitative PCR conditions

Direct specific qPCR was performed using a QuantStudio 6 Flex Real-Time PCR System (Life Technologies Holdings Pte. Ltd., Singapore), Light Cycler 480 Probes master (Roche Diagnostics Nederland BV, Almere, the Netherlands) and primers and probes specific to *H. fraxineus* (Chandelier et al. 2010). The cycling conditions followed the master mix manufacturer's instructions and the setting optimised by (Chandelier et al. 2010): preincubation: 10 min, 95 °C followed by 45 cycles of denaturation: 10 sec, 95 °C; annealing: 30 s, 60 °C; extension: 1 s, 72 °C. Composition of the reaction mixture was following: 0,2 µl of each primer (final concentration 400 nM), 0,2 µl of TaqMan probe (200 nM), 5 µl of TaqMan Universal PCR Master Mix, 1,4 µl of sterile deionized water and 3 µl of template DNA. Every reaction was performed in two technical repetitions together with a negative control containing the master mix without template DNA.

Absolute quantification

The concentrations of *H. fraxineus* DNA in the samples were expressed as numbers of copies of the target sequence in 1 µl of template DNA (further only *CN*). These values were obtained using a standard curve generated from reactions with different *CN*s (2.5×10^{2} to 2.5×10^{-2}) of plasmid pCR 2.1 TOPO TA vector (Invitrogen, Carlsbad, California, USA) by QuantStudioTM Real-Time PCR System Version 1.3 (Thermo Fisher Scientific). Plasmids contained species - specific insert (PCR products amplified with Cf-F and Cf-S primers). DNA was extracted from pure cultures of *H. fraxineus* (collection of Mendel University in Brno).

To express the absolute amount of ascospores in every analysed sample, an absolute quantification of ascospore suspension was performed. For that purpose ten apothecia were collected from ash leave rachises and immersed in 1 ml of distilled water in a 2-ml microtube. The following day, the clear liquid upper part without apothecia and other debris was transferred into a clean 2 ml microtube. Vortexed ascospore suspension was quantified in Bürker chamber by microscope. Ten-fold serial dilutions from 18750 to 1.875 ascospores in 100 µl of distilled water were transferred into clean 2 ml microtubes and DNA was extracted with the same protocol as for the spore trap samples. Extracted DNA samples were used as standards for a qPCR absolute quantification of the plasmids previously used for the quantification of the environmental samples. The lowest detectable concentration which turned positive in all three technical repetitions of the sample was 18.75 ascospores per sample ($Ct = 36.328$, SD = 0.862).

Statistical analysis

To describe the influence of meteorological factors on the ability of the inoculum to spread vertically, meteorological variables were averaged for three particular days of every sampling. Furthermore, the factor of riseability (*FR*) has been defined. It is expressed as the ratio of the ascospore concentration recorded at the highest sampler (*R5*) to the concentration at the lowest sampler (*R1*). It takes values lower than 1.0 in case the *R5* ascospore concentration is lower than *R1* concentration.

In order to describe the relationship between *CN* and the character of the weather described by explanatory variables (air temperature, precipitation, sunshine, air humidity, wind speed and air pressure), generalised linear regression models were constructed with explanatory variables measured on the same day as the dependent variable *CN*, or with explanatory variables recorded during previous four samplings (i.e. one sampling lag = period of preceding two days before the sampling started, two samplings lag = two to four days before, three samplings lag = four to six days before and four samplings lag = six to eight days before sampling) to simulate the lag of the pathogen's reaction on the changing weather. Due to the nature of the dependent variable (a strictly positive variable showing a positive skew for *R1*, *R5* and *FR*), we used the gamma distribution with a logarithmic link function when

Figure 2. Vertical profiles of spore concentrations recorded during the ten samplings.

fitting the regression models. To select variables for individual models, we used the procedure described in Morgan and Tatar (1972) implemented in the "bestglm" library for the R software (R Core Team 2018). This approach allowed the selection of the best subset of explanatory variables for particular GLMs. However, because of the low number of observations, in some cases the models did not converge numerically, and it was not possible to obtain the optimal regression model. The selection of suitable models was made with regard to the AIC values achieved (the smallest the best).

At the same time, we tried to model the relationship between *CN* and the height of the sampler. Due to convergence problems with an exponential model based on a differential equation, we used a somewhat simpler empirical exponential model with the following analytical form:

$$CN = \beta_0 \cdot \exp(-\beta_1 \cdot h)$$

where β_0 and β_1 are the estimated regression coefficients, and h is the height in meters. All numerical calculations were performed using the R 4.2.0 programming environment (R Core Team 2018).

Results

Fresh apothecia were observed on infected rachises at the sampling locality from the beginning of July until the end of the sampling (6th August, 2020). Consequently, all ten 48-h samplings showed presence of *H. fraxineus* in the air (Fig. 2).

Every sampler positively detected inoculum during every sampling. The lowest positively detected spore concentration was detected in samples from the highest sampler *R5* from the sampling started on 4th August (*CN* = 0.018, *Ct* = 37.428). This least concentrated sample contained 1.89 ascospores; however, this amount was calculated extrapolating the ascospore suspension standard curve, hence the exact amount cannot be taken in consideration. The highest concentration was recorded at *R1* during the following sampling started on 6th August (*CN* = 659.063, *Ct* = 27.181). Taking in account the sampling rate 52 l/min and sampling period of 48 h, we detected average spore concentration in a range from 0.013 to 462.12 spores per m^3.

Most of the detected spore concentrations showed a clear decreasing trend following the height of the sampling point. The resulting nonlinear regression model (Fig. 3; residual SE = 99.85, DF = 96), corresponding to the aforementioned parameterization, describing the generally decreasing trend, was estimated as follows:

$$CN = 150.3243 \cdot \exp(-0.2155 \cdot h)$$

GLM analyses resulted in 10 significant models to estimate *CN*s from meteorological variables measured during the sampling and lagged of 2–4, 4–6 and 6–8 days. Their parameters can be found in supplementary file “Parameters of GLM models”. The calculated *p* and positive or negative meaning of each parameter are displayed in Table 1.

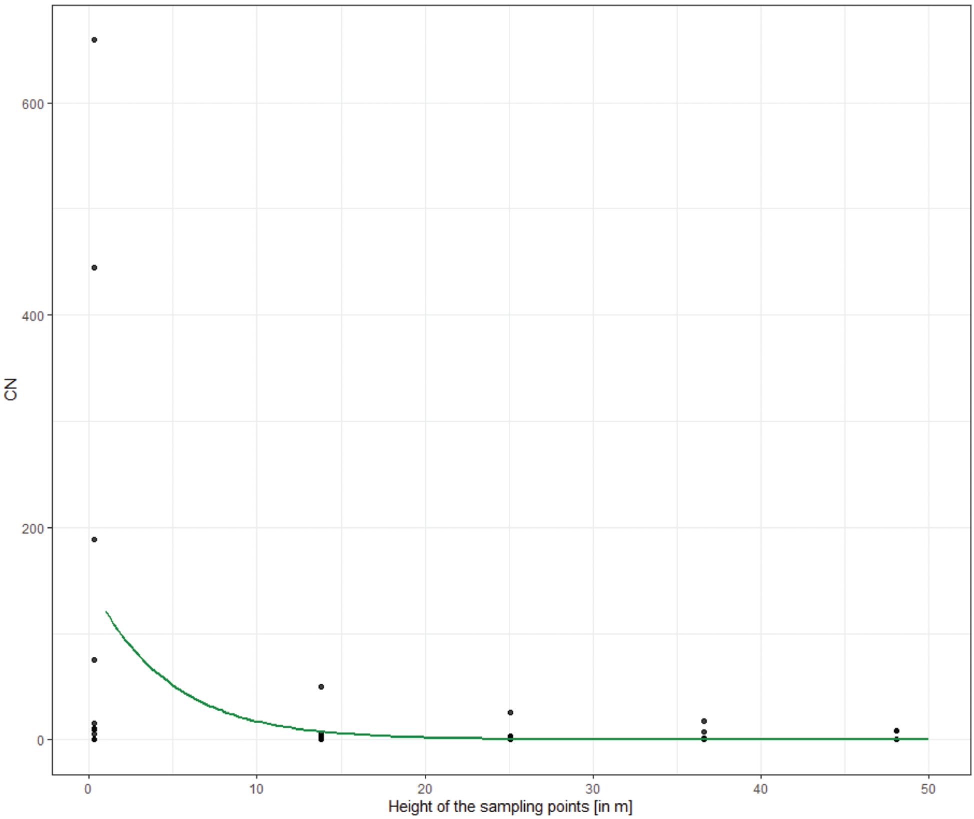

Figure 3. Model of the vertical spread of the inoculum. The nonlinear regression model described the decreasing trend of spore concentrations with increasing height of sampling.

Discussion

Our results prove for the first time, that propagules of *H. fraxineus* are reliably detectable at almost 50 meters above ground, where they have their main source in infected rachises (Gross et al. 2012). Repeated sampling revealed *H. fraxineus* DNA's presence to be more than twice as higher as in an aerobiological study, where air was sampled by 7-day volumetric spore traps (Hirst 1952) of RNSA (Aguayo et al. 2021).

The results of this study also confirm statements of other authors (Chandelier et al. 2014) who described decreasing trend of ascospore concentrations up to three meters, regardless of the site and time of the sampling. This was a general trend among our samplings. The ground sampling spot *R1* always showed higher *CN* than the top spot *R5*. In one case even more than 20,000 times.

However, not in every sampling did the *CN* values descend with increasing height. The sampling spot *R4* showed lower values, than *R5* for samplings 5, 6 and 7. This was probably due to overloading the sampling rods with dust, which is a critical handi-

Table 1. Meteorological variables as parameters of GLM models and their *p* during sampling and 0–8 days before sampling (d.b.s.).

R1	**6–8 d.b.s.**	**4–6 d.b.s.**	**2–4 d.b.s.**	**0–2 d.b.s.**	**sampling**
Air temperature				.014	.026
Sunshine				.009	.034
Precipitation			.032		.002
Air humidity			.032	.045	
Wind speed		.01	.031		
Air pressure			.012		
R5	**6–8 d.b.s.**	**4–6 d.b.s.**	**2–4 d.b.s.**	**0–2 d.b.s.**	**sampling**
Air temperature		.043		.040	.018
Sunshine				.001	
Precipitation		.001		.001	.002
Air humidity		.006	.004		
Wind speed					
Air pressure					
FR	**6–8 d.b.s.**	**4–6 d.b.s.**	**2–4 d.b.s.**	**0–2 d.b.s.**	**sampling**
Air temperature			.016		
Sunshine		.009	.029		
Precipitation	.034	.018		.009	.048
Air humidity				.011	
Wind speed			.016	.015	
Air pressure					
Legend:		Influence			
		positive	negative		
Significant parameter (*p* < .05)		*p*	*P*		
Highly significant parameter (*p* < .01)		*p*	*P*		

cap of rotorods (Lacey and West 2006; Chandelier et al. 2014; Dhingra and Sinclair 2017). The dust apparently came from outlets of ventilators of the silo adjacent to *R4* while depositing the harvested grain.

Furthermore, the sampling site *R3* gave higher *CN*s than it could be expected to follow the descendent trend during samplings 8 and 9. Similarly, *CN*s at *R5* from samplings 1, 5 and 6 were also not lower than *CN* of lower sampling points. An explanation for this anomaly could be the effect of horizontal air currents which could bring a higher amount of inoculum from more distant localities, influencing the results of sampling only in these cases of low local concentrations. Generally, abnormalities in the trend of decreasing *CN* with the height of sampling can be interpreted as a consequence of the fact that inoculum detected in heights above 10 m might be representative for areas within a perimeter of at least tens of kilometres (Aguayo et al. 2021). Study focused on long distance spread of pollen grains found correlation of pollen data from the sampler (elevated 10–20 m) and land use even 200 km apart (Oteros et al. 2015, 2017). In the case of our experiment, samplers *R2*, *R3*, *R4* and *R5* were mounted higher than 10 meters; *R5* even in more than 48 m. Taking into account the previously mentioned studies (Oteros et al. 2015, 2017, Aguayo et al. 2021) there is a theoretical presumption that the detected inoculum can be related to sources at a distance of up to hundreds of kilometres. Also fungal spores' long distance proofs are known. Based on spatial air sampling and modelling it was revealed that tobacco blue mould outbreak was caused by *Peronospora tabacina* spores blown from sources several hundred km away (Aylor et al. 1982). Another example of long distance transport of spores was studied by Vasaitis and Enderle (2017) considering a possible way of ash dieback introduction to Great Britain. Agricultural silo used for this study is located very beneficially from this point. Its height is almost three times bigger than the canopy layer of surrounding vegetation, which acts as a natural barrier for the vertical spread of the inoculum (Aylor 1999). Taking into account the geomorphology of surrounding landscape and predominant wind direction (NW), it is highly likely exposed to air masses from an adjacent plane area called "Českobudějovická pánev" (České Budějovice Basin), which is a tectonic ditch oriented NW – SE, elevated 380–410 m a. s. l., almost 70 km long and 10–12 km wide. From personal experiences we know that infected ashes are very frequently present there, especially in alleys along roads, riverside plantations and municipal green spaces. We assume that inoculum detected in *R2* – *R5* may originate in this area, not only from the sources adjacent to the sampling point. Transfer of spores by air masses up to 100 km has been already proven (Grosdidier et al. 2018b), which is enabling this hypothesis.

Long distance transfers of *H. fraxineus* by air masses have always been an important issue. It was considered that *H. fraxineus* had been introduced into Great Britain between 2008 and 2011 via long distance transfer of the air inoculum from mainland Europe. This statement was strongly supported with a model (Vasaitis and Enderle 2017). Recently, *H. fraxineus* was found for the first time in Spain (Asturias, NW Spain) on matured trees and its surrounding regeneration (Stroheker et al. 2021). The fact that it has not yet been observed in NE Spain, which is adjacent to the closest distribution of the pathogen, implies the possibility of long-distance transport of the inoculum from France via the Bay of Biscay. However, the introduction of the pathogen through plant material import cannot be overlooked.

Results of the GLM modelling of the determination of the detected spore concentrations by meteorological factors discovered numerous relations. Although the low number of repetitions decreases the reliability of the results' interpretation, we would like to highlight some of them:

Air temperature proved to have significantly positive effect two days preceding the sampling at both *R1* and *R5*. This confirms previous observations of Chandelier et al. (2014), who found a positive influence of higher temperatures on spore concentrations. On the contrary, sooner than two days before and directly during the sampling, the effect of air temperature was significantly negative. This pattern indicates, and confirms, that for the ascospore development and release the high air temperature is not favourable (Grosdidier et al. 2018a). Since the entire sampling period can be characterized like very warm with maximal temperatures exceeding 30 °C, this biological limitation of the pathogen was more apparent within our experiment.

The daily amount of precipitation showed different effects at different sampling heights. *R1* was significantly positively affected by precipitations two to four days before sampling. Through the enhanced humidity of the ground surface, rain proved to be essential for successful ascospore maturation and release (Timmermann et al. 2011; Hietala et al. 2013; Dvořák et al. 2016) and the consequent progress of ash dieback (Chumanová et al. 2019). On the other hand, concentrations at *R5* and riseability were significantly negatively affected by rain during the days before the sampling, and even highly significantly during the sampling. Two explanations for this phenomenon are probable: i) ascospores currently present in the air are washed out by rain from the aerosol which is a known factor (Aylor 1999), and ii) trapped ascospores are partly splashed from the sticky sampling surface. For the significantly positive effect of precipitation on riseability during sampling we do not have any reasonable explanation; however, the p for this parameter is approaching the limit of significance (p = .048) hence its importance is ambiguous.

Daily duration of sunshine showed highly significantly negative effect on spore concentration at *R1* and *R5* two days before the sampling and on riseability during the preceding four to six days. However, the sunshine was significantly positive during the sampling at *R1* and *R5* and during the preceding two to four days it was significantly positively affecting the riseability. This partly confirms and partly neglects results of Burns et al. (2022) who found a clear determination of *H. fraxineus* spore release after five-day-average net radiation and leaf moisture.

Air humidity was found to be an important factor through our sampling. At *R5* the humid air was significantly determining the inoculum load after two to six days; similarly, at *R1* there was a significantly positive effect on inoculum concentrations lagged by two to four days. The promoting effect of air humidity on the disease spread and establishment has already been emphasised many times (Timmermann et al. 2011; Hietala et al. 2013; Dvořák et al. 2016; Čermáková et al. 2017; Chumanová et al. 2019; Volke et al. 2019). However, up to two days before the sampling, the air humidity had an opposite effect at *R1* and negatively affected also the riseability. This ambiguity of the air humidity effect for sporulation emphasizes the necessity for more detailed study of the ascospore discharge mechanism such as in Ingold (1999).

Wind speed proved to have a significantly negative influence on ascospore concentrations at *R1* after two to six days. This effect is probably due to the desiccation of leaf rachises and growing apothecia which are not able to mature and sporulate under such conditions (Timmermann et al. 2011; Hietala et al. 2013). The significantly negative effect of wind speed was also found to affect the sampling directly. Since the wind speed is measured in horizontal direction, it is logical that strong wind cannot be beneficial for vertical spore transfer. Horizontally, moving air masses are supposedly continuously removing the local inoculum, which would otherwise reach higher air levels by convection (Garratt 1994; Lacey and West 2006).

Air pressure did not show much importance in our experiment. It was a significant parameter with negative influence on spore concentrations at *R1* after two to four days. At the same period and sampling height two other factors had positive influence on the spore concentrations: precipitations and air humidity. Naturally, rainy and humid weather is characterized by low air pressure, which we assume to be a reason for this result.

From a methodological point of view, rotating arm spore trap ROTTRAP 52 proved to be a reliable tool for the detection of *H. fraxineus* inoculum. All samplers successfully completed all ten 48h samplings at five sampling spots without any blackout even in hot or rainy weather. This reliability has been improved compared to previous experiments (Dvořák et al. 2016, 2017; Čermáková et al. 2017). Rotorods combined with quantitative real-time PCR detection and quantification of *H. fraxineus* in the air continuously produce robust and valuable data (Chandelier et al. 2014, Dvořák et al. 2016; Čermáková et al. 2017). Similarly, in our study all samplings yielded positive results. On the other hand, the possibility of overloading by other, more concentrated particles (in this study's case it was dust) must be always taken into consideration in experiment planning and interpretation of the results.

Conclusion

Our study revealed the permanent presence of the *H. fraxineus* inoculum up to 48 meters above the ground during the whole sampling period. Its concentration is continuously changing depending on previous and current weather, and decreases with height. It poses a persistent threat to ash trees, either at local or landscape scale. This finding supports a sceptical outlook for the future of ashes in European forests, but also confirms the important role of high height air sampling of the propagules of this invasive alien pathogen to ensure its reliable monitoring.

Acknowledgements

This work was funded by a HORIZON 2020 project (agreement no. 771271): Holistic Management of Emerging Forest Pests and Diseases (HOMED). We would like to thank to EUROPASTA SE company for cooperation and full access to their silo in Boršov nad Vltavou, namely to the director Ing. Josef Maška, Jaroslav Mrkvička, Ladislav Hakl and Jaroslav Hnilička. Furthermore, we thank Dr. Leticia Botella Sánchez for preparing the plasmid for absolute quantification.

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NeoBiota 84: 247–266 (2023)
doi: 10.3897/neobiota.84.90843
https://neobiota.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
NeoBiota
Advancing research on alien species and biological invasions

Duplex real-time PCR assay for the simultaneous detection of *Ophiostoma novo-ulmi* and *Geosmithia* spp. in elm wood and insect vectors

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Academic editor: Christophe Orazio | Received 25 July 2022 | Accepted 13 February 2023 | Published 18 May 2023

Citation: Pepori AL, Luchi N, Pecori F, Santini A (2023) Duplex real-time PCR assay for the simultaneous detection of *Ophiostoma novo-ulmi* and *Geosmithia* spp. in elm wood and insect vectors. In: Jactel H, Orazio C, Robinet C, Douma JC, Santini A, Battisti A, Branco M, Seehausen L, Kenis M (Eds) Conceptual and technical innovations to better manage invasions of alien pests and pathogens in forests. NeoBiota 84: 247–266. https://doi.org/10.3897/neobiota.84.90843

Abstract

Dutch elm disease (DED) is a destructive tracheomycosis caused by *Ophiostoma novo-ulmi*, an ascomycete probably originating in East-Asia that is devastating natural elm populations throughout Europe, North America and Asia. The fungus is mainly spread by elm bark beetles that complete their life cycle between healthy and diseased elms. Recently, it has been highlighted that some fungi of the genus *Geosmithia*, which are similarly well associated with bark beetles, seem to also play a role in the DED pathosystem acting as mycoparasites of *O. novo-ulmi*. Although some relationship between the fungi is clear, the biological cycle of *Geosmithia* spp. within the DED cycle is still partly unclear, as is the role of *Geosmithia* spp. in association with the bark beetles. In this work, we tried to clarify these aspects by developing a qPCR duplex TaqMan assay to detect and quantify DNA of both fungi. The assay is extremely sensitive showing a limit of detection as low as 2 fg μl^{-1} for both fungi. We collected woody samples from healthy and infected elm trees throughout the beetle life cycle. All healthy elm samples were negative for both *Geosmithia* spp. and *O. novo-ulmi* DNA. *Geosmithia* spp. are never present in infected, but living trees, while they are present in frass of elm bark beetles (EBB – *Scolytus* spp.) and at each stage of the EBB life cycle in much higher quantities than *O. novo-ulmi*. This work provides a better understanding of the role and interactions occurring amongst the main players of the DED pathosystem.

Keywords

DNA quantification, duplex qPCR, Dutch Elm Disease, *Geosmithia* spp. life cycle, *Ophiostoma novo-ulmi*, *Scolytus multistriatus*

Introduction

Dutch elm disease (DED) is a destructive tracheomycosis that has devastated natural elm populations throughout Europe, North America and Asia. The disease is caused by two subspecies of *Ophiostoma novo-ulmi* Brasier, i.e. ssp. *novo-ulmi* and ssp. *americana*, previously known as Eurasian (EAN) and North American (NAN) races, respectively (Brasier and Kirk 2001). These ascomycetes are responsible for the ongoing DED pandemic; since the 1970s, they have replaced the less aggressive *O. ulmi* (Buisman) Nannf. that caused the first DED pandemic at the beginning of the last century (Spierenburg 1921).

The fungus is mainly spread by species of elm bark beetles (Coleoptera, Curculionidae, Scolytinae) that complete their life cycle between healthy and diseased elms. Bark beetles belonging to the genus *Scolytus* Geoffroy are the main vectors of *O. ulmi* s.l. (Webber and Brasier 1984). Specifically, *S. scolytus* (F.) and *S. multistriatus* (Marsham), the large and small elm bark beetles (EBB), respectively, are the most common and important species spreading the pathogen worldwide (Webber and Kirby 1983; Webber and Brasier 1984; Webber and Gibbs 1989; Webber 1990, 2000; Faccoli 2001, 2004). The small EBB is the main vector in the Mediterranean area (Santini and Faccoli 2015). During spring, at the time of beetle flight, host plants are more prone to be infected and temperatures are favourable for fungal growth in plant tissue, enhancing the pathogen's aggressiveness (Santini and Faccoli 2015). Callow adults, carrying the *O. novo-ulmi* conidia, feed at the crotches of 1–2 years-old twigs of adult healthy elm trees to complete their sexual development, thus inoculating the pathogen. Once inoculated, the spores germinate into a growing mycelium and reach the xylem, where the fungus moves into the vessels (Webber and Brasier 1984), inducing the formation of tyloses and gels in the xylem vessels (Stipes and Campana 1981; Rioux et al. 1998; Ouellette et al. 2004a, b; Et-Touil et al. 2005) as a defence response. Later, the beetles move to dying elms to lay eggs in the inner bark of the trunks or branches, which provide the optimal environment for larval development (Rudinsky 1962) and fruiting of the pathogen (Webber and Brasier 1984). New contaminated beetles emerge from the bark to complete the cycle.

Recently, it has been highlighted that other organisms also play roles in the DED pathosystem (Pepori et al. 2018). Some fungi of the genus *Geosmithia*, a monophyletic morphogenus of anamorphic ascomycetes mainly associated with phloem-feeding bark beetles (Kolařík et al. 2004, 2005, 2007, 2008; Kubátová et al. 2004; Kolařík and Jankowiak 2013; McPherson et al. 2013; Jankowiak et al. 2014; Machingambi et al. 2014; Huang et al. 2019; Crous et al. 2022; Meshram et al. 2022), are consistently found in infected elms (Kolařík et al. 2004, 2005, 2007, 2008; Pepori et al. 2015; Huang et al. 2019; Strzałka et al. 2021; Crous et al. 2022).

Geosmithia spp., like *O. novo-ulmi*, are associated with elm bark beetles (Pepori et al. 2015, 2018) and can similarly be found in beetle larval galleries – thus sharing habitat with *O. novo-ulmi* – but the ecological niches of these fungi are different. A widespread horizontal gene transfer of the *cerato-ulmin* gene between *O. novo-ulmi* and *Geosmithia* species has been reported (Bettini et al. 2014).

Pepori et al. (2018) demonstrated the existence of a close and stable relationship, which can be classified as mycoparasitism by *Geosmithia* spp. towards *O. novo-ulmi*. There are still several gaps in defining the life cycle and lifestyle of elm-related *Geosmithia* species, especially when they cross and interact with the life cycle of DED fungi.

Previously, several methods of biocontrol of *O. novo-ulmi* have been investigated and have appeared promising under experimental conditions, although their practical application in the field has been limited (Webber and Gibbs 1984; Webber and Hedger 1986; Sutherland and Brasier 1995; Brasier 2000; Griffin 2000; Hintz et al. 2013; Ganley and Bulman 2016).

An accurate description of the life cycle and identification of the key factors that can enhance the attitude of *Geosmithia* spp. to act as effective biocontrol agents against *O. novo-ulmi* may be strategic in controlling the further spread of the disease.

In this study, a new, ad hoc duplex real-time PCR assay, based on TaqMan probe chemistry genus-specific for *Geosmithia* and species-specific for *O. novo-ulmi*, for the simultaneous quantification of both fungi from different matrices, was developed. Application of this molecular approach will fill the knowledge gaps related to the life cycle of *Geosmithia* spp. and will uncover the tripartite interactions amongst *O. novo-ulmi*, *Geosmithia* spp. and EBBs.

Materials and methods

Fungal strains

The duplex qPCR assay was validated using 12 isolates of *Geosmithia* spp. belonging to nine different species (*G. fassiatiae*, *G. flava*, *G. funiculosa*, *G. langdonii*, *G. lavendula*, *G. obscura*, *G. omnicola*, *G. pallida* and *G. putterillii*) and eight isolates of *Ophiostoma* from five species (*O. himal-ulmi*, *O. novo-ulmi* ssp. *novo-ulmi*, *O. novo-ulmi* ssp. *americana*, *O. quercus* and *O. ulmi*). Two ubiquitous species were also included as outgroups (Table 1). All fungal strains were obtained from the Institute for Sustainable Plant Protection – National Research Council (IPSP-CNR, Florence, Italy) collection (Table 1). Fungal isolates were grown on 300PT cellophane discs (Celsa, Varese, Italy) on 1.5% Malt Extract Agar (MEA; Difco Laboratories, Detroit, MI) in 90 mm Petri dishes and incubated in the dark at 20 °C. After 10 days, the mycelium was scraped from the surface of the cellophane and stored in 1.5 ml microfuge tubes (Sarstedt, Verona, Italy) at -20 °C. Fungal mycelium (ca. 100 mg fresh weight) was transferred into a 2-ml microfuge tube (Sarstedt) with two tungsten beads (3 mm) (Qiagen, Hilden, Germany) and ground with a Mixer Mill 300 (Qiagen) (2 min; 20 Hz). DNA extraction was performed using the E.Z.N.A. Plant DNA Mini Kit (Omega Bio-tek, Norcross, GA, USA), following the manufacturer's instructions. The concentration of extracted DNA was measured using a Nanodrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

Table 1. Fungal strains used in this study.

Species	Isolate Code	Host	Origin	Duplex qPCR[a] (*O. novo-ulmi/ Geosmithia* spp.)
Geosmithia fassiatiae	CCF3334	*Quercus pubescens*	Czech Republic	(-/+)
G. flava	MK1551	*Pteleobius vittatus* (on *Ulmus laevis*)	Czech Republic	(-/+)
G. funiculosa	IVV7	*U. minor*	Italy	(-/+)
G. funiculosa	CNR28	*U. minor*	Czech Republic	(-/+)
G. langdonii	MK1643	*Scolytus multistriatus* (on *U. laevis*)	Czech Republic	(-/+)
G. langdonii	MK1644	*Scolytus multistriatus* (on *U. laevis*)	Czech Republic	(-/+)
G. lavendula	CCF3394	*Chaetopyelius vestitus* (on *Pistacia terebinthus*)	Croatia	(-/+)
G. obscura	MK86	*Scolytus intricatus* (on *Quercus robur*)	Czech Republic	(-/+)
G. omnicola	CNR5	*U. minor*	Czech Republic	(-/+)
G. omnicola	CNR21	*U. minor*	Czech Republic	(-/+)
G. pallida	MK1622	*S. kirschii* (on *U. minor*)	Spain	(-/+)
G. putterillii	CCF3342	*Scolytus rugulosus* (on *Prunus* sp.)	Czech Republic	(-/+)
Ophiostoma himal-ulmi	CBS374.67	*U. wallichiana*	India	(-/-)
O. novo-ulmi ssp. *novo-ulmi*	CKT11	*Ulmus* sp.	Iran	(+/-)
O. novo-ulmi ssp. *novo-ulmi*	R64	*Ulmus* sp.	Romania	(+/-)
O. novo-ulmi ssp. *americana*	H172	*Ulmus* sp.	USA	(+/-)
O. novo-ulmi ssp. *americana*	H363	*Ulmus* sp.	Ireland	(+/-)
O. quercus	CBS722.95	*Quercus* sp.	Austria	(-/-)
O. ulmi	E2	*Ulmus* sp.	Netherlands	(-/-)
O. ulmi	R21	*Ulmus* sp.	Romania	(-/-)
Epiccoccum sp.	F15	*Q. suber*	Italy	(-/-)
Cladosporium sp.	F11	*Q. suber*	Italy	(-/-)

[a] + = positive qPCR amplification ; – = no amplification products.

Sampling on elm trees and bark beetle collections

Elm bark beetle (EBB) here means exclusively *Scolytus multistriatus* (Marsham), as it is the most common, active and effective DED vector in Italy and the only one found during sampling.

A total of 123 samples were collected from: i) wood of healthy elm trees; ii) dying elm trees showing DED symptoms (wood from newly-DED infected tissues, wood from old DED infections, living EBB larvae, living EBB pupae and wood frass from maternal and larval galleries); iii) EBB callow adults in flickering traps; and iv) adult females in galleries after oviposition (Table 2). All samples were collected in 1.5 ml microfuge tubes (Sarstedt), frozen in liquid nitrogen and immediately brought to the IPSP-CNR laboratory facilities to be stored in a -80 °C freezer before DNA extraction.

DNA extraction from woody samples and insects

Each woody sample (approx. 100 mg fresh weight from each collected tree and frass) and each insect sample (approx. 5.4 mg fresh weight –containing up to 4 larvae or pupae collected alive) was transferred into 2-ml microfuge tubes (Sarstedt), each containing two tungsten beads (Qiagen) and ground with a Mixer Mill 300 (Qiagen) (2 min; 20 Hz). DNA extraction was performed by using the E.Z.N.A. Plant DNA Minikit (Omega Bio-tek), according to the manufacturer's instructions.

Table 2. List of samples collected and tested in this study.

Source	N° of collected samples	Species	Sample	Geographic orgin (Lat., Long.)
Healthy trees	8	*Ulmus minor*	Wood	Florence, Italy (43.772402°N, 11.176578°E)
	6	*U. minor*	Wood	Sesto Fiorentino, Italy (43.817554°N, 11.188349°E)
New DED infection	7	*U. minor*	Wood	Siena, Italy (43.317361°N, 11.306896°E)
	4	*U. minor*	Wood	Castelnuovo Berardenga, Italy (43.341865°N, 11.519271°E)
	3	*U. minor*	Wood	Asciano, Italy (43.296617°N, 11.460314°E)
Old DED Infections	6	*U. minor*	Wood	Bagno a Ripoli, Italy (43.734871°N, 11.324844°E)
	4	*U. minor*	wood	Montelupo Fiorentino, Italy (43.720481°N, 10.988996°E)
	3	*U. minor*	Wood	Florence, Italy (43.811942°N, 11.240917°E)
	2	*U. minor*	Wood	Castagneto Carducci, Italy (43.194141°N, 10.567814°E)
	2	*U. minor*	Wood	Asciano, Italy (43.296617°N, 11.460314°E)
	2	*U. minor*	Wood	Poggibonsi, Italy (43.476425°N, 11.180486°E)
	2	*U. minor*	Wood	Castelnuovo di Val di Cecina, Italy (43.267503°N, 10.960795°E)
	1	*U. minor*	Wood	Asciano, Italy (43.296617°N, 11.460314°E)
	1	*U. minor* 'CEM187'	Wood	Bagno a Ripoli, Italy (43.734871°N, 11.324844°E)
	1	*U. minor* 'CEM370'	Wood	Bagno a Ripoli, Italy (43.734871°N, 11.324844°E)
	1	*U. minor*	Wood	Chiusdino, Italy (43.163653°N, 11.088422°E)
	1	*U. minor*	Wood	Castelnuovo Berardenga, Italy (43.341865°N, 11.519271°E)
Frass from EBB galleries	4	*U. minor*	Wood frass	Poggibonsi, Italy (43.476425°N, 11.180486°E)
	3	*U. minor*	Wood frass	Castelnuovo di Val di Cecina, Italy (43.267503°N, 10.960795°E)
	3	*U. minor*	Wood frass	Sesto Fiorentino, Italy (43.817554°N, 11.188349°E)
	2	*U. minor*	Wood frass	Chiusdino, Italy (43.163653°N, 11.088422°E)
EBB larvae	5	*Scolytus multistriatus*	Larvae	Montelupo fiorentino, Italy (43.720481°N, 10.988996°E)
	3	*S. multistriatus*	Larvae	Castelnuovo di Val di Cecina, Italy (43.267503°N, 10.960795°E)
	2	*S. multistriatus*	Larvae	Chiusdino, Italy (43.163653°N, 11.088422°E)
EBB pupae	4	*S. multistriatus*	Pupae	Castelnuovo di Val di Cecina, Italy (43.267503°N, 10.960795°E)
	2	*S. multistriatus*	Pupae	Montelupo fiorentino, Italy (43.720481°N, 10.988996°E)
	2	*S. multistriatus*	Pupae	Chiusdino, Italy (43.163653°N, 11.088422°E)
EBB in the galleries	5	*S. multistriatus*	Insect	Asciano, Italy (43.296617°N, 11.460314°E)
	2	*S. multistriatus*	Insect	Castagneto Carducci, Italy (43.194141°N, 10.567814°E)
	1	*S. multistriatus*	Insect	Florence, Italy (43.811942°N, 11.240917°E)
	1	*S. multistriatus*	Insect	Montelupo fiorentino, Italy (43.720481°N, 10.988996°E)
EBB callow adult	11	*S. multistriatus*	Insect	Sesto Fiorentino, Italy (43.817554°N, 11.188349°E)
	6	*S. multistriatus*	Insect	Montelupo fiorentino, Italy (43.720481°N, 10.988996°E)
	4	*S. multistriatus*	Insect	Florence, Italy (43.811942°N, 11.240917°E)
	4	*S. multistriatus*	Insect	Castagneto Carducci, Italy (43.194141°N, 10.567814°E)
	2	*S. multistriatus*	Insect	Chiusdino, Italy (43.163653°N, 11.088422°E)
	1	*S. multistriatus*	Insect	Bagno a Ripoli, Italy (43.734871°N, 11.324844°E)
	1	*S. multistriatus*	Insect	Florence, Italy (43.772402°N, 11.176578°E)
	1	*S. multistriatus*	Insect	Vaglia, Italy (43.890112°N, 11.339246°E)

Total DNA from each adult *S. multistriatus* beetle collected from flickering traps, as well as in mother and larval galleries, was extracted singly or in batches of four when it came to the beetles collected in the multi-funnel trap. No surface sterilisation was carried out. Beetles were ground by using Mixer Mill 300 (Qiagen) and DNA from the insect's body was extracted by using the E.Z.N.A. Insect DNA Minikit (Omega Bio-tek), following the manufacturer's instructions.

Total DNA was checked by agarose gel electrophoresis and was quantified using the Nanodrop ND-1000 spectrophotometer (NanoDrop Technologies). The quality of DNA extracted from elm woody tissue was checked using a SYBR-Green real-time PCR endogenous control for the actin gene, following Pepori et al. (2019).

TaqMan MGB probes and primer design

Two sets of primers and TaqMan minor groove binding (MGB) probes were newly designed to obtain genus-specific *Geosmithia* and species-specific *Ophiostoma novo-ulmi* qPCR markers.

The recently-described *G. funiculosa* (Crous et al. 2022) is associated with a broad spectrum of bark beetle species that feed on coniferous and deciduous host plants, including elms and it is phylogenetically close to other *Geosmithia* species found on elm (Pepori et al. 2015; Crous et al. 2022). *Ophiostoma novo-ulmi* ssp. *americana* and ssp. *novo-ulmi* do not differ at the chosen ITS1 target region. These features made these isolates suitable for their use as standard strains for qPCR assay validation.

Primer and TaqMan MGB probes were designed using Primer Express Software 3.0 (Applied Biosystems Foster City, CA, USA), on the basis of the internal transcribed spacer (ITS2) region of *Geosmithia funiculosa* (accession n. KR229885 – isolate CNR28) and ITS1 region for *O. novo-ulmi* ssp. *americana* (accession n. EF429091 – isolate 182E). The TaqMan MGB probes were labelled with the reporter dyes 6-carboxyfluorescein (FAM) and VIC at the 5’ end and a minor groove binder non-fluorescent quencher (MGBNFQ) at the 3’ end. Primers and probes sequences were reported in Table 3.

Homology of the amplicon sequence (both for *Geosmithia* spp. and *Ophiostoma novo-ulmi*) with the sequences of other species in the NCBI database was performed using standard nucleotide BLAST (BLASTn) (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Primers were synthesised by Eurofins Genomics (Ebersberg, Germany) and probes by Applied Biosystems (Foster City, CA, USA). Specificity of the primers and probes was also tested by qPCR on DNA from axenic cultures (Table 1), as reported below.

Duplex qPCR assay

Real-time PCR was assayed in MicroAmp Fast 96-well Reaction Plates (0.1 ml) closed with optical adhesive and using the StepOnePlus Real-Time PCR System (Applied Biosystems, Life Science, Foster City, CA, USA). Singleplex and duplex qPCR mixtures and thermocycler conditions were tested in this study (data not shown) in order to determine optimal qPCR conditions for the two target pathogens, which were finally set up as follows.

Duplex qPCR was performed in a 25 µl final volume containing: 12.5 µl TaqMan Universal Master Mix (Applied Biosystems,), 300 nM each forward primer (OphF and GeoF), 300 nM each reverse primer (OphR and GeoR), 200 nM each TaqMan MGB probe (OphPr and GeoPr) and 5µl genomic DNA. Each DNA sample was assayed in three replicates. Three wells, each containing 5 µl of sterile water, were used as the no-template control (NTC). For singleplex qPCR assay, only one primer set and one TaqMan MGB probe were used and sterile ddH_2O was added to reach the final volume (25 µl). The PCR protocol was 50 °C (2 min), 95 °C (10 min), 45 cycles of 95 °C (30 s) and 60 °C (1 min).

Data results were analysed using the software SDS 1.9 Sequence Detection System (Applied Biosystems) after manual adjustment of the baseline and fluorescence threshold.

Table 3. Primer and TaqMan MGB probes used in the duplex qPCR assay.

Target	Primers and probes	Sequences (5’-3’)[a]	Amplicon length (bp)	Tm (°C) [b]
Ophiostoma novo-ulmi	OphF (Forward primer)	GCCGCCCGAACCTTTT	60	58 58 68
	OphR (Reverse primer)	TGGCTGTTTTTGTTTGTTTCTCA		
	OphPr (TaqMan MGB probe)	**VIC**-AAACCAGTAACGAAACGT-**MGBNFQ**		
Geosmithia spp.	GeoF (Forward primer)	CGCCGTAAAACCCCAACTT	61	59 58 69
	GeoR (Reverse primer)	GTTCAGCGGGTATTCCTACTTGA		
	GeoPr (TaqMan MGB probe)	**FAM**-ACCAAGGTTGACCTCG-**MGBNFQ**		

[a]VIC=fluorescent label (Applied Biosystems); MGBNFQ= minor groove binder non-fluorescent quencher; FAM= reporter dyes 6-carboxyfluorescein. [b]T_m= melting temperature.

qPCR specificity and sensitivity assay and standard curve

The specificity of primers and probes (genus-specific for *Geosmithia* and species-specific for *Ophiostoma novo-ulmi*) were tested both in singleplex and duplex qPCRs using DNA (at final concentration of 5 ng μl^{-1}) from axenic cultures of other strains and species of the target organisms, as well as of closely-related species associated with elm and ubiquitous species (Table 1).

The standard curve was generated using DNA from strain CNR28 (*G. funiculosa*) and strain 182E (*O. novo-ulmi* ssp. *americana*) as standards. For each target species, standard points (ranging from 5 ng μl^{-1} to 2 fg μl^{-1}) were made using ten 1:5 serial dilutions of standard DNA of both target fungi. Each standard curve was built with standards run in both singleplex and duplex qPCR. The minimum amount of template DNA (limit of detection, LOD) that yielded 100% positive results with the singleplex and duplex assay (qPCR sensitivity) was determined. Three replicates of each dilution were analysed and reactions were repeated at least twice. Quantification of both fungal species DNA in unknown samples was made by interpolation from standard curves generated with *O. novo-ulmi* and *G. funiculosa* DNA standards that were amplified in the same PCR run. Reproducibility of the qPCR assay was assessed by computing the coefficient of variation (CV) amongst the mean values in eight independent assays. PCR efficiency was calculated against the slope of the standard curve (Eff = $10^{-1/slope}$ -1) (Bustin et al. 2009), from eight independent experiments.

Validation of qPCR assay in plant tissues

To evaluate the possible interference of plant DNA extract in the newly-designed qPCR assay, the same ten 1:5 serial dilutions (ranging from 5 ng μl^{-1} to 2 fg μl^{-1}) of fungal DNA (*O. novo-ulmi* or *Geosmithia* spp.) were mixed with DNA extracted from healthy elm woody tissue (at 20 ng/tube final concentration) and run on the same qPCR plate of the standard curve (fungal DNA diluted in sterile ddH_2O). All samples were run in triplicate as previously described.

Linearity and sensitivity of qPCR on DNA from ascospore serial dilution

To test the linearity and the sensitivity of each qPCR TaqMan protocol, two different ascospore serial dilutions were obtained from mycelium of axenic culture of *Ophiostoma novo-ulmi* (strain 182E) and *Geosmithia funiculosa* (strain CNR28). Fungal isolates were grown on MEA media and, after five days, the presence of the ascospores was observed using a Zeiss Axioskop 50 optical microscope. Each ascospore suspension was obtained by scraping the surface of mycelium with a sterile scalpel and then placing it in 1 ml of sterile water. The number of ascospores per ml was determined in a Burker Chamber and, for each pathogen, six 1:10 serial dilutions (1:1 *O. novo-ulmi* 1.3×10^7 ascospores per ml; 1:1 *G. funiculosa* 5.6×10^6 ascospores per ml) were prepared. All suspension dilutions were centrifuged for 3 min at 12,000 rpm, the excess water was removed and the ascospore pellets were ground in a 1.5-ml Eppendorf tube using a micropestle (Eppendorf, Hamburg, Germany) in 500 µl of lysis buffer AP1 (EZNA Plant DNA, Omega Bio-tek) and DNA extraction continued with the recommended protocol provided by EZNA Plant DNA kit (Omega Bio-tek, Inc). For each ascospore dilution, 2.5 µl of extracted DNA was assayed using the StepOnePlus Real-Time PCR System (Applied Biosystems) as previously described.

Statistical analysis

For each fungal pathogen (*Ophiostoma novo-ulmi* and *Geosmithia* spp.), pairwise comparison of Cq values of standard points was conducted between duplex and singleplex using the chi-square ($\chi 2$) test. The Bland-Altman plot was used to determinate the agreement between the two assays (Bland and Altman 1986, 2007). The amount of fungal DNA in insects' bodies and elm tissues was expressed as pg fungal DNA/µg total DNA extracted. Differences in *Geosmithia* spp. and *O. novo-ulmi* DNA were detected by the analysis of variance (ANOVA), followed by Tukey's HSD post-hoc test. The significance was evaluated at the 0.05 p-level. Statistical analysis was carried out using XLSTAT (Addinsoft New York, USA).

Results

Specificity and sensitivity of qPCR assays

BLAST search in NCBI showed 95–100% homology between the designed amplicon sequences and the sequence of *Geosmithia* species and *Ophiostoma novo-ulmi* deposited in GenBank.

All DNA from *Geosmithia* spp. isolates (Table 1) were positively amplified after qPCR, using the *Geosmithia*-genus-specific assay. The *Geosmithia* genus-specific assay did not generate any amplicon with DNA from any of the other species tested, such as *O. quercus*, *O. ulmi*, *O. novo-ulmi*, nor with *Epiccoccum* spp. and *Cladosporium* spp.

Ophiostoma novo-ulmi-specific assay successfully amplified DNA from all the *O. novo-ulmi* strains and it did not generate any amplicon DNA with other *Ophiostoma* species tested, including *O. ulmi*, *Geosmithia* spp. or any of other fungal species tested (Table 1). No differences in terms of specificity between singleplex and duplex were observed for the tested isolates.

The standard curves generated with the singleplex and duplex assays did not significantly differ for *Geosmithia* spp. (χ^2 = 0.612; df = 1; P = 0.43) or for *O. novo-ulmi* (χ^2 = 0.167; df = 1; P = 0.68) (Fig. 1). The high level of agreement between singleplex and duplex platforms was confirmed by Bland-Altman plots (Fig. 1). In general, similar levels of agreement between singleplex and duplex for each target gene were reported, with most C_q differences in each comparison falling within the limits of agreements.

The amplification efficiency of duplex qPCR assay was calculated from the slope value of the standard curves according to the equation previously described (Kubista et al. 2006). The slopes of the standard curves were 3.522 for *O. novo-ulmi* and 3.507 for *Geosmithia* spp. and these values corresponded to amplification efficiencies ranging from 92.3% to 92.8% (Table 4). The correlation coefficient (r^2) was 1 and 0.998 for *O. novo-ulmi* and *Geosmithia* spp., respectively, indicating a strong linear relationship between the Cq value and the logarithm of the fungal DNA concentration (Table 4).

The limit of detection (LOD) of both duplex and singleplex qPCR assays were as low as 2 fg μl^{-1} for both *Geosmithia* spp. and *O. novo-ulmi*.

The duplex assay revealed no amplification difference between pure fungal DNA (*Geosmithia* spp. or *O. novo-ulmi*) in sterile water and the same amounts diluted in a mixture containing DNAs of different organisms (*Geosmithia* spp., *O. novo-ulmi* and DNAs from elm wood and insect).

Table 4. Efficiency, linear correlation and assay precision of duplex qPCR assay for the detection of *Geosmithia* spp. and *O. novo-ulmi*.

Fungi and variability experiment	Efficiency (%)	Linear correlation (R^2)	Coefficient of variation %
***Geosmithia* spp.**			
Intra assay	95.3	0.999	1.18 ± 0.13
Inter assay	92.8	0.999	1.3 ± 1.07
Ophiostoma novo-ulmi			
Intra assay	96.8	0.999	1.19 ± 0.01
Inter assay	92.3	0.999	1.06 ± 0.66

Duplex real-time qPCR from plant tissues and bark beetles

All DNA samples were analysed by duplex qPCR for the quantification of *Geosmithia* spp. and *O. novo-ulmi.* No DNA of *Geosmithia* spp. or *O. novo-ulmi* was detected in any of the healthy elm samples analysed. Elm samples with recent or previous seasons' infections showed the exclusive presence of *O. novo-ulmi*, with increasing amounts of the pathogen according to the stage of infection (from 18 pg DNA/µg total DNA in recent infections to 140 pg DNA/µg total DNA in older infections) (Fig. 2).

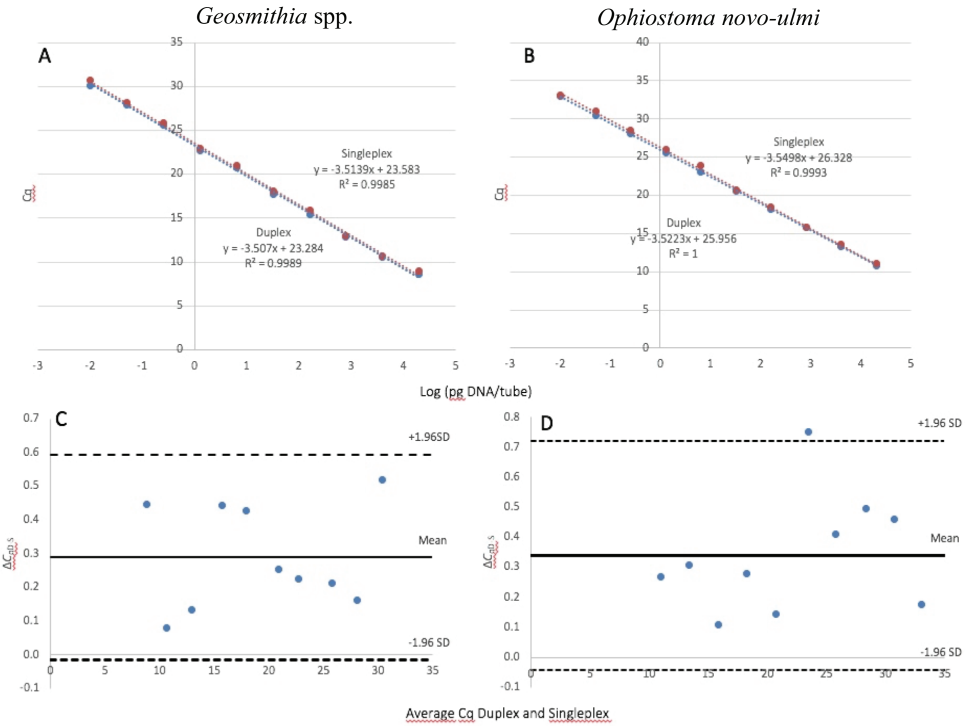
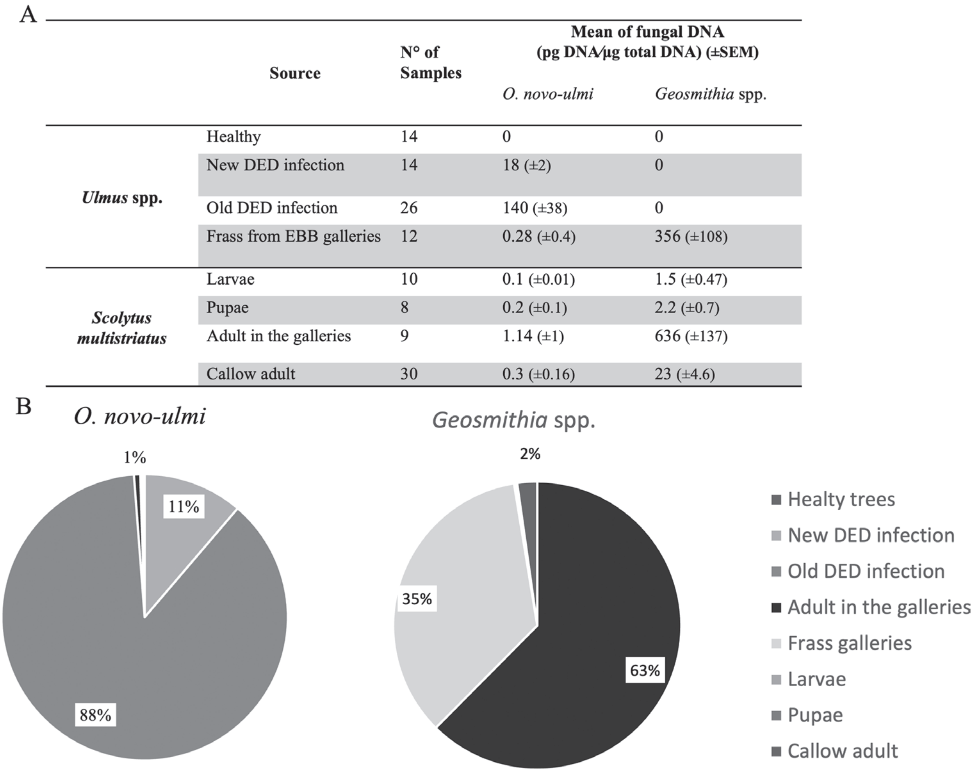


Figure 1. Comparison between singleplex and duplex qPCR **A** standard curve of *Geosmithia* spp. and **B** *Ophiostoma novo-ulmi* generated with the singleplex (blue dots) and duplex (red dots). For each targeted gene, ten different 1:5 serial dilutions (ranging from 5 ng μl^{-1} to 2 fg μl^{-1}) of *Geosmithia* spp. and *O. novo-ulmi* standard DNA were assayed in triplicate. Standard curves were generated by plotting the threshold quantification cycle value (C_q value) versus the logarithmic genomic DNA concentration of each dilution series. The Bland-Altman plot for *Geosmithia* spp. (**C**) and *O. novo-ulmi* (**D**) are shown for the same serial dilutions. The C_q difference between the two methods ($\Delta C_{qD\text{-}S}$) is plotted against the average of both methods (x-axis) for every individual pair of measurements. The interval of the mean of the difference ± 1.96 times the standard deviation (SD) defines the 95% interval of the limits of agreement.

Duplex qPCR results revealed the presence of both fungi in all EBB samples, collected in different stages of their biological cycle (including samples from frass collected in the galleries). In particular, significantly higher quantities of *Geosmithia* spp. DNA compared to *O. novo-ulmi* were found on female EBB collected after ovideposition ($p < 0.0001$, Fig. 2A), corresponding to 63% of the amount of *Geosmithia* found inside the insect galleries (Fig. 2B). The presence of *Ophiostoma* detected was significantly lower ($p = 0.05$) than *Geosmithia* in all EBB samples analysed, especially in the insects present in the galleries (Figs 2, 3). The quantity of *Geosmithia* DNA in wood frass and callow adult insects was significantly higher than in pupae and larvae ($p < 0.0001$; Fig. 2A).



A

	Source	N° of Samples	Mean of fungal DNA (pg DNA/µg total DNA) (±SEM)	
			O. novo-ulmi	*Geosmithia* spp.
***Ulmus* spp.**	Healthy	14	0	0
	New DED infection	14	18 (±2)	0
	Old DED infection	26	140 (±38)	0
	Frass from EBB galleries	12	0.28 (±0.4)	356 (±108)
Scolytus multistriatus	Larvae	10	0.1 (±0.01)	1.5 (±0.47)
	Pupae	8	0.2 (±0.1)	2.2 (±0.7)
	Adult in the galleries	9	1.14 (±1)	636 (±137)
	Callow adult	30	0.3 (±0.16)	23 (±4.6)

Figure 2. Fungal DNA of *O. novo-ulmi* and *Geosmithia* spp. on analysed samples by using duplex qPCR assay **A** Mean of fungal DNA (pg DNA/µg total DNA) ± SEM (Standard Error of the Mean) **B** percentage presence of *O. novo-ulmi* and *Geosmithia* spp. DNA in plant tissues and EBB samples analysed.

Linearity and sensitivity of qPCR on DNA from ascospore serial dilution

DNA extracted from ascospore serial dilution showed a linear relationship for *O. novo-ulmi* (R^2 = 0.999) and *Geosmithia* spp. (R^2 = 0.999) (Fig. 4). Fungal DNA quantification for *O. novo-ulmi* ranged from 31 pg μl^{-1} to 10 fg μl^{-1} corresponding to 10^7 to 10^2 ascospore/ml; while for *Geosmithia* spp. from 5.8 pg μl^{-1} to 3 fg μl^{-1} corresponding to 10^6 to 10 ascospore/ml.

Discussion

Dutch Elm Disease is still causing massive damage in Europe and the death of elms is still catastrophic in ecological and economical terms through the loss of genetic diversity and trees lost from urban and natural forest stands (Santini and Faccoli 2015).

The detection of fungi by traditional methods, such as isolation from plant tissues and insect bodies, may be sometimes challenging and time-consuming, seriously

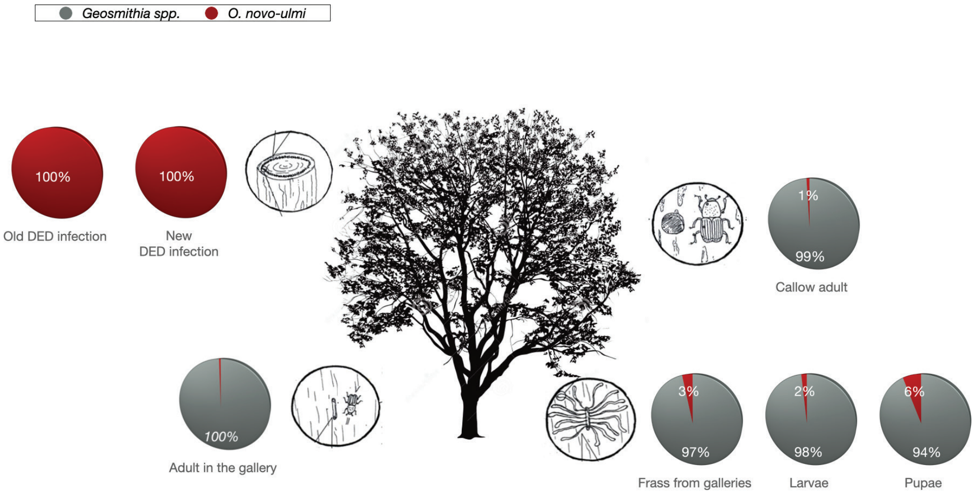


Figure 3. Proportion of target DNAs (%) at different DED infection stages.

impairing our knowledge of their biological cycles. In addition, these methods do not allow quantification of the target organism. DNA sequence-based molecular tools, such as real-time PCR, digital PCR or, even if indirectly, LAMP (Hardinge and Murray 2020) and HTS, are increasingly used to enable accurate and specific detection and quantification from any substrate (Lindahl et al. 2013).

Multiplex qPCR is an increasingly utilised method (Bonants and te Witt 2017; Luchi et al. 2018; Rizzo et al. 2020) allowing simultaneous detection of different microorganisms in the same reaction, thus significantly reducing both the quantity of samples and the overall cost of the analysis. The use of a multiplex assay may prove particularly important to distinguish pathogens that cause similar symptoms, as in the case of *Fusarium circinatum* and *Caliciopsis pinea*, which cause comparable symptoms on *Pinus radiata* (Luchi et al. 2018) or the study of the four European species of *Heterobasidion* that attack conifers (Ioos et al. 2019).

In this study, the developed and validated duplex qPCR assay was able to detect and quantify the presence of *Geosmithia* spp. and *O. novo-ulmi* from different matrices (frass and plant tissue; adults, larvae and pupae of bark beetles) collected from healthy and DED-symptomatic elms.

This duplex qPCR assay showed high reproducibility and specificity for both genus-specific *Geosmithia* spp. and species-specific *O. novo-ulmi* and high sensitivity (LODs 2 fg μl^{-1}, for both fungi). This assay allowed the detection in elm trees of *O. novo-ulmi* infections before symptoms had fully developed, as well as the presence of *Geosmithia* spp. in different host tissues and on the insect body.

Our results confirm that *Geosmithia* is closely associated to EBB galleries, as also reported by Kolařík et al. (2008), showing extremely high amounts on the EBB female bodies and in maternal gallery frass.

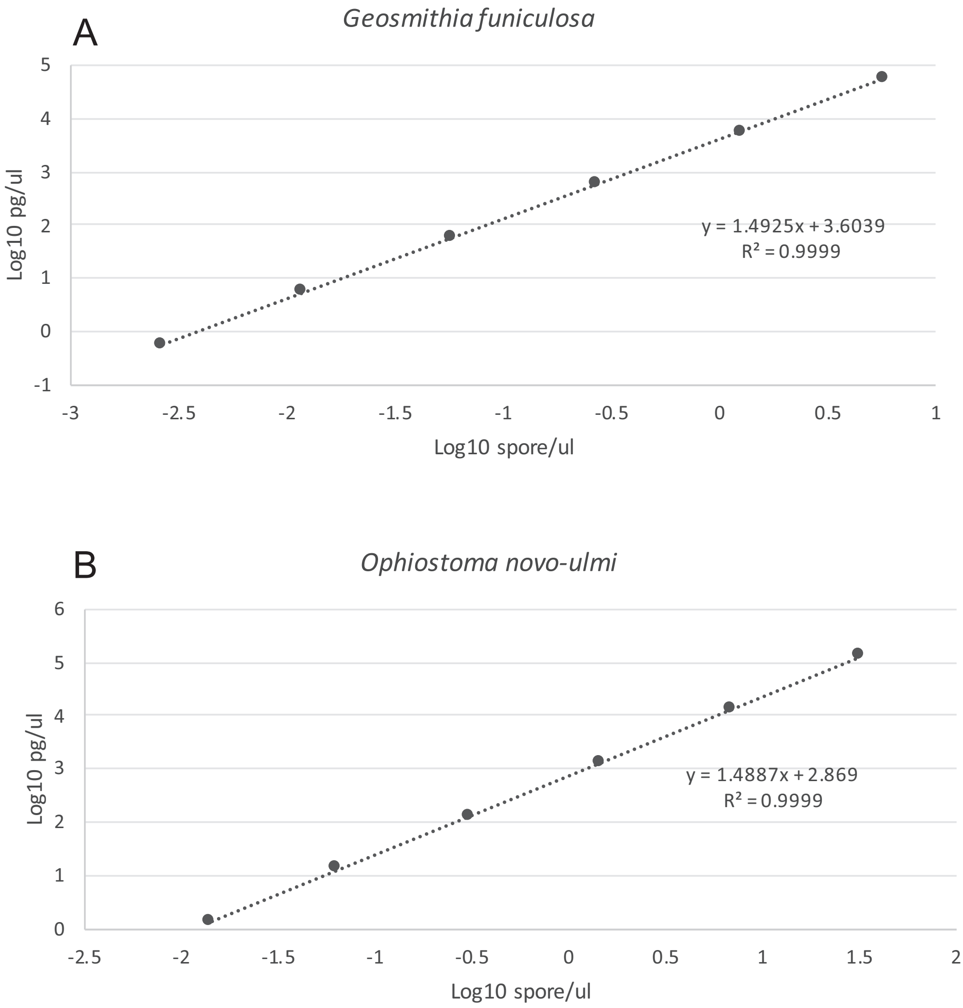


Figure 4. The quantification of **A** *Geosmithia funiculosa* and **B** *Ophiostoma novo-ulmi* extracted from ascospore dilutions. For each sample, dilution data were reported as the median value of triplicates ±SD.

Our observations indicate that the humidity and temperature conditions within the subcortical galleries seem to promote the fitness of the fungi studied here, particularly *Geosmithia*. In addition, the results show that *Geosmithia* is always present in beetle galleries along the studied period, but the detected DNA quantity decreases significantly as the insect's maturation progresses, i.e. from the time of ovideposition until the callow adults flicker.

This study confirms the association between bark beetles and *Geosmithia*, as also reported by other studies (Kolařík et al. 2008, 2017; Pepori et al. 2018; Huang et al. 2019) and highlights that this association is constant throughout the life of the bark beetle and is not only specific to the subcortical developmental stage. Moreover, fungi benefit from this association because the beetles transport them to new host plants (Paine et al. 1997; Six 2003; Six and Wingfield 2011) and prepare a suitable habitat for their growth. In the galleries dug by insects, the fungi become metabolically more active because they have access to a constant supply of nutrients such as decaying wood (Stokland et al. 2012).

The elm bark beetles are generally unable to digest the lignin, cellulose and hemicellulose components that make up xylem tissues (Dadd 1970; Geib et al. 2008) and feed primarily on the phloem. However, for some phloem-feeding beetles, phloem tissues remain relatively low in usable nitrogen and sterols and, thus, the associated fungi can serve as a complementary source of nutrients (Six 2012). It has been observed that symbiotic fungi are able to access nitrogen stored in the sapwood and translocate it into the phloem where the larvae and pupae of bark beetle feed (Stokland et al. 2012). Bark beetles and ambrosia beetles, as reported also by Kolařík and Kirkendall (2010) and Veselská and Kolařík (2015), use these fungi as principal nutritional symbionts and recently new *Geosmithia* species associated with ambrosia beetles have been described in a tropical forest in Costa Rica (Kolařík and Kirkendall 2010).

EBBs are the main vectors of *Geosmithia* spores on their body and maybe use the fungus as a complement to their nutrition, especially during the larval and pupal stages of their life cycle that takes place within the galleries under the elm bark. However, more studies are needed to confirm this hypothesis.

The callow adults complete their maturation over a few days by digging short feeding burrows in the phloem of the twig and sapwood of healthy elms (Fransen 1939; Webber and Brasier 1984), where they deposit the DED fungal spores. This study shows that these insects carry large quantities of *Geosmithia* and much less of *O. novo-ulmi* (Fig. 3). The spores of the latter reach the xylem and move in the vessels through a phase of yeast multiplication (Webber and Brasier 1984), giving rise to the infection process. *Geosmithia*, at least in this first phase, is not detectable and this could mean that it does not find optimal conditions to spread or it is translocated in other parts of the plant. In fact, although the new insects flicker from the bark of dying elms carrying 99% *Geosmithia* spores, to the xylem of elm trees experiencing new attacks, we found only the presence of the DED pathogen. These results are in contrast with those reported by Pepori et al. (2018), who found that the artificial inoculation of both fungi in the same elm clone resulted in significantly lower symptoms than single inoculations of *O. novo-ulmi*. Maybe the reason lies in the fact that artificial inoculations, generally performed in the internodal section of the twig, circumvent the natural plant reaction, while beetles dig their burrow at the twig crotches (Santini and Faccoli 2015).

None of the target DNAs was detected in healthy elm tissues and only *O. novo-ulmi* DNA was detected in DED-symptomatic plants, confirming that *Geosmithia* does not adapt to the conditions of living plants tissues or even in xylem of plants with early DED symptoms (Pepori et al. 2018).

These findings show that this fungus is not an endophyte, at least in elm. Instead, *Geosmithia* was detected in abundance on EBB bodies and in EBB tunnels in decaying plants. Our analyses suggest that the presence of this fungus is mostly associated with the breeding activity of the vector insect on elm trees as already observed in other studies (Kolařík et al. 2007, 2008; Kolařík and Jankowiak 2013).

In conclusion, the duplex qPCR technique developed in this work is extremely sensitive and able to specifically identify and quantify the presence of both *O. novo-ulmi* and *Geosmithia* spp. in plants with different levels of DED symptoms, on EBB larvae, pupae and wood frass from maternal and larval galleries and on the body of callow adult insects, providing better insight into the dynamics of this complex fungus-fungus association mediated by *S. multistriatus*. This work provided solid data on the actual DNA quantity of the two fungi at the different steps of the DED cycle, thus gaining a better understanding of the role and interactions occurring amongst all the pathosystem players.

Dutch elm disease continues to be extremely damaging on planted and natural elm stands in Europe. Critical thresholds comparable to those that led to the decline of the first epidemic do not appear to have been reached and the current disease dynamic seems likely to continue.

Moreover, an increasingly warming climate could have a great influence on beetle epidemics, their aggression, population dynamics and migration (Bentz and Jönsson 2015), allowing the expansion of the DED epidemic to more northern latitudes (Jürisoo et al. 2019, 2021).

Several aspects of *O. novo-ulmi-Geosmithia-Scolytus* interactions within the DED pathosystem need to be further studied and more in-depth information on the biological cycle of *Geosmithia* spp. during the flickering period of new generations will be essential to use this fungus as a biocontrol agent of DED and finally allow European elms to re-populate the landscape.

Acknowledgements

This study was supported by the HOMED project (http://homed-project.eu/), which received funding from the European Union's Horizon 2020 Research and Innovation Programme under grant agreement No. 771271. We thank Dr. Miroslav Kolařík for providing *Geosmithia* spp. isolates from Czech Republic, Croatia and Spain. Authors wish to warmly thank Dr. Matthew Duncan Haworth for English language editing. Authors wish to thank the anonymous referees who helped to significantly improve the manuscript.

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NeoBiota 84: 267–279 (2023)
doi: 10.3897/neobiota.84.95692
https://neobiota.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
NeoBiota
Advancing research on alien species and biological invasions

Testing early detection of pine processionary moth *Thaumetopoea pityocampa* nests using UAV-based methods

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Academic editor: Alberto Santini | Received 26 September 2022 | Accepted 9 December 2022 | Published 18 May 2023

Citation: Garcia A, Samalens J-C, Grillet A, Soares P, Branco M, van Halder I, Jactel H, Battisti A (2023) Testing early detection of pine processionary moth *Thaumetopoea pityocampa* nests using UAV-based methods. In: Jactel H, Orazio C, Robinet C, Douma JC, Santini A, Battisti A, Branco M, Seehausen L, Kenis M (Eds) Conceptual and technical innovations to better manage invasions of alien pests and pathogens in forests. NeoBiota 84: 267–279. https://doi.org/10.3897/neobiota.84.95692

Abstract

Early detection of insect infestation is a key to the adoption of control measures appropriated to each local condition. The use of remote sensing was recommended for a quick scanning of large areas, although it does not work well with signals bearing low intensity or items that are difficult to detect. Unmanned Aerial Vehicle (UAV, or drone) may help in getting closer to individual trees and detect atypical signals of small dimensions. The larvae of the pine processionary moth (PPM, *Thaumetopoea pityocampa* (Denis & Schiffermüller, 1775, Lepidoptera, Notodontidae) build conspicuous silk nests on the external parts of the host plants at the beginning of the winter and their early detection may prompt managers to adopt management techniques. This work aims at testing two deep learning methods (Region-based Convolutional Neural Network - R-CNN and You Only Look Once - YOLO) to detect the nests under three different conditions of host plant species and forest stands in southern Europe. YOLO algorithm provided better results and it allowed us to achieve F1-scores as high as 0.826 and 0.696 for the detection of presence / absence and the individual nests, respectively. The detection of all the nests that can be present on a tree is not achievable with either UAV scanning or traditional ground observation, therefore the integration of the methods may allow the complete efficiency of the surveillance. The use of UAV combined with Artificial Intelligence (AI) image analysis is recommended for further use in forest and urban settings for the detection of the PPM nests. The recommended methods can be extended to other pest systems, especially when specific symptoms can be associated with an insect pest species.

Keywords
AI algorithm, forest, Insecta, Lepidoptera, Notodontidae, pest, PPM, UAV, urban

Introduction

The use of remote sensing can provide evidence of abnormal biological activity in forest ecosystems (Forzieri et al. 2021). New satellite constellations with more frequent flyovers and multispectral cameras were used to provide accurate images of forest cover and to better detect isolated patches of tree mortality (Senf et al. 2017; Sebald et al. 2021) and infer putative responsible biotic factors based on spatiotemporal dynamics patterns (Senf et al. 2015). Remote sensing techniques are efficient to detect crown discoloration of mature trees or patches of killed trees as well as temporary defoliation events (Sangüesa-Barreda et al. 2014). However, the images used so far still have coarse spatial resolution (40–50 cm) to detect damage within an individual tree and even less on parts of a tree. These very localised anomalies are low intensity and are difficult to detect at far distances and that is the main advantage of using Unmanned Aerial Vehicle (UAV, or drone), i.e., to get closer to individual trees to scan and detect atypical signals of small dimensions.

Two recent systematic reviews underline the growing use of UAV for forest health surveys (Duarte et al. 2022; Ecke et al. 2022) and address applications for mapping tree defoliation, and trees damaged by pine wood nematode and bark beetles. Specific assessments of individual tree defoliation caused by the pine processionary moth (PPM, *Thaumetopoea pityocampa*, Lepidoptera: Notodontidae) were done in Spain with an accuracy of about 80% (Cardil et al. 2017, 2019). Otsu et al. (2018, 2019) combined UVA image acquisition with novel image classification techniques to achieve 95% overall accuracy in detecting defoliation. The detection of individual PPM winter nests, however, was not attempted before our study. Winter nests start to be visible in the outer parts of the trees as soon as the larvae moult to the third instar (Uemura et al. 2021) and before significant damage is caused. Early detection would therefore be especially useful to predict tree defoliation and health risk to humans and domestic animals and thus suggest when to apply control methods preventively (Battisti et al. 2017). As long as the PPM is expanding to upper latitudes and higher elevations because of climate change (Roques 2015), an early detection – early action system should be the most recommended method to adopt, especially in urban forests that are next to be infested and where the perception of the risk by citizens and managers is not yet of high concern.

Beyond the acquisition of images by UAVs, the detection of objects on these images is mainly limited by the performance of analytical tools. The primary objective of this study was to compare different deep learning algorithms to meet the challenge of accurately counting objects in UAV images, such as the PPM winter nests that can be partially hidden in the tree canopy and may show blurred contours. With Region-based Convolutional Neural Network (R-CNN) algorithms (Nugroho 2018), significant improvements were achieved in the most important computer vision problems such as segmentation and object detection. The latest updated version of this deep learning network is Faster R-CNN (Ren et al. 2015). You Only Look Once (YOLO) algorithm is another widely used deep learning system for real-time object detection and is considered much faster than the previous one (Redmon et al. 2016). Comparing both models, Wu et al. (2021) demonstrated similar accuracy but higher running speed for YOLO in the frame of pine wilt disease surveys with UAVs.

Materials and methods

Because our goal was to propose a method applicable in different conditions of development of PPM infestations, we conducted our studies in three sites in France, Italy and Portugal differing by the nature of the terrain and climatic conditions. The sites also allowed to test three major host-plants of PPM in the Mediterranean region, i.e., *Pinus nigra* Arnold, *P. pinaster* Aiton, and *P. pinea* L., which are characterised by different crown architecture. In doing so, we were also able to quantitatively test the performance of PPM nest detection in relation 1) to nest size (small vs large) and location in the tree crowns (periphery vs centre) than for nests located at the top of the tree crowns, and 2) to decreasing density of pine trees in the stand.

Study sites and ground assessment

Three study sites were selected in south-western Europe (France, Italy, and Portugal) to ensure a maximum of variability of conditions (Fig. 1). In Portugal (Arez - Alcácer do Sal, 38.315665°N, 8.493764°W, 39 m), we selected one pure even-aged *Pinus pinea* stands of 12 years old with a density 100 trees/ha (tree height 0.82–6 m, tree diameter at breast height (dbh) 9 cm). In France (Cestas, 44.779308°N, 00.795404°W; 61 m), we selected several *Pinus pinaster* plots in an even aged experimental plantation of 10 years old (ORPHEE), which had the advantage of comparing several stand densities (tree height 9.80 m, dbh 16 cm). The UAV survey was conducted on 4 blocks (c.a. 1.5 ha), each including 4 plots of 400 m^2 with a density of 2500, 1250, 825 and 625 trees/ha, respectively. In Italy (Lavini di Marco Trento, 45.8467°N, 11.0365°E, 680 m), we selected a site composed mainly by *Pinus nigra* in a natural uneven aged forest in the Southern Alps, growing on steep slopes with several isolated and relatively short trees (mean density of about 100 trees/ha, tree height 1.5–10 m, tree dbh 10–30 cm). It was thus less dense than the plantation forests.

All three sites were surveyed for visual abundance inventory of PPM nests from the ground (two observers looking at both sides of the trees) and each tree was identified and geo-localised (Suppl. material 1: fig. S1). In addition to ground counts, on the French site, PPM nests were visually counted from a mobile platform (Fig. 1b) at 2 m above canopy of all trees of the 16 sampled plots. Several characteristics were

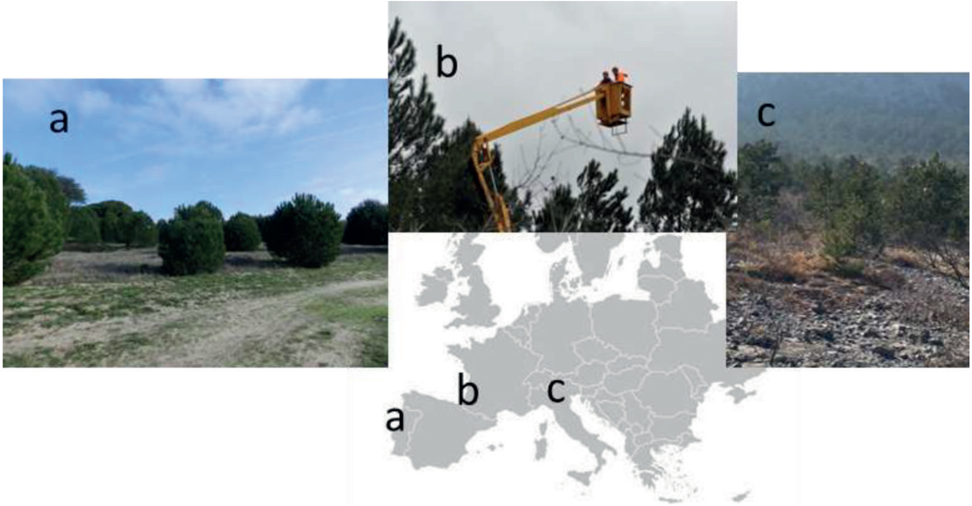

Figure 1. Location of the study sites and types of habitat **a** stand of *Pinus pinea* in Portugal **b** stand of *Pinus pinaster* in France **c** stand of *Pinus nigra* in Italy.

also recorded such as the position on terminal shoot vs. lateral branch and the size of the nest by distinguishing between small and lightly woven nest (grey colour, weave not much beyond the needle clusters) vs. medium to large and well woven nest (white colour, weave enveloping the needle clusters).

UAV survey

We conducted preliminary surveys to test the optimal flight conditions with the high definition (HD) camera (RGB HD SONY Alpha 7R). Test flights in 2019 and operational flights during the winter 2020–2021 on different terrain conditions in France, Italy, and Portugal led us to choose RGB HD sensors with focal length of 35 mm and a definition at least equal to 36 Mpix. A multirotor UAV platform of type DJI Matrice 300 was used and flights were planned with an overlapping of 80% along and across tracks. The spatial resolution of the images is a key point of interest in the context of single tree damage detection. For image processing, it is usually required to have at least 9 pixels within a targeted object. We, therefore, focused on the acquisition of subcentimetric images to detect PPM nests of about 5 cm in diameter. For a given sensor, the flight altitude directly defines ground spatial resolution. An operational trade-off must be found between Ground Sample Distance (GSD) and the ability of photogrammetric software to find correlation points between two subsequent images in order to generate an orthomosaic. Using Simactive Correlator3D (SimActive High-End Mapping Software Home Page. Available online: https://www.simactive.com/correlator3d-mapping-software-features) or Pix4Dphotogrammetric (Professional Drone Mapping and Photogrammetry Software Home Page. Available online: https://www.pix4d.com/product/pix4dmapper-photogrammetry-software) commercial software led us to define a minimum of 30 m flight altitude above the canopy to reach an image resolution of 0.7 cm GSD. We used the Simactive Correlator3D software due to its capacity to create an orthophoto for each image of a UAV flight.

Deep learning models

Two advanced architectures of deep learning model were implemented for single nest detection on UAV images. The first model was based on the two-stage detector Faster RCNN inception Resnet V2 (Ren et al. 2015) and the second on a single stage detector based on the YOLO v5 framework (Redmon et al. 2016) (Suppl. material 1: fig. S3). Those deep learning models were tested and trained to reach an optimal solution for automatic nest detection on UAV images using the open source frameworks built by TensorFlow (Abadi et al. 2016), which is the TensorFlow Object Detection API. Data augmentation was applied to artificially raise the training dataset by changing the level of brightness, hue, noise, or image compression. The models were finally trained using random crop sampling of raw images. Model training was performed for approximately six hours on a personal computer that has an NVIDIA GeForce GTX1060 Graphical Processing Unit (GPU). The datasets were split into 80% for training and 20% for testing, which is a widely used split for testing a detector's accuracy, especially in cases where limited datasets are available (Rácz et al. 2021).

Looking at the UAV orthophotos sequence over a unique tree reveals that some nests are only visible from side view angle. The orthomosaic phase of the photogrammetric process which aims to select parts of images closest to the nadir (i.e., Dji_0159 in Suppl. material 1: fig. S2) will lead to omission. In order to consider these lateral positions, the AI-based nest detection model was consequently applied to each individual orthorectified UAV images and not to the global orthomosaic image of each study site. In addition, an exhaustive visual assessment of each tree on each photo was independently made to inventory the number of nests on each image by a single observer (AG). This visual assessment has been set up to distinguish the monitoring performance of the AI based model from the performance of the UAV monitoring itself. A spatial geodatabase was set up to further assign detected nests to trees. For each single tree, the image with the maximum number of nests detected was retained. The results of the AI based nest detection model were evaluated by crossing the visual photointerpretation of UAV images with ground surveys and in-situ canopy inventories when available.

Data analysis

We calculated the classical metrics for evaluating the prediction quality of machine learning models, which combine numbers of True Positive (TP, detection of a PPM nest in the presence of a PPM nest), True Negative (TN, no detection of a PPM nest in the absence of a PPM nest), False Positive (FP, detection of a PPM nest in the absence of a PPM nest) and False Negative (FN, no detection of a PPM nest in the presence of a PPM nest). We estimated the precision, which measures the extent of error caused by False Positives ($P = TP/(TP+FP)$), and the recall, which measures the extent of error caused by False Negatives (R = TP/(TP+FN)). However, we used the F1-score as main evaluation metrics to maximise both precision and recall (eq1) considering that errors caused by false negatives and false positives were equally undesirable. The F1 score ranges from 0 to 1 and the higher the F1 score, the better the model.

$$\text{F1-score} = 2^*(\text{Precision} \times \text{Recall}) / (\text{Precision} + \text{Recall}) \qquad \text{equation (1)}$$

F1-score was used to compare the performance of the two architecture models (FRCNN and YOLO) in comparison with human eye detection on aerial photographs and from the ground by using paired *t*-tests on all trees grouped together or using countries as replicates. Paired t-tests were also used to compare the performance of nest detection between small (<10 cm diameter) vs. big nests (≥10 cm diameter) and lateral vs terminal nests in the 16 plots of the French site. An ANOVA was used to test the effect of pine density on detection performance from interpreted UAV images. All statistical analyses were performed with XLSTAT 2022.1.2.1288 (Addinsoft).

Results

A total of 936 trees were inventoried at the three sites, simultaneously from the ground and from UAV images, and they showed considerable differences in the rate of colonization. A total of 665 PPM nests were visually inventoried from the ground over the entire study and 222 nests were detected by human eyes on UAV images of the same trees (Table 1).

A total of 22,904 images composed the UAV database leading to 2,858 nests being visually assessed on the images due to multiple views of the same nest.

The performance of AI model architectures (FRCNN vs YOLO) was compared with human interpretation of UAV images for all images gathered on all trees from the three countries. This dataset included all trees counted from UAV images, and not only trees observed from the ground. A total of 1,477 trees were inventoried on UAV images (803 in France, 459 in Italy and 215 in Portugal). This dataset was used for comparing human visual interpretation of UAV images with AI models estimates, considering both presence of nests and their number per tree. YOLO architecture performed better than FRCNN with similar precision but better recall (less omission) and thus higher F1-score. Similar results were obtained for the presence of nests and the number of nests per tree (Fig. 2).

Table 1. Summary of pine trees and PPM nest sampled in the study simultaneously from the ground and from UAV images.

Country	No. trees (ground)	% infested trees (ground)	No. PPM nests (ground)	% infested trees (UAV - human eye)	No. PPM nests (UAV - human eye)	% infested trees (UAV - FRCNN)	No. PPM nests (UAV - FRCNN)	% infested trees (UAV - YOLO)	No. PPM nests (UAV - YOLO)
France	803	23.4	354	11.3	99	4.1	34	9.5	77
Italy	75	33.3	34	36.0	34	32.0	30	32.0	30
Portugal	58	96.6	277	72.4	93	63.8	58	75.9	83
Total	936		665		222		122		190

Using data from each country (i.e., different pine species) as replicates, we found significantly better F1-scores with YOLO than with FRCNN for both presence of nests (paired t-test, p = 0.02) and number of nests per tree (paired t-test, p = 0.03) (Fig. 3).

The use of YOLO algorithm to identify the number of nests per tree detected from the ground provided results that did not differ significantly from those obtained with human eye interpretation of UAV images (paired t-test using countries as replicates, p = 0.97). The mean F1-scores were 0.238 and 0.242 for YOLO and human eye, respectively, suggesting low performance of both methods. However, the F1 score was three-fold higher for the detection of infested trees, irrespective of the number of nests, with F1-scores of 0.648 (YOLO) and 0.676 (human eye), respectively.

When nest detection from the ground was combined with nest detection from a platform (803 trees in 16 plots, French site), the tree infestation rate was 23% for ground and 19% for platform observations. YOLO performed similarly (paired t-tests, n = 16, p = 0.08) to detect the number of nests from the ground or from the platform,

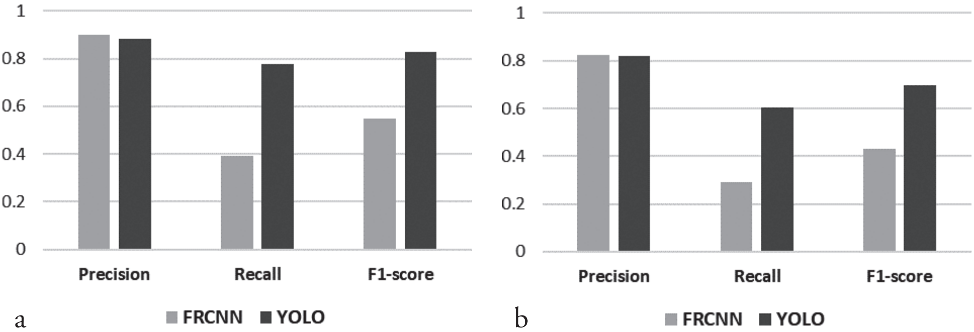

Figure 2. Performance of FRCNN and YOLO architectures for the detection of (**a**) presence / absence of PPM nest and (**b**) number of PPM nests per tree using the full dataset of 1,477 observed trees on UAV images in France, Italy, and Portugal.

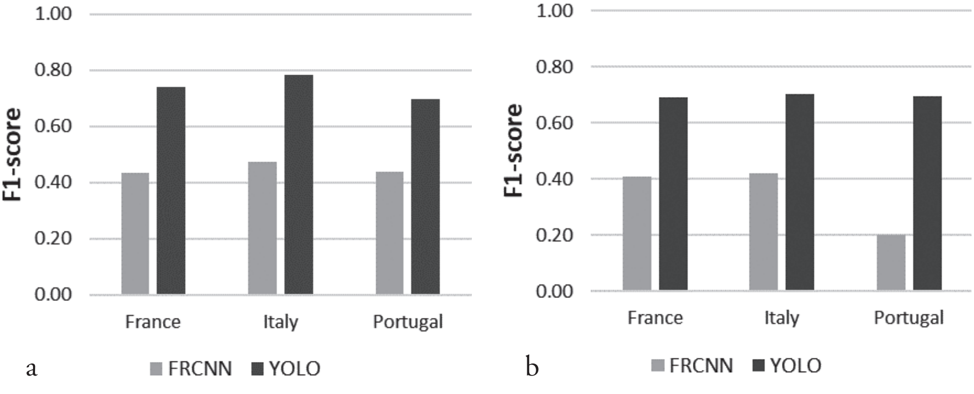

Figure 3. F1-score of FRCNN and YOLO architectures for the detection of (**a**) presence / absence of PPM nest and (**b**) number of PPM nests per tree using the full dataset of 1,634 observed trees on UAV images in France, Italy, and Portugal.

with mean F1 scores of 0.432 and 0.361, respectively. The same was observed for presence of nests (p = 0.11), with mean F1 scores of 0.526 and 0.438, respectively.

The performance of the YOLO algorithm was not significantly influenced by the density of maritime pine trees in the French site (ANOVA, n = 4, p = 0.83 and 0.56 for the number of nests and their presence, respectively), although the worst performance was obtained at the highest pine density (2500 trees/ha), where the canopy cover was 100% and the estimated percentage of infested trees the lowest (13%) (Fig. 4).

The performance of the algorithm was significantly influenced by the size of the nests (paired t-test, P = 0.008). The performance was not significantly influenced by the position of the nest (paired t-test, P = 0.442). The algorithm was performant at detecting the presence of nests more than 10 cm, irrespective of their localization on the terminal or lateral branches in the tree crown (Fig. 5).

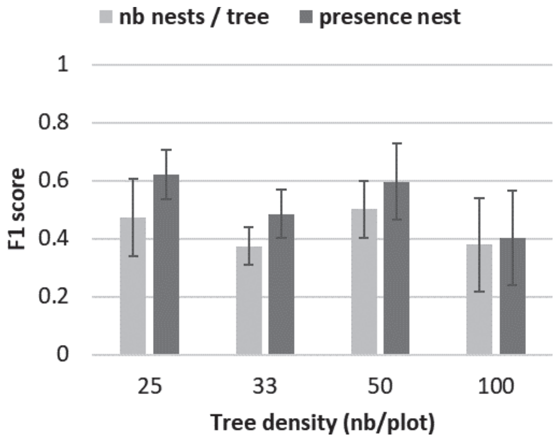

Figure 4. F1-score of YOLO for the number of PPM nests per tree and their detection according to tree density per plot (400 m^2) in the French site. Tree density corresponded to 2500, 1250, 825 and 625 trees/ha for the 100, 50, 33 and 25 trees per plot, respectively.

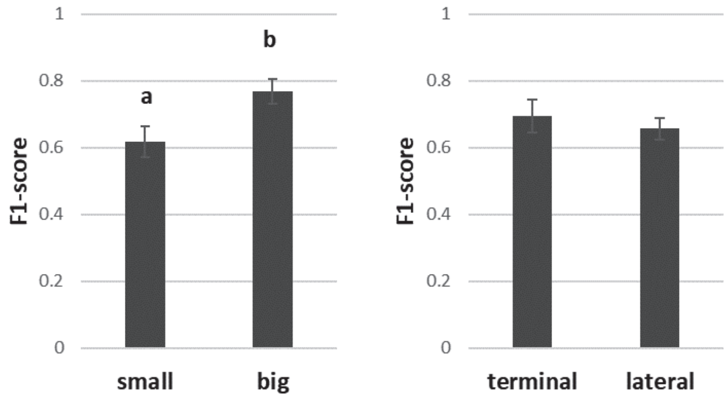

Figure 5. F1-score of YOLO for the detection of the presence of PPM nests according to the size of the nest (small < 10 cm diameter; big > 10 cm diameter) and position on the branch in the French site.

Discussion

The use of AI proved effective to detect the nests of PPM on trees of different species and sizes, even under variable densities. In particular, the YOLO algorithm was superior to R-CNN for this special application. This result did not allow an exhaustive detection of the nests occurring on trees. The study proved the advantage of using UAVs to document the presence of at least one nest per tree. It therefore represents a substantial step forward in the integration of the UAV survey with ground observations in the monitoring of the colonies of an important forest defoliating insect in the Mediterranean area. Furthermore, the study paves the way for early detection of symptoms associated with the presence of pests and pathogens on the canopy of forest and ornamental trees, which is essential to elicit specific and targeted management measures.

The use of remote sensing in the detection of biotic disturbances was implemented for achieving higher performance of surveillance and for addressing management measures (Lehmann et al. 2015; Hall et al. 2016). The latter case is especially true for pathogens and pests that may cause defoliation or discoloration of a group of trees (Duarte et al. 2022; Ecke et al. 2022) or even individual trees in a stand of trees (Näsi et al. 2018) or in an urban setting (Wagner and Egerer 2022). The winter nest of PPM is a special target for UAV detection. In this species, the silk is spun as long as the temperature is decreasing at the beginning of the winter (Uemura et al. 2021), well before the massive defoliation occurs and becomes detectable (Battisti et al. 2015). Tracking early nest formation may thus allow managers to decide which control measures can be adopted among the few available under the different growing conditions of the trees (Roques 2015). As expected, the detectability of the presence of the PPM increased with the size of nests and thus, as nest volume increases exponentially during the fall and winter (Branco et al. 2008), the period of image acquisition will be a relevant variable to analyse.

The AI analysis performed equally well with different host-pine species, percentage of infested trees, and local topography. The YOLO algorithm always yielded satisfactory results in maximising the detection power of nests. Even when compared with the human eye's careful inspection of each image, the YOLO algorithm performed equally well in identifying the trees carrying at least one PPM nest. The performance was different, however, when image data from UAVs were compared with ground/platform assessment of nest presence, which, of course, allows many more directions of observation than the one from above. The number of nests per tree counted from the ground often differed from the number of nests counted on the images, either by human eye or YOLO algorithm. It could be explained by a general underestimation or simply by counting different nests from the ground and from the UAV images taken from above. Overall, the quick flyover of a UAV over a forest stand or a city park largely outweighs the cost of detailed observations from the ground/platform, and in any case, the detection of nests from the UAV can inform people of the risk and the need to carry out more precise observations on the ground. Interestingly, the detection power was not affected by the stand density in the French site, except at the highest density of 2500 trees/ha for which tree crowns were overlapping. In contrast, nest size results to be the most important trait for detection.

As PPM is increasingly becoming a species of concern for forests and trees in relation to the rapid range expansion and large population growth in the areas of infested trees (Backe et al. 2021), the availability of a quick canopy scanning that can detect the early occurrence of nests seems to be a promising tool for pest managers, as shown for invasive alien species in forests (de Groot et al. 2020). With the refinement of small symptom detection from aerial images, especially when the contrast with the background is not as bright as in PPM, the method is potentially applicable to many organisms causing discoloration in tree canopies.

In conclusion, we demonstrate the potential use of IA on UAV images to detect at the tree level the presence of localised pests. Results significantly differ depending on IA algorithms, opening possibilities for further improvement. This technique can pave new avenues in the surveillance and management of emerging and non-native pests of trees, where early detection and early action should go together to achieve a satisfactory level of protection.

Acknowledgements

The study was conducted in the European project HOMED (Holistic management of emerging forest pests and diseases), which receives funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 771271. The team from the University of Lisbon was also funded by the Forest Research Centre, a research unit funded by Fundação para a Ciência e a Tecnologia I.P. (FCT), Portugal (UIBD/00239/2020). We thank Yannick Mellerin, Laurent Saléra and Rémy Dourthe for their help in the field work and Ricardo Cipriano for the access to pine stands. We thank INRAE - UEFP (Forest experimental Facility UEFP-https://doi.org/10.15454/1.5483264699193726E12) for the management of the ORPHEE site. We warmly thank two reviewers for comments on the manuscript.

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Supplementary material 1

Supplementary images

Authors: André Garcia, Jean-Charles Samalens, Arnaud Grillet, Paula Soares, Manuela Branco, Inge van Halder, Hervé Jactel, Andrea Battisti

Data type: images (PDF file)

Explanation note: Example of nest and trees carrying nests on different host plants: a. *Pinus pinea* in Portugal; b. *Pinus nigra* in Italy. Sequence of four orthoimages (Dji 159 to 162) taken from a drone on the Italian site where a nest can be seen only in three images (yellow oval) with a change on its relative position in relation to drone location. Another nest on the left is visible in all the four images. Nest detection boxes (green) of YOLO deep learning model on *Pinus pinaster* (France).

Link: https://doi.org/10.3897/neobiota.84.95692.suppl1

NeoBiota 84: 281–317 (2023)
doi: 10.3897/neobiota.84.95687
https://neobiota.pensoft.net

REVIEW ARTICLE

A peer-reviewed open-access journal
NeoBiota
Advancing research on alien species and biological invasions

Eradication programs against non-native pests and pathogens of woody plants in Europe: which factors influence their success or failure?

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Academic editor: Andrea Battisti | Received 28 September 2022 | Accepted 6 March 2023 | Published 18 May 2023

Citation: Branco S, Douma JC, Brockerhoff EG, Gomez-Gallego M, Marcais B, Prospero S, Franco JC, Jactel H, Branco M (2023) Eradication programs against non-native pests and pathogens of woody plants in Europe: which factors influence their success or failure? In: Jactel H, Orazio C, Robinet C, Douma JC, Santini A, Battisti A, Branco M, Seehausen L, Kenis M (Eds) Conceptual and technical innovations to better manage invasions of alien pests and pathogens in forests. NeoBiota 84: 281–317. https://doi.org/10.3897/neobiota.84.95687

Abstract

When a non-native species succeeds in establishing in a new habitat, one of the possible responses is to attempt its eradication. In the present study, we analysed European eradication programmes against non-native pests and pathogens of woody plants (PPWP) from 1945 to date. Our main goal was to identify which factors affect the success of an eradication programme, reinforcing guidelines for future eradication of PPWP. Data on eradication campaigns were obtained from online databases, scientific and grey literature, and Plant Protection Organizations' reports. Factors influencing eradication success for both arthropods and pathogens were analysed with LASSO regression and decision tree learning.

A total of 848 cases officially declared as eradication attempts were documented in our database (8-fold higher than previous reports). Both the number of programmes and their rate of success increased sharply over the last two decades. Only less than 10% of the non-native organisms affecting woody plants were targeted for attempted eradication despite the high economic and ecological impacts caused by some species for which no efforts were undertaken. Almost one-third of the officially declared cases of eradication concerned organisms that were still restricted to the material with which they were introduced. For these cases the success rate was 100%. The success rate of established species was only 50% for arthropods and 61% for pathogens. The spatial extent of the outbreak was the factor that most affected the outcome of eradication campaigns. The eradication success decreased abruptly above 100 ha for arthropods and 10 ha for pathogens. Additionally, other variables were shown to influence the outcome of eradication programmes, in particular the type of environment, with the highest eradication success rate found in nurseries and glasshouses, with successful outcomes increasing if quarantine measures were applied and when monitoring included asymptomatic plants. Particular species traits may reduce eradication success: parthenogenetic arthropods, saprotrophic pathogens, wind dispersal, the possibility to remain asymptomatic indefinitely, and the existence of resting spores or stages.

In conclusion, small affected areas, quick response, and efficient implementation of quarantine restrictions, together with particular species traits, may allow a high probability of eradication success. Preparedness at the country and European level would allow a larger number of target species to be included in future eradication programmes.

Keywords

Biological invasions, pest and pathogen management, surveillance

Introduction

The rate of biological invasions has sharply increased over the last century mainly due to globalization trends, including intensified travel, population growth, migratory fluxes, liberalisation of international trade and the consequent increase in global trade (Pimentel 2002; Ghelardini et al. 2016; Brockerhoff and Liebhold 2017; Seebens et al. 2017). Additionally, global climate change may also contribute to the increase of invasions by alien species (Sala et al. 2000). Forest ecosystems, including those close to nature, forest plantations, and urban forests have been highly affected by invasive species (Liebhold et al. 2012; Desprez-Loustau et al. 2016). The resulting negative impacts on biodiversity, ecosystem services, socio-economy, and human health provide compelling reasons to develop and implement the best management strategies to prevent biological invasions and to mitigate their consequences. In optimal circumstances, we need to know how to avoid these consequences through prevention or prompt eradication.

The first line of defence against biological invasions relies on preventing the introduction of non-native organisms into a new area. This is considered the most effective strategy for dealing with invasive species and is achieved through international quarantine measures, such as banning the import of goods from contaminated regions or requiring that these goods can only be imported after appropriate phytosanitary treatments (Haack et al. 2014; Sequeira and Griffin 2014; Sikes et al. 2018). Despite the biosecurity systems adopted by many countries to detect and intercept potentially dangerous organisms arriving through trade and travel routes (Sequeira and Griffin 2014), a huge proportion of them remains undetected (Brockerhoff et al. 2006; Meurisse et al. 2019). The situation is aggravated by the fact that phytosanitary measures generally target only known species on quarantine lists (Desprez-Loustau et al. 2016). Luckily, not all invading populations succeed in establishing in non-native habitats. In fact, most invasions fail, either because the new habitat climate is unsuitable or host plants are not available (Paap et al. 2022). Also, low-density populations may be subject to extinction due to environmental and demographic stochasticity and to Allee effects

(Liebhold and Bascompte 2003). Still, many populations of non-native organisms succeed to overcome phytosanitary measures and become established and potentially invasive in a new region. In such cases, adequate surveillance systems may allow for their early detection and implementation of appropriate suppression measures (Liebhold and Kean 2019). Eradication may then be the best option for alien species for which high impacts are expected, preventing the indefinite accumulation of deleterious effects and economic impacts. However, the costs of eradication may exceed its benefits if the area colonised by an established invading population is too large, requiring substantial resources while the likelihood of eradication success is low (Tobin et al. 2014). In such cases, containment might be a better alternative to stop or slow down the spread of an invasive organism (Myers et al. 2000; Brockerhoff et al. 2010; Goheen et al. 2017).

Several factors are well accepted as contributing to the success of an eradication programme, among which, early detection and quick response are crucial (Brockerhoff et al. 2010; Pluess et al. 2012b; Liebhold et al. 2016; Hansen et al. 2019). Further, enough resources must be allocated from the start to the end of the program. Funding agencies or governments may be inclined to abandon the efforts once the pest or pathogen densities are no longer causing significant economic or ecological impacts, although invasive populations may decline naturally towards extinction once they are suppressed below an Allee effect threshold (Simberloff 2002; Liebhold and Tobin 2008; Liebhold et al. 2016). In addition, eradication measures are doomed to fail if some stakeholders allow the invaders to persist on their properties. In these cases, mandatory cooperation is required to carry out the required procedures. Also, public awareness-raising campaigns are needed to increase community support (Pluess et al. 2012b). The availability of effective surveillance tools is crucial as they determine the capacity to accurately delimit the infested area, even when populations are at low densities. For instance, eradication of an invasive insect is estimated to be 20-fold more likely to succeed when an attractant is available (Tobin et al. 2014; Liebhold et al. 2016; Suckling et al 2021). Finally, success depends also on the availability of highly effective eradication techniques, either increasing mortality or reducing reproduction. The combination of more than one eradication technique is considered to guarantee better results (Blackwood et al. 2012, 2018).

Previous reviews have attempted to better identify which factors determine the success of an eradication programme (e.g., Brockerhoff et al. 2010; Pluess et al. 2012a, b; Tobin et al. 2014; Smith et al. 2017). Based on a data set of 136 eradication campaigns against invasive alien invertebrates, plants and pathogens, Pluess et al. (2012a) identified the area infested at the beginning of the eradication programmes as the sole factor significantly determining the success of eradication attempts. In another study, Pluess et al. (2012b), using a dataset of 173 eradication campaigns against 94 species worldwide (51% of which were successful), identified several other factors influencing eradication success and differences among taxonomic groups. Bacteria and viruses were the most likely, and fungi were the least likely to be eradicated. Infested area, reaction time, and application of sanitary measures, such as the prohibition of movement of possibly infested material or equipment, most affected the success or failure of programmes. Eradication in man-made habitats was also more likely to succeed than campaigns carried out in semi-natural or natural habitats. In another study, Tobin et al. (2014) analysed 672 programmes targeting 130 arthropod species. The authors also identified a negative association between the success rate and the size of the infested area. However, their analysis indicated that the detectability of the target pest was one of the most critical factors associated with eradication success. The method of detection and the primary feeding guild of the target species also contributed to the success or failure of eradication campaigns. A higher success rate was observed for Diptera and Lepidoptera and a lower one for Coleoptera, which was attributed to the existence of effective and cost-efficient semiochemical lures. Noteworthy was that data used by Tobin et al. (2014) were compiled into a web-based database – the Global Eradication and Response DAtabase ("GERDA", Kean et al. 2022 available for public consultation. This database includes information on eradication programmes targeting terrestrial arthropods and plant pathogens in 108 countries, and by the end of 2021, 1048 eradication programmes were reported. For pathogens, Smith et al. (2017) analysed GERDA data from 190 plant pathogen eradication programmes to identify treatment efficiency and found *in vitro* tissue culture in combination with thermotherapy as the most successful, to eradicate viral or bacterial pathogens. Although the information in GERDA is extremely valuable for accessing global trends and drivers of eradication success and failure, both the distribution of invasive pests and pathogens and the number of eradication attempts have continued to increase far beyond those reported in this database (Suckling et al. 2021).

In the present study, a systematic analysis of European eradication programmes against non-native pests and pathogens of woody plants (PPWP) is addressed. We note that in some cases of pests or pathogens, the species might be native to one region of Europe but non-native to other regions. An example is the oak processionary moth (*Thaumetopoea processionea*) which is native to Central Europe and non-native in the UK. For pathogens there are a few cases for which the species origin was unknown. The main goal of our analysis was to identify key determinants of eradication success/failure against non-native PPWP in the European region (considered all countries in the European Continent except for Russia) so that guidelines can be developed for countries that are subject to EU legislation. Explanatory variables applicable for the European region, and countries subject to common legislation, may differ from other world regions, so the results of previous studies may not be able to fully explain the causes of success or failure of eradication programmes in this specific region. To this aim we collected and made available a comprehensive dataset of eradication attempts against PPWP for the European region, with data not previously available in other databases as GERDA. A new methodological approach was also proposed, based on LASSO regression and decision trees.

Methods

Data sources

To identify introduced species of insects associated with woody plants in Europe, we used the list provided in Roques et al. (2016) complemented by a search of EPPO reporting services (1974–2021) and a search on Google scholar and Web of Science with many combinations of the keywords "alien (or non-native, or exotic) arthropod (or insect) species Europe", "alien insect arthropod (or insect) + each European country", "first report arthropod (or insect) Europe", "first report arthropod (or insect) + each European country". For pathogens, a list of invasive forest fungi and oomycetes detected in Europe from 1800 to 2008 was retrieved from Santini et al. (2013). Data on introduced species from 2008 until 2021 was retrieved from EPPO reporting services. We then looked for eradication programmes against each species. To identify eradication attempts against all pathogen groups (bacteria, nematodes, fungi + oomycetes and viruses or viroids), the EPPO A1 and A2 and alert lists were initially consulted for pathogens of woody plants, and all eradication programmes were searched in EPPO reporting services (1974–2020). To identify eradication attempts for both arthropods and pathogens, the EPPO Global Database (https://gd.eppo.int) was the main online database we consulted. This information was complemented with information from GERDA – Global Eradication and Response Database (http://b3.net.nz/gerda/index.php) and a search in the scientific and grey literature, including works published in scientific journals, conference proceedings, presentations, and books. Published information was searched through Google Scholar and Web of Science, using the "species name", "alien arthropod", alien insect", "plant (or tree) pathogen", "Europe (or individual countries)" and the words "eradication" and "containment" as keywords, in different combinations. Additionally, eradication reports or technical reports, pest alerts, and press releases from National and Regional Plant Protection Organizations (**NPPOs** and **RPPOs**) were consulted. The time range for eradication attempts ranged from 1945 to 2021. Finally, additional information was kindly provided by some countries' NPPOs and RPPOs. For detailed information on some of the eradication cases which were not available in the English language, advanced searches were conducted on Google by introducing the species name and limiting results to the country for which information was missing. The information and reports obtained from these searches were then translated to extract the information required for our analyses.

Terms criteria

Non-native and invasive species

We used the following definitions for the terms:

- Non-native (=non-indigenous) – an introduced species that does not occur naturally in an area, but was introduced as the result of deliberate or accidental human activities, or expanded its range as a result of human activities.
- Invasive – a species whose introduction and dispersal threatens ecosystems, habitats or species, with socio-economic and/or environmental damage and/or harm to human health (CBD 2008)
- Emerging – a species that has increased its population with time becoming injurious.

According to (ISPM no. 5) the term "alien" only applies to individuals or populations that have entered by human agency into the area. However, in some cases it is not clear whether the introduction was human-mediated or just the result of natural spread. Here we consider all the non-native species independently of the introduction pathways, which in some cases are unknown.

For both arthropods and pathogens, the full list of species for which eradication was considered also includes species that are native to parts of Europe, but non-native in the regions where eradication was attempted. For arthropods these include the species *Dendroctonus micans* (Kugelann), *Ips typographus* (Linnaeus), *Lymantria dispar* (Linnaeus) *Thaumetopoea processionea* (Linnaeus) and *Thaumetopoea pityocampa* (Denis & Schiffermüller) and for pathogens the species *Phytoplasma mali* (Seemüller & Schneider, 2004), *Phytoplasma pyri* (Seemüller & Schneider, 2004), Plum pox virus and *Dothistroma septosporum* (Dorogin) Morelet. For three species of fungi the origin is still unknown: *Cylindrocladium buxicola* (Henricot & Culham, 2002), *Dothistroma pini* Hulbary, and *Plenodomus tracheiphilus* (Petri) Gruyter, Aveskamp and Verkley. We included in the analysis all the pathogens for which an eradication programme was implemented, including non-native (either for Europe or for the region where the programme was implemented) and species of unknown origin.

Established and post-border interceptions

Although commonly referred to as "under eradication" in EPPO and NPPO reports and GERDA, some of the cases reported as "subject to eradication" corresponded to measures taken against a detected pest or pathogen that was still restricted to the material with which it was introduced or for which only adult insects were found. We considered these cases as post-border interceptions. According to FAO (2019), an establishment corresponds to a reproducing population that has already spread from the material in which it was introduced and is expected to perpetuate for the foreseeable future.

For both arthropods and pathogens, an establishment was considered "new" to an area if no report of the particular species was made previously from that area. Also, we considered an establishment as new if it occurred in an area previously infested, but where the population was assumed to have been previously eradicated, with an official declaration of eradication by the relevant authorities. For arthropods, we also considered an establishment as new when it was located within an isolated demarcated area – to guarantee non-overlapping demarcated areas between newly detected establishments. For pathogens, the demarcated area of the infected plants was often not reported, due to the high number of reported cases in nurseries and associated commercial confidentiality. We thus considered a new establishment when the pathogen was first detected in a given NUTS III unit (Nomenclature of territorial units for statistical purposes, created by Eurostat).

Infested/infected and demarcated areas

The infested/infected areas comprised the limited areas determined by the pest or pathogen presence. When the extent of these areas was numerically reported, we used the published values (in hectares). When only distribution maps were available, affected areas were measured using either ArcGIS online measure tool or by transposing the points of infested/infected plants to Google Earth Pro (version 7.3.4.8642) and measuring the area delimited by them.

The demarcated area corresponds to the area legally established by each national plant protection organization (NPPO) as subject to eradication and containment measures, and usually comprises an infested core zone, where the pest is present, and a buffer zone around the infested zone. We followed the ISPM no. 5 definition of a buffer zone (FAO 2019).

Datasets

A comprehensive database was constructed including the following information for each case (when available): i) species under eradication, ii) detection date, country, and location; iii) detection method, passive surveillance (i.e. casual observations reported by researchers, technician or citizens) or official survey conducted with that purpose; iv) establishment status (established or post-border interception); v) affected hosts; vi) host type (broadleaves, conifers, palms), vi) control methods used (chemical, host removal, biological, traps); vii) size of the infested area (as exact area information was not always available we defined it in categories ≤ 1 ha, > 1 ≤ 10, > 10 ≤ 100, > 100 ≤ 1000 or > 1000 ha); viii) environments infested (urban/peri-urban, protected green-houses, countryside); ix) climate, categorized as Temperate, Mediterranean or Continental according to Köppen classification system (Peel et al. 2007); x) programme start year, last detection, and date of eradication declared; xi) public education, and xii) the outcome, i.e. legal status (eradicated, under eradication, failure to eradicate). Categories used in each parameter are also described in Table 1 and Suppl. material 1.

For some parameters, information was not always available and so we defined additional criteria. For the establishment status, the pest or pathogen was considered established unless stated that it was found only on the imported plant material and not in other plants at that time or posteriorly to the destruction of the original plant material. For the outcome, we consider a pest or pathogen to be eradicated when there was an official confirmation, or if no further future records were reported. If the official status changed to restricted distribution or containment and it continued to spread, it was considered a failure. Otherwise, it was still considered under eradication.

For pathogens, in many cases, the exact location of detection was not known and thus, we used the NUTS3. If the pathogen was no longer detected during the next two-yearly surveys (or two consecutive surveys when surveys were separated by more than one year) in that region, it was considered eradicated.

Table 1. Variables used as predictors in the modelling analysis and their categories.

List of predictors for arthropods	
Control	
Control methods	Host removal; other (including methods such as chemical, biological or traps; or combo (combination of host removal with other methods)
Restrictions on the movement/ quarantine	Yes; or no
Monitoring method	Visual observation; or visual observation + traps
Response time	≤1 year; or > 1 year
Use of a semiochemical lure	Yes; or no
Environment	
Location	Island; or mainland
Initial infested area	≤1 ha; > 1 ≤ 10 ha; > 10 ≤ 100 ha; > 100 ≤ 1000 ha; or > 1000 ha
Main type of environment affected at start of program	Confined (nurseries, glasshouses and garden centers); urban/peri-urban (private and public gardens, along roadsides of habited areas, industrial areas, etc.); or countryside (orchards and woodlands or forests)
Climate	Mediterranean; Temperate; or Continental (according to Köppen classification)
Species traits	
Host type	Broadleaf; conifer; or palm
Phytophagous specialisation	Monophagous; oligophagous; or polyphagous
Feeding behaviour	External; or Internal feeders
Body size	small (≤ 2 mm); medium (> 2 mm ≤ 10 mm); or large (> 10 mm)
Voltinism	Multivoltine; univoltine; or semivoltine
Main reproduction method	Parthenogenesis; or sexual
Yearly flight duration	< 4 months; ≥ 4 months < 9 months; or ≥ 9 months
Existence of resistant stages	Yes; or no
List of predictors for pathogens	
Control	
Control methods	Host removal or combo (combination with other methods such as chemical or biological)
Restrictions on the movement/ quarantine	Yes; or no
Preventive felling conducted	Yes; or no
Surveys at least annual	Yes; or no
Response time	≤ 1 year or > 1 year
Environment	
Location	Island or mainland
Initial infested area	≤1 ha; > 1 ≤ 10 ha; > 10 ≤ 100 ha; > 100 ≤ 1000 ha; or > 1000 ha
Main type of environment affected at start of programme	Confined (nurseries, glasshouses and garden centers); urban/peri-urban (private and public gardens, along roadsides of habited areas, industrial areas, etc.); or countryside (orchards and woodlands or forests)
Native susceptible hosts in the area	Yes; or no
Species present in adjacent NUTSIII	Yes; or no
Climate	Mediterranean; Temperate; or Continental (according to Köppen classification)
Species traits	
Host type	Broadleaf; broadleaf + conifer; or conifer
Group	Fungi/oomycete; bacteria; nematode; or virus/viroid
Host range	Specialist (one or a few taxonomically related species); or generalist (which infect multiple hosts, and are transmitted efficiently in hosts from different species, often from unrelated taxa)
Incubation period	Time since infection until symptom development: ≤ 1 month; > 1 ≤ 12 months; or > 12 months
Possibility to remain asymptomatic for long periods or indefinitely	Yes; or no
Sporulation/replication ability	High; or low
Existence of resting spores or stages	Yes; or no
Main dispersal mechanism	Wind; biotic vectors; or water
Possible saprotroph	Yes; or no

Some of this information was used only for descriptive analysis whereas other parameters were used in the modelling analysis (Table 1). To obtain a sufficient number of replications per level in a factor, some levels were merged. Cramér's V correlation between variables was estimated with the software package R, for the cases for which information for all the variables were available. (Suppl. material 2).

Statistical modelling

The statistical modelling aimed to predict the probability that the species became established (i.e., no longer found only on primary material) and next, once established, the probability of successful eradication as a function of different explanatory variables. Three main categories of factors were distinguished: i) control options, ii) characteristics of the environment/location of the outbreak, and iii) biological traits of the species. All analyses were performed for arthropods and pathogens separately.

The combined effect of predictors on the probability of eradication successes

When testing how and which combination of predictors affect eradication success, we employed two different statistical methods: LASSO regression and regression trees. Both methods have two features that are important for our analysis: 1) they can handle collinearity between predictors – which is important because some variables might be confounded, for example because a certain management strategy is predominantly applied to particular groups of taxa, and 2) they both select variables based on the ability of the model to predict new outbreak cases (cases that were not seen by the model during the training phase through so-called holdout-validation). The LASSO binomial regression model adds a penalty that scales with the size of the regression coefficient. As a result, the parameter estimates will become smaller, and, importantly, the parameter values of the non-important predictors become zero (Tibshirani 1996). A range of penalties was tested and the penalty that minimises the deviance in the hold-out sample was chosen as optimal. Currently, to the best of our knowledge, no software package exists that performs LASSO regression with random effects. Therefore, for species that had five or more records, a species-specific fixed effect was added to account for inherent differences across species. Regression tree analysis was also employed to identify the main factors that explain eradication success. Regression tree analysis builds a decision tree by splitting the data into branches, and partitioning the data into smaller groups as the tree branches branch out. Each split (branching) represents a split in the explanatory variable with a given probability. The trees are optimized and pruned such that the smallest cross-validation error is obtained. As splits can be different from one branch to another, one can take the interaction between variables into account. In this analysis, every species was given equal weight and thus the records of the same species were weighted by the inverse of the number of records per species. Both methods were used to explain factors that explain the establishment and the eradication success of a species. All methods were fitted in the software package R using the packages 'glmnet' for the LASSO regression, and ''rpart, "partykit"' for the tree regression, respectively.

Results

Descriptive analysis

Eradications and post-border interceptions

A total of 848 cases officially declared as eradication attempts were documented in our database, 314 against arthropods and 534 against pathogens. These cases concerned 49 species of arthropods (47 insect and 2 mite species) and 34 species of pathogens (21 fungi and oomycetes, 8 bacteria, 2 nematodes, and 3 virus/viroids). A large number of reports corresponded to post-border interceptions. These cases represented 49% (154) of reports on arthropods and 19% (87) on pathogens.

In the case of insects, these data show that for only 9% of the compiled list of 487 non-native insect species of woody plants detected in Europe, eradication measures were taken (42/487, Fig. 1). Species that are native to parts of Europe but non-native in other European countries (e.g. UK) were not included in this analysis.

The total number of insect species for which "eradication" measures were taken (both established populations and post-border interceptions), increased in the last two decades (Fig. 1). Still, the numbers are very modest when compared with the total number of non-native species introduced in Europe, reaching a maximum of 18% of the total number of introduced species in the last twenty years (25/136). For invasive fungi and oomycetes in European forests, eradication was attempted in only 12% of the cases (17/146).

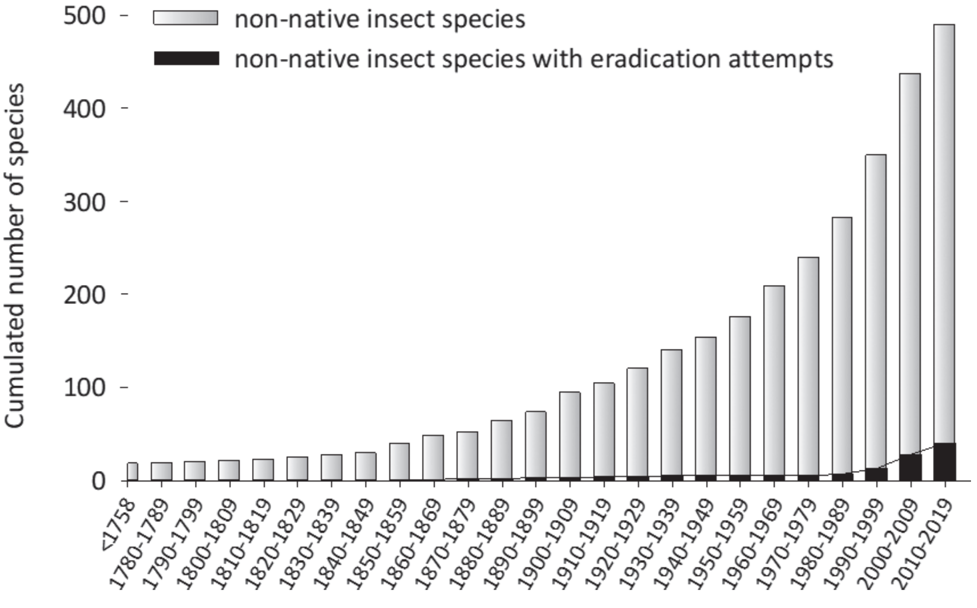

Figure 1. Cumulative number of non-native species of insects of woody plants for which eradication of established or intercepted populations was attempted, and the cumulated number of alien insect species reported for Europe until 2019.

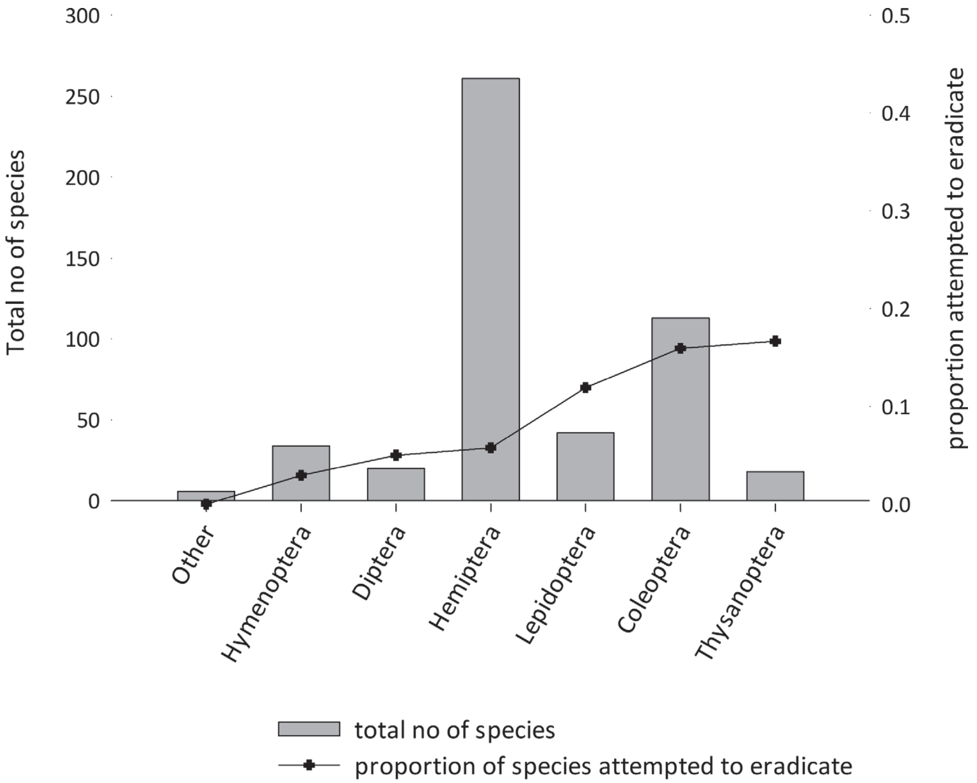

Figure 2. Total number of non-native insect species on woody plants reported for Europe by order (bars) and proportion of species attempted to eradicate (established species).

Post- border interceptions were observed for only a few species. For arthropods, 43% of reported interceptions are linked to the oak processionary moth (*Thaumetopoea processionea*, OPM) in the UK, outside of the containment area in London and South East England where the pest has established, after being accidentally introduced from mainland Europe (DEFRA 2022). OPM has been intercepted in all types of environments, from nurseries to urban and peri-urban areas and in recently established plantations. *Anoplophora chinensis* ranks second in the number of interceptions (21 cases). This beetle was mostly intercepted in nurseries and urban and peri-urban environments, associated with imported *Acer* spp. plants for planting and bonsais (Branco et al. 2021). The remaining interceptions are distributed among 20 species (Suppl. material 1), mostly found in nurseries, garden centres or other confined environments (68% of cases).

Concomitantly, there is a discrepancy between the number of alien insect species reported and the number of eradication attempts by taxa. Most of the non-native species (52%) are hemipteran sap suckers, but eradication was attempted for only 6% of these (Fig. 2). Coleopterans represent 42% of the reported eradication attempts against insects and 96% of these attempts were against wood borers (Cerambycidae, Curculionidae, Buprestidae).

For pathogens, 11 species have been intercepted outside of import-associated inspections, mostly in confined environments (72%). *Phytophthora ramorum* ranks first in the number of interceptions (63%), distributed among 12 European countries, followed by *Cryphonectria parasitica* (13%), the causal agent of chestnut blight, for which most interceptions were reported in the UK, where it has only recently established (Hunter et al. 2013; Romon-Ochoa et al. 2022).

Detection

For arthropods 49% of detections occurred during official surveys (53/108). The remaining cases were detected by passive surveillance which corresponded mostly to members of the public who reported symptoms of infested plants or sightings of adult insects to the competent phytosanitary authorities, by operators of nurseries and greenhouses and growers. In contrast, pathogen detections occurred mostly during official surveys, in 90% (247/275) of cases.

Success of eradication programmes in Europe

Eradication measures taken against organisms still restricted to the primary material with which they were introduced, here defined as post-border interceptions, were 100% successful. From here on we will consider only eradication programmes targeted at established populations in Europe. In total, 160 programmes were launched against 41 species of arthropods (Fig. 3) and 447 programmes against 31 species of pathogens (Fig. 4). The proportion of successes and failures varied greatly among species.

Arthropod species and feeding guilds

Attempts to eradicate arthropods were mostly concentrated on bark and wood borers, followed by sap-suckers, and defoliators. Other guilds were rarely targeted. In 50% of the concluded programmes (55/111), species were confirmed eradicated. Eradication is still in progress in 46 cases (29%). Three species rank the highest in the number of eradication attempts: *Anoplophora glabripennis* (39), *A. chinensis* (18) and *Rhynchophorus ferrugineus* (17). Eradication success differed greatly between species (Fig. 3). The highest eradication success was reported for *A. glabripennis* (100% – 23 cases), although many programmes (16) are still in progress. Eradication has never been successful for 13 arthropod species. Leading among these cases are sap-suckers, notably the psyllid *Trioza erytreae*, for which six programmes were launched, four of which failed and two are still ongoing (EPPO- Global database 2022). Although the area increased over which this pest is distributed, an effective biological control programme has been launched with the introduction of the parasitoid *Tamarixia dryi* (JC Franco, unpublished data).

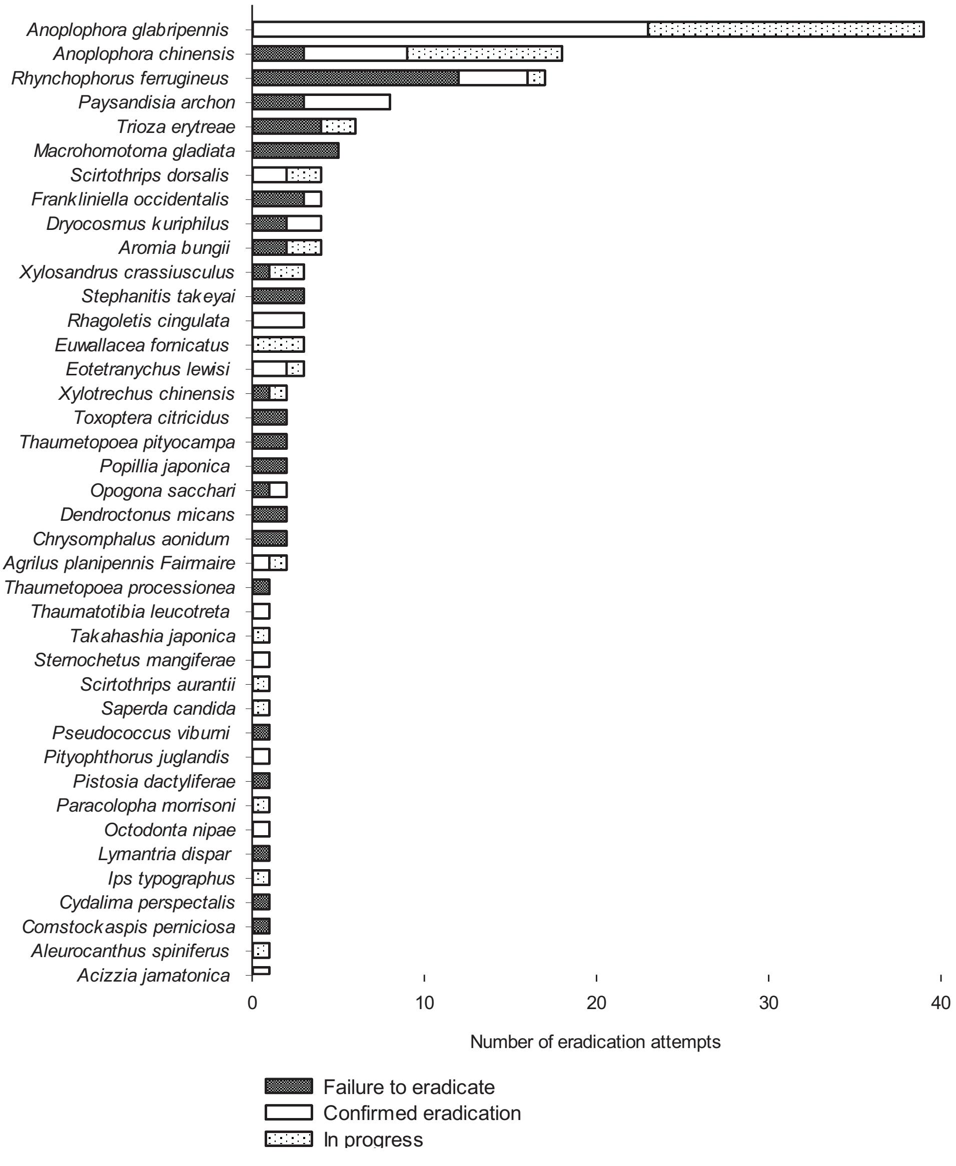

Figure 3. Arthropod species for which eradication was attempted in Europe.

Pathogen species and groups

Concluded programmes against established pathogens accounted for 359 cases. In addition, 80 cases are still in progress and for 8 cases the outcome is still unknown. Eradication programmes targeted 31 species, including fungi/oomycete, bacteria, nematodes and viruses (Fig. 4). The success rate for the concluded programmes is 61%, with little variation between groups.

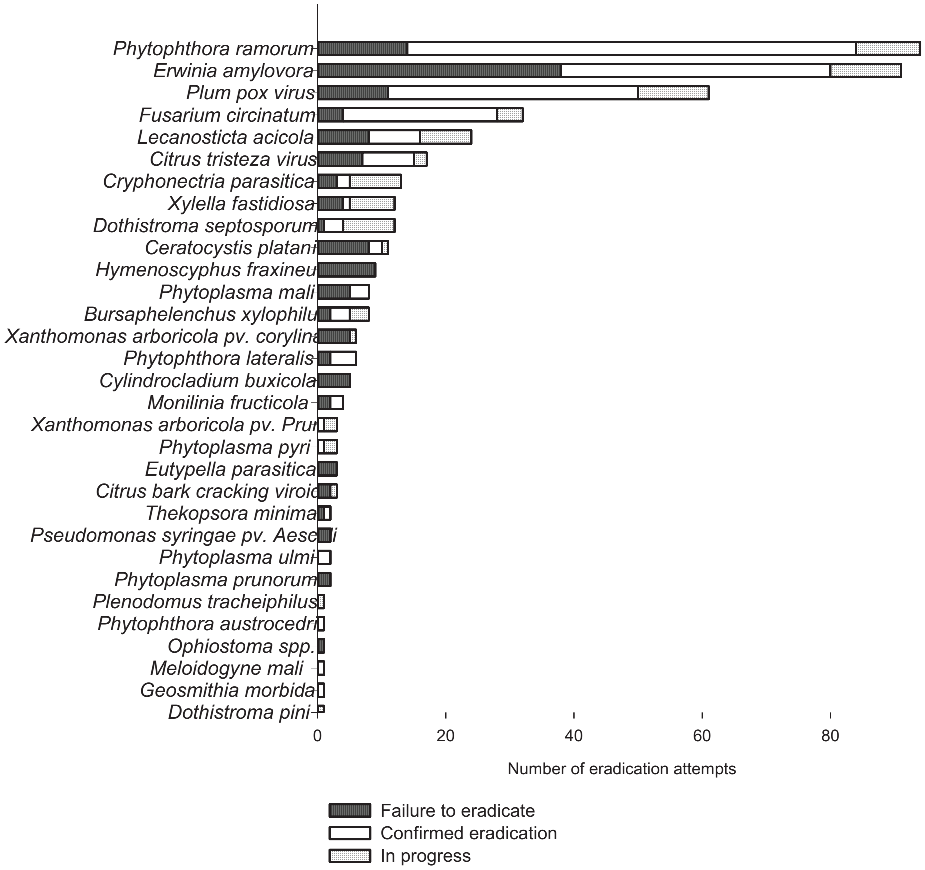

Figure 4. Pathogen species for which eradication was attempted in Europe.

As observed for arthropods, eradication of pathogens was mostly focused on a few species. Three species alone account for over half of total eradication attempts: *Phytophthora ramorum* (21%), *Erwinia amylovora* (21%) and *Plum pox virus* (PPV) (14%). *Phytophthora ramorum* (sudden oak death) was first detected in Europe on *Rhododendron* and *Viburnum* plants in nurseries (Werres et al. 2001) and later in infected Japanese larch trees, *Larix kaempferi*, in the United Kingdom (Brasier and Webber 2010). *Erwinia amylovora* or fireblight is a pathogen of plants in the family Rosaceae (CABI 2019). Plum pox virus disease, commonly known as sharka, is one of the most destructive diseases of stone fruits from the genus *Prunus* (CABI 2019).

Temporal and spatial trends

For both arthropods and pathogens, the total number of eradication programmes against established populations increased abruptly in the last two decades, (Fig. 5). The success rate of eradication attempts against arthropods reached 72% in the period 2011–2020. In contrast, all programmes that started before 2000 failed. For pathogens, the success

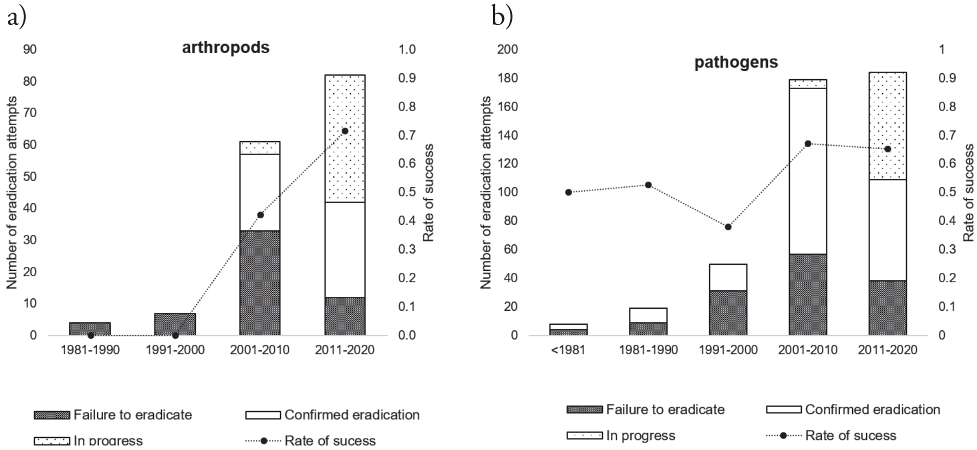

Figure 5. Eradication attempts in Europe by decade and corresponding rate of success of programmes targeting **a** arthropods and **b** pathogens.

rate has also increased, being highest for the last two decades (approximately 65%). In the decades prior to 2000, the success rate was moderate, ranging between 40% and 52%.

In terms of geographic distribution, the highest number of eradication attempts per country were reported for France (81), Spain (61), Italy (57) and Germany (56) (Fig. 6). A clear concentration of eradication programmes exists in Western Europe (Fig. 6), with many of these programmes still ongoing.

Reaction time and duration of programmes

For arthropods, most of the attempts were carried out within one year after first detection (84%), and 10.6% were carried out in the second year, with similar success rates (53% in both cases). All five programmes starting later than 2 years after detection failed. Similarly, for pathogens, 89% of the eradication programmes were launched within one year after the first detection, with a success rate of 66%. The rate of success dropped to 42% and 25% when they were launched in the second or third year, respectively. Of the 12 programmes launched more than three years after detection, one is still in progress and the remaining failed.

The duration of failed eradication programmes was on average 5.8 ± 4.5 years (mean ± SD) for arthropods and 6.5 ± 6.5 for pathogens. For successful programmes, the duration from the start until the last detection was shorter, with 2.0 ± 2.6 and 2.0 ± 3.2 years for arthropods and pathogens respectively. Still, monitoring could continue for several years after the last observations of the species.

Area affected

For both arthropods and pathogens, the success rate was the highest for infestations restricted to small areas (Fig. 7). For infested areas < 1 ha, the success rate was 82% and

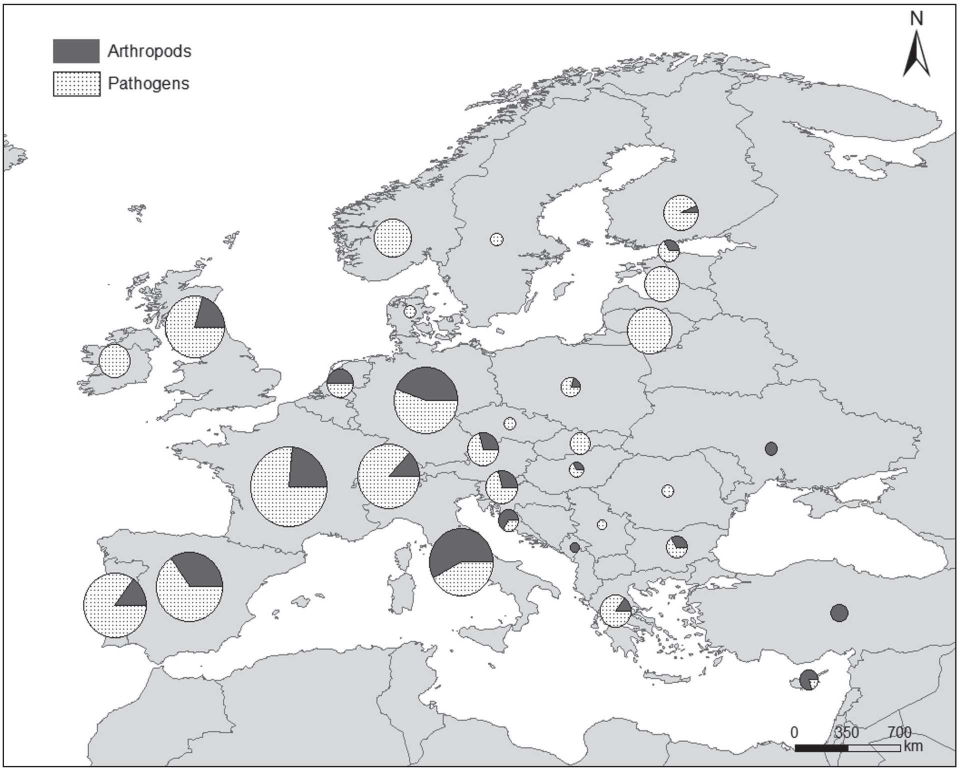

Figure 6. Eradication attempts against arthropods and pathogens in Europe, by country. The size of each pie is proportional to the number of eradication attempts in that country.

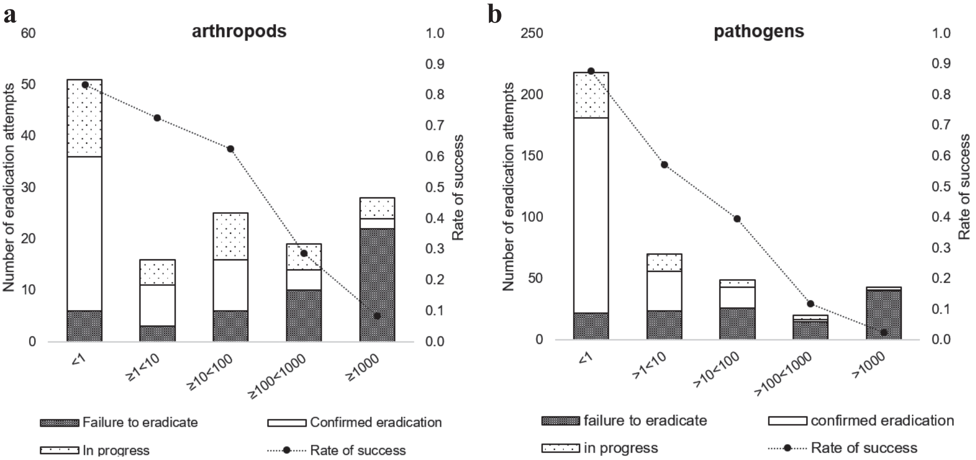

Figure 7. Eradication attempts conducted in Europe by infested area and corresponding rate of success against **a** arthropods and **b** pathogens. Information on the approximate area affected at the time of programme start was retrieved for 139/160 cases for arthropods and 408/447 cases for pathogens.

90%, for arthropods and pathogens, respectively (Fig. 7). Success decreased with an increase in the affected area, with the sharpest decrease observed for pathogens.

For areas above 1000 ha, success for pathogens was only achieved once out of 40 concluded programmes (2.5%). This unique success concerned *E. amylovora* in Norway. The programme started in 1986 in an infested area of 30,000 ha where all the hosts were removed (i.e., all *Cotoneaster*, *Sorbus* and *Pyracantha*). Within the quarantine area (70,000 ha), the production and sale of all common fire blight hosts was prohibited and bee hives were moved to areas that were free from hosts of *E. amylovora*. From 1993 to 2000, no new detections were made and the outbreak was declared eradicated in 1998. Although fire blight was again detected within the restriction zone in 2000, it is unknown whether a re-emergence or a new introduction occurred (Sosnowski et al. 2009).

Climate

Regarding the role of climate, for arthropods, the lowest eradication success was reported in Mediterranean climates (Köppen Csa, Csb) (29%), and higher success rates were observed for temperate (Köppen Cfa, Cfb) (63%) and continental climates (Köppen Dfb) (67%). For pathogens, the success rate related to climate varied depending on the group considered: i) for fungi and oomycetes the highest rate of eradication success was reported in Mediterranean climates (93%), intermediate for continental (71%), and the lowest for temperate climates (60%); ii) for bacteria the lowest success rate was again reported for temperate climates (34%), yet the highest success rate was registered in continental climates (77%), with an intermediate rate of success for Mediterranean climates (46%); iii) for viruses and viroids, the eradication success was low in the Mediterranean and temperate climates, with 62% and 64%, respectively, and high in continental ones (93%). Most attempts to eradicate nematodes were conducted in Mediterranean climates, with an overall success rate of 67%.

Detection site and affected hosts

New establishments of arthropods were most often detected in urban or peri-urban areas, including residential and industrial areas (65% of cases). The rate of eradication success was highest (74%) in confined environments, where the plant materials were delimited (nurseries, glasshouses and garden centres), intermediate in residential areas (52%), and lowest (26%) in the countryside (orchards, woodlands /forests). Pathogen detection, on the other hand, occurred in the countryside in 50% of cases (mostly orchards, Fig. 8), followed by confined environments (30% of cases), and only 20% of cases were first reported in urban and peri-urban areas. The rate of pathogen eradication success was also high in confined environments (86%) and similar for the remaining environments (50% for countryside and 51% for urban/peri-urban).

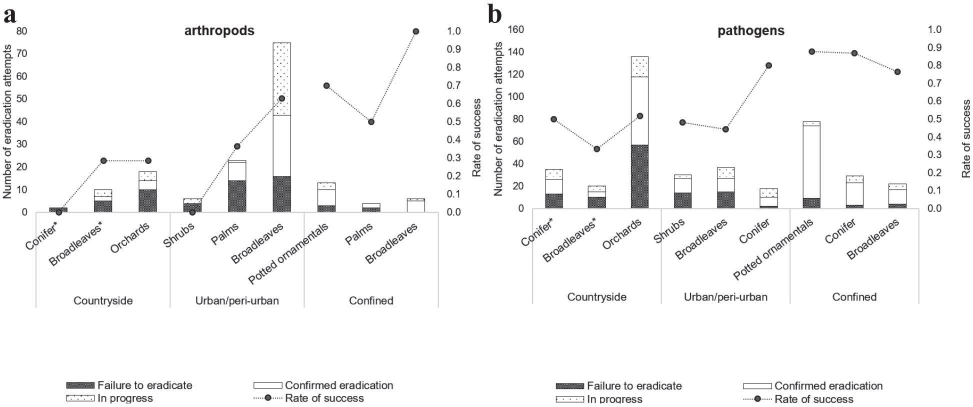

Figure 8. Eradication attempts conducted in Europe against **a** arthropods and **b** pathogens, by detection site and with the corresponding rate of success. Information on the main type of environment affected at the time of the programme start was retrieved for 157/160 cases for arthropods and 405/447 cases for pathogens. * in woodlands or forests.

Most eradication programmes targeted PPWPs attacking broadleaves (79%, both for arthropods and pathogens). For arthropods attacking broadleaves in urban and peri-urban areas, the eradication success rate was 63%, while in the five cases reported in confined environments, the success rate was 100%. The lowest success rates were reported for pests on countryside woodland and forest conifers and on urban and peri-urban shrubs, where all six launched eradication programmes have failed (Fig. 8). For pathogens, the eradication success rate on different host plants was overall similar in countryside and urban and peri/urban environments (Fig. 8), albeit slightly lower on broadleaves in woodlands and forests (30%) and on ornamentals in urban environments (44%). The only exception was the high success rate reported for pathogens attacking conifer pests in urban environments with an 80% success rate.

Eradication methods

Information on the eradication methods applied was available for 149 out of 160 cases for arthropods and for 427 out of 447 cases for pathogens. Eradication methods consisted mainly of host removal or destruction of host plants, which was used in 81% and 99.8% of the programmes against arthropods and pathogens, respectively. For arthropods, this proportion increases to 94% when only wood borers were considered.

When host removal was used alone, or in combination with other methods, the rate of success was 58% (48/82) for arthropods and 62% (217/350) for pathogens. Host removal was commonly combined with quarantine or movement control restrictions imposed by legislation, preventing the movement of host plants or potential host plant material outside of the demarcated areas. For nurseries, these measures usually implied that for a given period of time, neither potentially affected, nor susceptible plants, could be traded. In the field, the quarantine area usually included the infested zone and a buffer zone delimited around the infested/infected zone, which together represented the demarcated area of the outbreak. When host removal was combined with quarantine measures the success rate increased to 70% for arthropods and 65% for pathogens. When treatment of a surrounding area of predefined extent around the focus zone was imposed, either by removal of all or part of sensitive hosts or by chemical control measures, the success rate was overall higher, 67% for arthropods and 70% for pathogens, than when no such measures were applied, with 38% for arthropods and 46% for pathogens.

For arthropods, the combination of host removal with chemical treatments was reported in 31% of concluded cases, with an overall success rate of 37%. Chemical treatment without host removal was reported only in 20 cases with low success (25% success rate). Other control methods such as biological control or traps were seldom used, alone or in combination with other methods (used in 8% and 9% of programmes, respectively).

Against pathogens, disinfection of associated material, such as production machinery and tools used was reported for *E. amylovora* and *F. circinatum* (EPPO- Global database 2022), which in combination with other methods resulted in a 76% eradication success rate. The use of chemical control with either fungicides or antibiotics was only reported for 6 concluded cases (50% success rate). For pathogens transmitted or potentially transmitted by insects, vector control was used in several cases (28 concluded), and against *E. amylovora* the prohibition of beehive movement was often imposed.

Monitoring

For arthropods, visual observation was the only monitoring method used in 43% of the eradication programmes. Detection dogs were used in 29 eradication programmes against the two *Anoplophora* species, and tree climbers were further used for monitoring *A. glabripennis*, for which these methods were frequently used simultaneously, with a 100% success rate. However, it is important to note that 16 eradication programmes against this species are still in progress.

For pathogens, monitoring consisted of visual observation for symptoms and the sampling of plant material for laboratory analysis, either by morphological or, more commonly, by molecular methods. For some species, such as *P. ramorum* and the pine wood nematode (PWN), only symptomatic plants were commonly sampled, whereas for others, such as Citrus Tristeza Virus (CTV) and *F. circinatum* sampling of asymptomatic hosts is regularly conducted. For *E. amylovora* and PPV for example, an overall higher success rate of eradication was observed when asymptomatic plants were also sampled (60% and 92%, vs 51% and 74%, respectively). Annual surveys at places of production or other specified areas are mandatory in some cases, and were conducted in 84% of concluded cases. Conducting annual surveys provided a higher success rate (67%, 185/276) than when surveys were conducted less frequently (44%, 16/36). For *P. ramorum* in the UK, in addition to visual inspection in nurseries and ground surveys, aerial surveys were also used in forested areas with larch (*Larix* spp.), looking for visible symptoms. This method was also used for *Phytophthora lateralis* in UK forests. When symptoms were detected, confirmation was then attained by laboratory analysis of plant samples. In many European countries, traps were also commonly used to monitor vectors of pathogens transmitted or potentially transmitted by insects.

EPPO recommendations

Of the eradication attempts reported, the vast majority were against species present in EPPO Alert, A1 or A2 lists (EPPO 2021) with or without legal mandatory measures. Only 14% of cases against arthropods and 9% against pathogens were against species not included in EPPO lists. The success rate in these cases was only 10% and 38%, respectively.

Society and citizens' engagement

Information about the citizens' education and the engagement of stakeholders during the eradication programmes is scarce. The involvement of citizens was reported for 75/160 cases of arthropod eradication programmes. In approximately half of these cases (51%), involvement was limited to the reporting of insects or symptoms to the phytosanitary authorities. For the remaining cases, involvement was compulsory, imposed by legislation, such as the obligation to report sightings or to cut infested/infected trees. Targeted species were *A. glabripennis*, *A. bungii*, *D. kuriphilus*, *R. cingulata*, *R. ferrugineus*, *S. dorsalis*, *Toxoptera citricidus*, and *T. erytreae*. A volunteer collaboration was recorded in 15 cases, concerning *A. chinensis*, *A. glabripennis*, *D. kuriphilus* and *R. ferrugineus*. By contrast, a mainly negative attitude was recorded against the eradication of *A. glabripennis* in Kent, UK. The negative perception was due to unwillingness to cut historical trees or because citizens were angry claiming that contractors were cutting the wrong trees (Porth et al. 2015). Actions for the education of citizens and public information about the ongoing eradication program were expressed in half of the programmes, whereas for the other half, no information was available. Public education for signs and symptoms of pathogens was reported for 62% of the programmes, mostly through the availability of web pages and leaflets given to producers and owners of nurseries and garden centres. Public involvement was mandatory for many of the targeted species. A negative attitude against a programme was only expressed in one case, targeting *Xylella fastidiosa* in Italy. The growers expressed resistance to the massive olive tree culling imposed by European Union containment regulations, including old, historical trees and to the use of insecticides for vector control in organic farming (which prohibits the use of chemical pesticides) (Nadeau 2015). Prosecutors in southern Italy accused the researchers of spreading the disease and halted the European Union – ordered cull of olive trees (Abbott 2015).

Correlation between variables

The results of the Cramers' V index for the nominal variables highlighted a strong correlation between some of the variables (Suppl. material 2). For arthropods strong associations (V > 0.5) were estimated for variables mainly related with species traits, such as feeding guild and a number of other species traits (Suppl. material 2). Also, for pathogens strong associations were estimated between species traits. These correlations are solved by LASSO and regression trees, as both methods select variables based on their added predictive ability.

Statistical modelling

Results for establishment probability are given in Suppl. material 3. Here the results of LASSO regression and decision tree analysis are reported for the eradication success of programmes against established populations.

Arthropods

The LASSO regression results showed that the area affected at the start of the eradication programme was the most important factor affecting the outcome of eradication success. For areas ≤ 1 ha and > 1 ≤ 10 ha, success is similar, but above this threshold, there is a negative relationship between the area affected and the probability of a successful eradication (β = -0.72 for > 10 ≤ 100 ha; β = -1.29 for > 100 ≤ 1000 ha and β =-2.69 for > 1000 ha; coefficients are reported at a log odds scale). Other environmental factors affecting the outcome of eradication success were the main type of environment affected at the start of the program, with a higher success rate in confined environments than in the countryside (β = 0.66), and a slightly higher success rate in mainland than in island locations (β = 0.01). Regarding control measures, only the implementation of quarantine/movement restrictions was positively associated with eradication success (β = 0.52). For species traits, internal feeders had a higher probability of eradication success than external feeders (β = 0.66), oligophagous species had a lower probability of eradication success (β =-0.22), and the group of fruit/seed feeders and gall makers had higher eradication success (β =-0.32). Considering the species targeted, *A. glabripennis* was associated with a higher eradication success (β = 2.35) than species for which less than five cases were reported. The optimal penalty value (λ) for the model was 0.028.

In the regression tree analysis, the optimised tree resulted in only one split, with higher eradication success for areas below 10 ha than for larger infested areas (82.6% vs 28.6%)**.** When the area as explanatory factor was removed, a secondary tree was obtained (Fig. 9) for which eradication success was higher when quarantine/movement restrictions were implemented (69%) than when they were not (31%). The variables host type, location and phytophagous specialisation were also excluded from this tree construction due to association with the affected area. Further tree divisions highlighted the main type of environment affected, the main reproduction method, and climate as explanatory variables affecting eradication success.

Pathogens

The LASSO regression estimated that the affected area was the most important factor associated with eradication failure and the higher the area the stronger the association

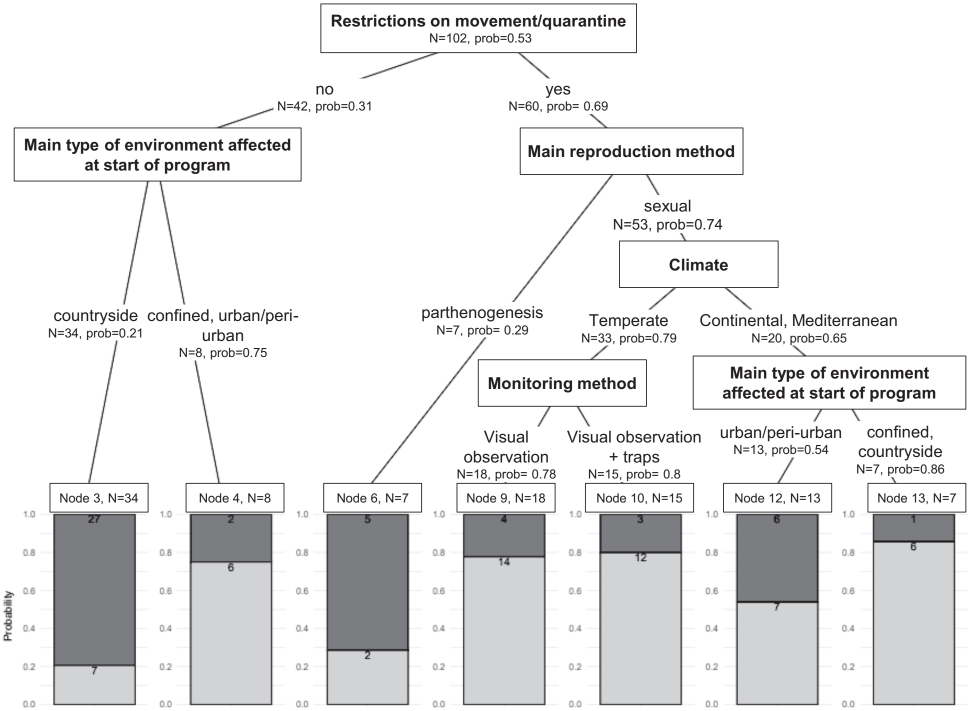

Figure 9. Optimal classification tree (after removing the size of the affected area) for factors affecting eradication success and failures of 102 eradication programmes against non-native arthropods of woody plants in Europe. In the model, every species was given equal weight and thus the records of the same species were weighted by the inverse of the number of records per species. Light grey in bars represents successful eradication, dark grey represents failure to eradicate.

(>1 ≤ 10 ha: β = -0.798; > 10 ≤ 100 ha: β = -1.825; > 100 ≤ 1000 ha: β = -3.246; > 1000 ha: β = -4.334). The type of environment affected also influenced the outcome, with success more likely in confined than in urban/peri urban (β = -0.196) and countryside environments (β = -0.237). Eradication success was more likely when the eradication programme started within the first year after detection (β = 0.369), in temperate than Mediterranean climates (β = 0.434), when surveys were conducted at least annually (β = 0.566), and when host removal alone was used compared to combined methods (β = 0.063). Possible saprotrophic species were harder to eradicate, although the effect was small (β = -0.050). At the species level, *Fusarium circinatum* (β = 0.771), Plum pox virus (β = 0.438) and *Cryphonectria parasitica* (β = 0.223) were easier to eradicate than species with lower than five eradication attempts, and *Hymenoscyphus fraxineus* (β = -2.013), *Phytoplasma mali* (β=-0.998), *Xanthomonas arboricola* pv. *Corylina* (β = -2.453), *Xylella fastidiosa* (β = -0.511), Citrus tristeza virus CTV (β=-0.059) and *Lecanosticta acicula* (β = 0.196) were harder to eradicate. The optimal penalty value (λ) for the model was 0.011.

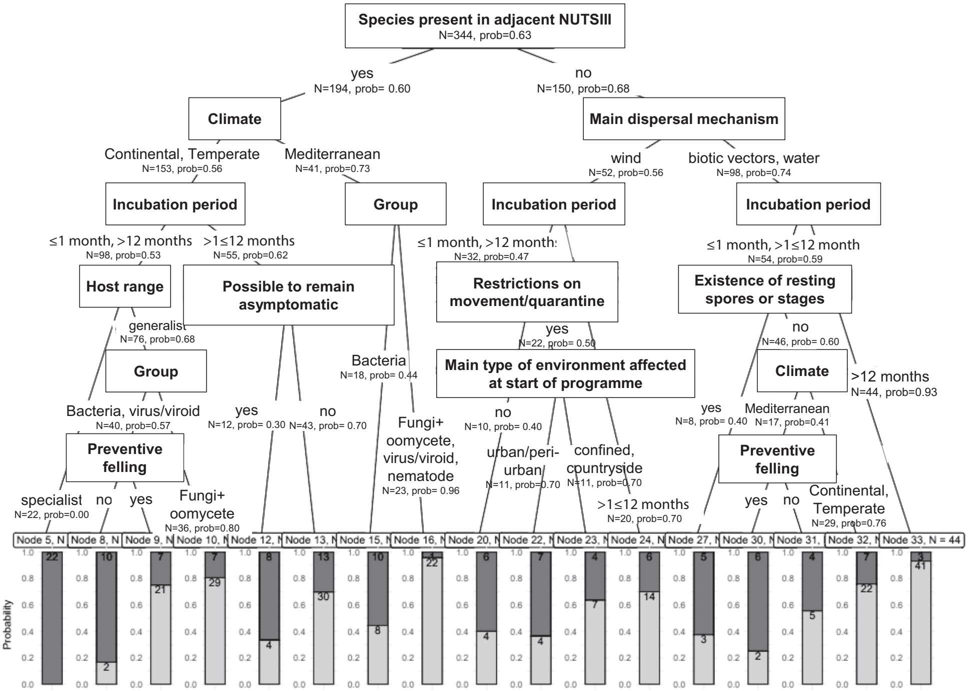

Figure 10. Optimal classification tree (after the area affected removed) for factors affecting eradication success and failures of 344 eradication programmes against pathogens of woody plants in Europe. In the model, every species was given equal weight and thus the records of the same species were weighted by the inverse of the number of records per species. Light grey in bars represents successful eradication, dark grey represents failure to eradicate.

In the regression tree analysis, the optimized tree resulted in only one split where the area was the only variable included, like the results for arthropods. However, here the separation occurred for areas below 1 ha, which had higher eradication success than larger areas (88.1% vs 34.6%). When the area was removed from the model (Fig. 10), the presence of the species in adjacent NUTSIII units resulted in the first split, with higher eradication success when the species was not yet present in adjacent NUTS. The variables host type and location were also removed due to their close association with area. Other environmental factors affecting the outcome of an eradication programme included the climate and the main type of environment affected at the start of the program. Several species traits were shown to influence the probability of a successful eradication, namely the main dispersal mechanism, the host range, the incubation period, the possibility to remain asymptomatic for long periods or indefinitely and the existence of resting spores or stages. Differences in the influence of these factors between groups were observed. Regarding control and monitoring options, the implementation of restrictions on movement/quarantine and preventive felling affected the outcome of an eradication programme.

Discussion

Temporal and spatial trends

An increasing number of non-native forest pests and pathogens was observed in the last century in Europe (Santini et al. 2013; Roques et al. 2016, 2020; Ghelardini et al. 2017). Here we report a concomitantly increasing number of eradication programmes conducted in Europe against pests and pathogens affecting woody plants. However, eradication was attempted for only 9% of the non-native insect species and for 12% of the pathogens. In contrast, for hundreds of species eradication was ever attempted. Nevertheless, these figures are better than those reported for alien insects in North America, where eradication was attempted for only 1.8% of established species (Liebhold et al. 2016).

We may deduce that species with higher economic or ecological impacts were those selected for eradication programmes. A low benefit: cost ratio has been suggested as one of the reasons for eradication not to be attempted (Kean et al. 2022). Still, there are species with high economic impact, such as *Gonipterus platensis* in the Iberian Peninsula (Cordero-Rivera et al. 1999; Valente et al. 2018) for which eradication was never attempted. Several possible reasons for not attempting to eradicate an invasive population have been proposed (Liebhold et al. 2016; Kean et al. 2022): 1) the fact that the pest or pathogen was already too widespread or abundant, or that spread was too rapid; 2) an underestimation of the potential impacts of the pest or pathogen; 3) the lack of adequate surveillance mechanisms to detect an invasion early enough; 4) the lack of effective control tools; 5) the existence of open pathways for re-introduction; 6) or policymakers did not consider eradication to be a realistic option. Since the decision to carry out an eradication programme is typically taken at the national level, and frequently imposed at the European level, this leads to responsibilities in the scientific community in transferring information to policymakers so that more species could be targeted. We hope this revision work may contribute in this regard.

At the European level, eradications were more concentrated in Western regions with minor numbers in the northern and eastern European countries. In part, this distribution coincides with the hotspots of first detections in Europe (Branco et al. 2019), that is the countries where most incursions of high-impact invaders occurred. But there is still a contrast between the high number of first detections in central-eastern countries and the low number of eradication attempts.

Overall eradication success

An optimistic conclusion of our study is that the overall rate of eradication success has been increasing over time for both pathogens and arthropods, and especially for the latter. In the last decade, eradication success attained levels of 76% for arthropods and 68% for pathogens. Yet, these figures include officially declared eradication measures taken against PPWP on imported materials or against adult insects, i.e. before establishment. The success for arthropods is similar to that reported by Tobin et al.

(2014) (78%), whereas for pathogens it is higher than the 55% reported by Smith et al. (2017), with both of these studies being based on data from the Global Eradication Database GERDA. However, care should be taken with the interpretation of these results, as we found they might be overestimated. In fact, a significant part of successful programmes commonly referred to as "eradication" in EPPO and NPPO reports, and consequently in GERDA, concern non-established species; i.e. arthropods or pathogens that were still restricted to the materials with which they were introduced. We propose that these cases could be more accurately defined as interceptions not associated with import inspections. Cases in which pests and pathogens were still restricted to the materials with which they were introduced were mostly found in nurseries and in greenhouses, but a few of them were also on trees already planted in the field. An example of this latter situation was reported in 2019 on oak trees recently planted in the UK, imported from the Netherlands and Germany, which carried oak processionary moth caterpillars. These trees were destroyed and this was considered a successful eradication (UK GOV 2019). In the present study, we treated these cases as "post-border interception". In total, we counted 241 cases in this category and the eradication success rate in these cases was 100%. This outstanding result confirms that the surveillance of plant materials imported and moved inside countries should be done with incessant efforts. A higher investment in surveillance and detection has been identically defended by many authors (Simberloff 2003; Tobin et al. 2014; Ganley and Bulman 2016; Liebhold et al. 2016). Many previous studies considered these cases as eradications as in the GERDA database. However, if one considers eradication in *sensu stricto* only for the programmes against established species, the rate of eradication success is significantly lower. In fact, when we consider only the established species, the overall eradication success rate decreases to 50% for arthropods and 61% for pathogens. However, despite being lower than in other reviews, this value is still relatively high. Furthermore, the success rate of programmes has been increasing in recent decades. Therefore, more optimism is justified about the likelihood of eradication programmes being successful and worthwhile.

On the other hand, eradication success relies on external drivers, and some species might be particularly difficult to eradicate which may be related to environmental factors or species traits. With this aim, we tried to understand the main factors determining eradication success.

Eradication success: Time and space

One of the most consensual conclusions of previous studies is that eradication success is greater the smaller the affected area. Tobin et al. (2014) reported that eradication was 1.3 times less likely for every log10 increase in the infested area for arthropods, and Pluess et al. (2012a) identified a critical threshold of 4905 ha for the infested areas of alien invertebrates, plants, and plant pathogens, above which the probability of successful eradication reduced to half (66.7% vs 32.5%). Not surprisingly, our results are consistent with this conclusion, although our thresholds are different. Additionally, our analyses indicated lower thresholds than in previous studies: below thresholds of 10 ha for arthropods and below 1 ha for pathogens, the success rate is very high, corresponding to 82% for arthropods and 90% for pathogens. But above these thresholds eradication success significantly decreases. Still, an eradication success of 60% was attained between 10 ha and 100 ha for arthropods and between 1 ha to 10 ha for pathogens. This means that for infested areas up to these sizes, eradication may still be considered feasible. Nevertheless, if circumstances are favourable, even eradication of infestations over larger areas may be successful, provided sufficient resources are available. Interestingly, the decreasing slope was steeper for pathogens than for arthropods, which suggests that the factor area is likely to be even more important for the former group. The length of time elapsed since the first detection also led to a decrease in the success rate, as previously mentioned by Pluess et al. (2012b). However, it is difficult to separate the factor time from the area (as the affected areas usually increase over time). Therefore, the main recommendation is that governments and organizations involved should invest in surveillance and detection to achieve detection as soon as possible. Furthermore, preparedness in terms of having tools and plans available is critical to enable timely responses to newly detected incursions to start eradication programmes quickly and increase their likelihood of success. This also implies being well informed about new potential risk organisms and how to eradicate them when they arrive, prior to their invasion.

We observed that species with a higher number of eradication attempts are also those with the highest eradication success. This may reflect increased knowledge on how to deal with these particular invasive species, accumulated in the previous eradication attempts, which would also facilitate a quick reaction before its spreading and becoming then impossible to eradicate.

Blackburn et al. (2011) proposed a conceptual framework for biological invasions which has recently been updated by Paap et al. (2022) to accommodate forest pathogens. Both frameworks are composed by a series of stages, namely transport, introduction, establishment and spread, each stage having a particular barrier that a population needs to overcome to reach the next stage. Different management options may apply at different stages, and early detection of the invader and a fast response time, increase the feasibility of successful eradication. For instance, in nurseries and greenhouses PPWP are usually detected in the introduction phase, when populations still must overcome the limited distribution barrier. This applies to the reported cases still restricted to the primary material in which it was introduced, which had 100% eradication success. By contrast, ash dieback, caused by *H. fraxineus*, is an example of a species that was identified as invasive too late, when large areas of European forests were already invaded and there was no possibility to react anymore (Pautasso et al. 2013).

The similarity of symptoms to native or previously introduced species can mask the presence of invasive species for long periods, as occurred for *Phytophthora cinnamomi* and *Heterobasidion irregulare*, with similar symptoms as *Phytophthora* × *cambivora* and *Heterobasidion annosum*, respectively (Brasier et al. 1993; Vettraino et al. 2005; Garbelotto et al. 2022). For these cases, the development and/or implementation of new genomic biosurveillance tools is critical, so that the taxonomy of the invasive organism can be clarified unequivocally and effective eradication program can be started rapidly (Hamelin and Roe 2020; Luchi et al. 2020).

All these cases reinforce the concept that we should be able to identify potential invaders before they leave the country of origin to be prepared in advance. A good example is the case of *P. ramorum*, the causal agent of sudden oak death in California. The high potential risk identified early for this species, and the fear of having a similar epidemic in Europe, boosted the early detection and the rapid implementation of containment measures. However, not all potential invaders with high economic and ecological impacts have demonstrated this potential in its native region or other invaded regions. Frequently, an organism only becomes emergent in the invaded range, since resistance of native host plants and the communities of natural enemies keep them at low or imperceptible levels in its native range (Elton 1958; Jeffries and Lawton 1984; Wolfe 2002).

Environmental drivers and species traits

As expected, species found in confined or limited environments, usually subject to frequent intervention, such as greenhouses, are easier to eradicate. The same results were found by Pluess et al. (2012b). Both pathogens and arthropods became established more easily, and thus more difficult to eradicate, in urban or peri-urban areas and in the countryside (woodlands, forests or orchards) than in confined environments. An interesting outcome from our work is that urban and peri-urban areas have similar establishment probabilities and equal difficulties of eradication as countryside. The relevance of urban forests and urban trees for the establishment and spread of invasive forest species has been gaining relevance (Poland and McCullough 2006; Paap et al. 2017; Branco et al. 2019; Dale et al. 2022; Nunes et al. 2023) which is reinforced by our results.

Climate may play a role in the success of eradication programmes. Warmer climates may favour higher growth rates for arthropod populations. On the other hand, Mediterranean climates with harsher summer conditions, or a continental climate with severe winters may explain a lower probability of establishment and a higher probability of eradication success for some groups of insect pests and pathogens in these conditions. Still, a general trend of climate in the eradication success did not emerge from our analysis. The differences in the climate of origin and the one of the invaded range could have played a role in the eradication of specific species. Additional studies could address this hypothesis. Further, our dataset does not completely allow to disentangle climate effects from other factors, namely cultural and socio-economic ones.

Regarding species traits, we could associate some traits with a higher difficulty of eradication. For arthropods, the most remarkable outcome is the extremely low success in eradicating Hemipteran species. This is probably explained by several traits shared by many hemipteran species, such as the high dispersal ability, frequently mediated by wind and their difficulty of detection at low densities due to their often small size and cryptic stages, high fecundity and short life cycles. Concomitantly, in the LASSO models, species traits associated mostly with hemipterans in our group of species, such as parthenogenesis, were found to be relevant. An example is the psyllid *T. erytreae*, for which six eradication programmes were launched, and none succeeded, despite the huge effort invested in it. For pathogens, as expected, eradication proved to be harder for species with high saprotrophic abilities, for species dispersed by wind, for species that may remain indefinitely asymptomatic and for species with resting spores or stages. Unexpectedly, however, species with intermediate incubation periods (>1 ≤ 12 months) were overall easier to eradicate than those with shorter (≤ 1 month) or longer periods (>12 months). Short incubation periods may lead to faster population growth and dispersal thus challenging eradication efforts. The concomitant harder difficulty to eradicate species with incubation periods longer than one year may be associated with poor detection before planting infected material: if disease symptoms may appear after plantation, with a lag that may reach several years for some pathogens, the infected area may become large, hampering eradication success (Migliorini et al. 2015). This highlights the need for detection attempts on asymptomatic plants that indeed improved the eradication prospect (see later).

When calculating correlation between variables, most strong associations (V = 0.5) were obtained between pairs of different species traits, both for arthropods and pathogens. These correlations are justified given the high number of cases concentrated on only a few species. The statistical modelling used were able to deal with collinearity to an extent: for the LASSO regression when multiple variables are correlated they will be penalized leading to one unique predictor becoming important; for the tree-regression it chooses the variables that lead to the best split in the data. Nevertheless, it is important to note, that potentially some of the correlated variables could have been used as surrogate in the LASSO or tree regression.

Management options

An outstanding result of our study is that management options did not emerge as a relevant predictor variable of eradication success. This might be due to the fact that host plant removal, almost always combined with other treatments, was the commonly used management strategy for both arthropods and pathogens. Chemical control alone leads to very low success rates (25%). Other management options are very species-specific, such as the use of tree climbers for *A. glabripennis* monitoring, nest removal for oak and pine processionary moth control, and vector control for several vector transmitted pathogens and thus, do not allow extrapolation to general guidelines. Also, generally similar eradication measures were applied everywhere for a given species, because frequently these measures are mandatory according to European regulations.

Another main significant outcome of our review is the importance of quarantine measures for the success of arthropod eradication. For pathogens, however, the implementation of such measures was relevant only when the target organisms were wind-borne. The intensification of surveys, at least in an annual rhythm, was shown to be relevant both for the detection of pathogen infection before establishment and for the success of eradication. Giving up the efforts of surveillance and control after a while, especially when the populations are under low levels and difficult to detect, is a common error leading to unsuccessful eradication campaigns (Simberloff 2002;

Liebhold and Tobin 2008; Tobin et al. 2014). Therefore, persisting in monitoring for several years after the last detection can be crucial for the success of programmes. Tobin et al. (2014) reported that the existence of a sensitive monitoring tool (such as pheromone traps) was one of the most important predictors in the success of an eradication campaign against arthropods. However, in our study, the use of a semiochemical lure neither affected the outcome of an eradication campaign nor the probability of establishment, and the use of traps for monitoring only slightly increased the eradication success. In the study by Tobin et al. (2014), however, the authors highlighted that when *Lymantria dispar* and *Ceratitis capitata* were excluded from the analysis, the use of targeted traps or lures was no longer significant in the outcome of an eradication attempt. The high efficiency of the available semiochemical lures for these two species may contrast with the lures currently available for the arthropod species targeted for eradication in Europe. For *A. glabripennis*, for e.g., a recent study has shown that the available pheromone traps, although recommended for monitoring and mass trapping of this insect, are inefficient at intercepting the pest (Marchioroand Faccoli 2021). For pathogens, we observed that an overall higher success rate of eradication was observed when asymptomatic plants were also sampled (60% to 92%), compared to when only symptomatic trees were sampled 51% to 74%.

Conclusions

We conducted a thorough review of the eradication programmes carried out in Europe against arthropods and pathogens of woody plants and their successes or failures. Contrary to the general scepticism regarding the potential success of eradication measures, our review demonstrates that eradication programmes can be very successful, especially when detections occurred at an early stage of invasions and when the infested areas were still small. Difficulties in eradication are naturally higher in the countryside conditions in comparison with confined environments. In this respect, pests and pathogens of woody plants are as difficult to eradicate in urban and peri-urban areas as in rural forests and orchards.

We should be aware that the high success reported in previous studies and databases results in part from the inclusion of cases in which pests and pathogens were still restricted to the primary plant material with which they were introduced. After removing these cases the overall success dropped to 50%. Thus, particular attention should be paid to imported primary plant materials, involving the awareness of different actors and not only Plant Protection Inspectors.

It is surprising that eradication efforts in Europe targeted only a small group of non-native species (<10% of the non-native organisms affecting woody plants). Since the decision to carry out an eradication program is taken at the national level and frequently imposed also at the European level, we believe that more species could be considered for eradication if policymakers would be better informed about the advantages of eradication measures and actions taken quickly to ensure success of eradication.

This leads to responsibilities for the scientific community in transmitting these pieces of information to policymakers.

Management strategies used in eradication programmes are very species specific and there is no general golden rule in this respect. Still, most of the successful programmes invested in integrating multiple methods combined with relentless and persistent monitoring.

Acknowledgements

This work was funded by the European Union's Horizon 2020 Program for Research and Innovation under grant agreement no. 771271 "HOMED"

S. Branco, M. Branco, and J.C Franco were supported by Forest Research Centre (CEF), (UID/AGR/00239/2019 and (UIDB/00239/2020). and by the Laboratory for Sustainable Land Use and Ecosystem Services–TERRA (LA/P/0092/2020), research units funded by the Foundation for Science and Technology (FCT), Portugal.

We would also like to thank the plant protection organizations of the following countries (or regions), for kindly providing additional information on the reported eradication attempts: Austria- Federal Ministry Of Agriculture, Regions And Tourism; Belgium- Federal Agency for the Safety of the Food Chain; Croatia- Agency for Agriculture and Food; Finland- Plant Health Unit Ruokavirasto / Finnish Food Authority; France- Département de la Santé des Forêts (C. Husson); Germany- Julius Kühn-Institute – Federal Research Centre for Cultivated Plants, Institute for National and International Plant Health; Hungary- National Food Chain Safety Office; Italy (Piemonte)- Settore Fitosanitario e Servizi Tecnico-Scientifici, Regione Piemonte; Lithuania- State Plant Service under the Ministry of Agriculture; Netherlands- Netherlands Plant Protection Organization; Slovenia- Ministry of Agriculture, Forestry and Food; Spain- Área de Sanidad Vegetal y Forestal de Sanidad e Higiene Vegetal y Forestal; Switzerland- Federal Office for Agriculture, Plant Health and Varieties; United Kingdom – Department for Environment, Food and Rural Affairs.

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Supplementary material 1

Eradication database

Authors: Sofia Branco, Manuela Branco

Data type: List of eradication attempts against non-native pests and pathogens of woody plants in Europe (excel document)

Explanation note: Database including the following information for each case (when available): i) species under eradication, ii) detection date, country, and location; iii) detection method (passive surveillance (i.e. casual observations reported by researchers, technician or citizens) or official survey conducted with that purpose; iv) establishment status (established or post-border interception); v) affected hosts; vi) host type (broadleaves, conifers, palms), vi) control methods used (chemical, host removal, biological, traps); vii) size of the infested area (as exact area information was not always available we defined it in categories ≤ 1 ha, > 1 ≤ 10, > 10 ≤ 100, > 100 ≤ 1000 or > 1000 ha); viii) environments infested (urban/peri-urban, protected green-houses, countryside); ix) climate, categorized as Temperate, Mediterranean or Continental according to Köppen classification system (Peel et al. 2007); x) programme start year, last detection, and date of eradication declared; xi) public education, and xii) the outcome, i.e. legal status (eradicated, under eradication, failure to eradicate.

Link: https://doi.org/10.3897/neobiota.84.95687.suppl1

Supplementary material 2

Correlation matrix

Authors: Jacob C. Douma, Sofia Branco

Data type: Results of statistical analysis (excel document)

Explanation note: Correlations between predictor variables, using Cramers' V.

Link: https://doi.org/10.3897/neobiota.84.95687.suppl2

Supplementary material 3

Establishment success analysis, using LASSO regression and decision trees

Authors: Jacob C. Douma, Sofia Branco

Data type: Statistical analysis (word document)

Explanation note: Analysis of the factors affecting establishment success for non-native species of arthropods and pathogens of woody plants in Europe, using LASSO regression and decision trees.

Link: https://doi.org/10.3897/neobiota.84.95687.suppl3

NeoBiota 84: 319–347 (2023)
doi: 10.3897/neobiota.84.90549
https://neobiota.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
NeoBiota
Advancing research on alien species and biological invasions

Conditions of emergence of the Sooty Bark Disease and aerobiology of *Cryptostroma corticale* in Europe

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Academic editor: Christelle Robinet | Received 19 July 2022 | Accepted 10 March 2023 | Published 18 May 2023

Citation: Muller E, Dvořák M, Marçais B, Caeiro E, Clot B, Desprez-Loustau M-L, Gedda B, Lundén K, Migliorini D, Oliver G, Ramos AP, Rigling D, Rybníček O, Santini A, Schneider S, Stenlid J, Tedeschini E, Aguayo J, Gomez-Gallego M (2023) Conditions of emergence of the Sooty Bark Disease and aerobiology of *Cryptostroma corticale* in Europe. In: Jactel H, Orazio C, Robinet C, Douma JC, Santini A, Battisti A, Branco M, Seehausen L, Kenis M (Eds) Conceptual and technical innovations to better manage invasions of alien pests and pathogens in forests. NeoBiota 84: 319–347. https://doi.org/10.3897/neobiota.84.90549

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Abstract

The sooty bark disease (SBD) is an emerging disease affecting sycamore maple trees (*Acer pseudoplatanus*) in Europe. *Cryptostroma corticale*, the causal agent, putatively native to eastern North America, can be also pathogenic for humans causing pneumonitis. It was first detected in 1945 in Europe, with markedly increasing reports since 2000. Pathogen development appears to be linked to heat waves and drought episodes. Here, we analyse the conditions of the SBD emergence in Europe based on a three-decadal time-series data set. We also assess the suitability of aerobiological samples using a species-specific quantitative PCR assay to inform the epidemiology of *C. corticale*, through a regional study in France comparing two-year aerobiological and epidemiological data, and a continental study including 12 air samplers from six countries (Czechia, France, Italy, Portugal, Sweden and Switzerland).

We found that an accumulated water deficit in spring and summer lower than -132 mm correlates with SBD outbreaks. Our results suggest that *C. corticale* is an efficient airborne pathogen which can disperse its conidia as far as 310 km from the site of the closest disease outbreak. Aerobiology of *C. corticale* followed the SBD distribution in Europe. Pathogen detection was high in countries within the host native area and with longer disease presence, such as France, Switzerland and Czech Republic, and sporadic in Italy, where the pathogen was reported just once. The pathogen was absent in samples from Portugal and Sweden, where the disease has not been reported yet. We conclude that aerobiological surveillance can inform the spatial distribution of the SBD, and contribute to early detection in pathogen-free countries.

Keywords

Acer pseudoplatanus, aerobiology, airborne fungal spores, climate change, drought-induced forest disease, heat wave, invasive pathogen, maple bark disease, quantitative species-specific PCR

Introduction

Emerging infectious diseases threaten human health, agriculture and biodiversity (Jones et al. 2008). The occurrence of emerging diseases in forest ecosystems has exponentially increased over the last four decades in Europe (Santini et al. 2013), and the number of fungal plant diseases has shown a 13-fold worldwide increase in 15 years (Fisher et al. 2012). The most common drivers of new forest disease emergence are the introduction of exotic pathogens in new geographic areas and climate change (Ghelardini et al. 2016). Exotic pathogens have coevolved with hosts from their native range under particular environmental conditions and with particular associated microorganisms (Desprez-Loustau et al. 2007; Stenlid and Oliva 2016). The introduction of exotic pathogens to new geographic areas can potentially lead to severe disease outbreaks due to their encounter with naïve hosts, to the release of natural enemies, and to more favourable environmental conditions. Moreover, climate change can result in nonlinear range shifts of infectious forest diseases, as it can simultaneously affect the host's and pathogen's ecological niches (Dudney et al. 2021). This multifactorial nature of disease emergence may hinder proper epidemics' prediction and hence the establishment of appropriate disease management programmes.

Examples of forest diseases linked to climate extremes that are increasing in Europe are Diplodia tip blight in pine species (Brodde et al. 2019) and the Sooty Bark

Disease (SBD) in sycamore maple (*Acer pseudoplatanus*) trees (Bencheva 2014; Koukol et al. 2015). The SBD is caused by the ascomycete *Cryptostroma corticale*, putatively native to eastern North America (Ellis and Everhart 1889). In Europe, it was first reported in England in 1945 (Gregory and Waller 1951) and, in continental Europe, in France in 1951, followed by sporadic records in other European countries (Wilkins 1952; Cazaubon 2012). After 2000, SBD has been more frequently reported in Europe (Bencheva 2014; Cochard et al. 2015; Koukol et al. 2015; Oliveira Longa et al. 2016). The fungus has been described as an opportunistic pathogen, which particularly develops under high summer temperatures and drought stress (Abbey 1978; Ogris et al. 2021). It seems to remain in host tissues asymptomatically (Kelnarová et al. 2017) and to invade the cambium and the phloem of affected trees (Gregory and Waller 1951) when those extreme weather conditions occur. The infection can progress slowly for several years, but extensive tissue colonisation and damage have been reported one to two years after a very warm summer weather in England (Abbey 1978). The SBD is characterised, in its early stages, by generic symptoms such as wilt, branch dieback and epicormic shoots (Gregory and Waller 1951). But, in advanced stages, bark shedding exposing the fungal black stroma with a mass of spores is a typical symptom of SBD (Gregory and Waller 1951). According to the observations made by Gregory and Waller (1951) and Abbey (1978), the main mass of spores may be discharged after a heat wave or drought episode, but the SBD could develop at low intensity in the limb of the tree before becoming acute. However, the aetiology of the disease is not fully elucidated. The interest in studying SBD arises, on the one hand, from its increasing presence in Europe and its association with climate warming. On the other hand, the spores of *C. corticale* cause hypersensitivity pneumonitis in humans (Braun et al. 2021), currently called Maple Bark Stripper Lung (WHO 2022). This human disease was previously called Maple Bark Disease (MBD) and was first described in 1932 on woodmen, foresters and mill workers in eastern North America that were in contact with logs of *Acer* species with the presence of the fungus (Emanuel et al. 1962; Plate and Schneider 1965; Braun et al. 2021). The concern for a possible increasing risk of the disease in humans as a result of a greater presence of SBD in Europe, as the One Health approach anticipates (Destoumieux-Garzón et al. 2018), calls for the need to study the actual progress of the epidemic.

The SBD spread is likely to be limited by the occurrence of drought and heat wave episodes, that promote the infection process of the introduced pathogen itself. Monitoring SBD presence therefore requires good surveillance methods that are not dependent on the identification of symptoms in the host as those occur mainly after extreme weather and in the advanced stages of the disease. The conidia of *C. corticale* have been speculated to disperse by wind (Gregory and Waller 1951). Thus, aerobiology, which studies biological particles in the air, seems an appropriate approach to monitor the disease epidemic and to detect the disease in new areas before the appearance of symptoms on local sycamore maple trees. Particularly, we aim at testing the suitability of aerobiological samples from the pollen-monitoring network existing in Europe to assess *C. corticale* presence, as they proved adequate for other forest pathogens (Aguayo et al. 2020).

The objectives of the present study are therefore: (1) to develop a real-time PCR assay for the detection of *C. corticale* spores in aerobiological samples; (2) to analyse the conditions of emergence of the SBD in Europe through the study of time-series data of SBD occurrence and climatic data from France and Switzerland; (3) to analyse the dispersion of the pathogen *C. corticale* by wind at a regional scale, and (4) to study its presence on aerobiological samples at a continental scale.

Materials and methods

Study of pathogen emergence in France and Switzerland

Time series data collection

To analyse the emergence of SBD and its potential link to climate, we analysed complete time-series data of disease occurrence in France and Switzerland during the last three decades, from 1990 to 2021 and modelled this occurrence as a function of different climatic variables. The French disease records during these three decades were obtained from the database of the French Forest Health Department (DSF, French acronym). This database contains annual records of forest health problems observed in France by a network of foresters trained for the diagnosis of abiotic, entomological or pathological damages. The Swiss data were obtained from the forest protection reports generated by the Swiss Federal Institute for Forest, Snow and Landscape Research (WSL) (Queloz et al. 2020). The records in this database are based on the specific symptoms of the disease and diagnostic in the respective laboratories when symptoms are not conclusive. To account for potential sampling bias in our database (i.e. different monitoring intensities across time and regions), we standardised our data following a procedure commonly-used in medical epidemiology (Lawson 2001), that has also been applied in forest pathology (Fabre et al. 2012). Briefly, we computed a record rate $RR_{ij} = NSBD_{ij}/NRef_{ij}$ where $NSBD_{ij}$ and $NRef_{ij}$ are, respectively, the number of SBD cases and the number of other reported health problems concerning sycamore maple other than SBD, for year *i* and country *j*. The $NSBD_{ij}$ is used here as a proxy for both the observation pressure and the density of the host which cannot be separated in the dataset. This report rate (RR_{ij}) was then standardised by dividing by the report rate over the entire data set, i.e. including all years (Eq. 1).

$$SRR_{ij} = \frac{NSBD_{ij}}{NRef_{ij}} \cdot \frac{NRef}{NSBD} \qquad \text{Eq. 1}$$

where *NSBD* and *NRef* are, respectively, the total number of SBD and reference cases for the entire data set. Thus, a value of X for SRR_{ij} means that the report rate is X times the average report rate. Therefore, we assumed that a SRR_{ij} higher than 1 was an outbreak of the disease, as the reported number of cases exceeds the the basal level of the disease (considered to be the global average of our database, i.e. *NSBD*/*NRef*). The distribution of the SBD records in France and Switzerland are shown in Suppl. material 1.

The climatic data were obtained from Météo-France (SAFRAN database) computed on a daily basis on an 8-km resolution grid throughout France and Switzerland (except for the Tessin region, where these data were not available) (Suppl. material 1). We selected all points of the climate grid close to each SBD record made in the 1989–2021 period. We computed eight variables, related to high summer temperature and drought (Ogris et al. 2021) and to limiting winter conditions, to be used as predictors of the SBD occurrence: the average daily maximal temperature in summer (July-August, $T_{Xsummer}$), in spring (April-June, $T_{Xspring}$) and in the vegetative season (April-August, T_{Xveg}), the water balance calculated as the sum of the daily difference between rainfall (P) and Penman-Monteith evapotranspiration (ETP) in summer (July-August, $P\text{-}ETP_{summer}$), in spring (April-June, $P\text{-}ETP_{spring}$), in the vegetative season (April-August, $P\text{-}ETP_{veg}$), the number of days in the year where the temperature exceeds 25 °C (*n25*) and the average daily minimum temperature in winter (January-March, $\mathrm{T}_{\mathrm{Nwinter}}$). We chose the threshold of 25 °C, as it has been reported as the optimal growth temperature of *Cryptostroma corticale* (Ogris et al. 2021). The eight variables were computed for one, two and three years preceding each disease record, which potentially contributed to or impeded disease development. We did not include the year when the disease was recorded, because disease records occurred throughout the year and not only after summer.

Aerobiological study: experimental design, sample collection and data sources

The samples used as starting material in our aerobiological study consisted of microscope slides with a ca. 48-mm portion of Melinex tape (corresponding to 24 h ± 2 h, depending on the sampling time) from Hirst-type volumetric air samplers used to monitor airborne pollen grains and fungal spores by the aerobiology networks of the involved European countries. The Hirst-type air samplers (Hirst 1952) are active vacuum-pumped suction traps with a rotating drum containing the Melinex tape covered by an adhesive solution which captures the particles present in the air (Fig. 1). Further details can be found in Lacey and West (2006) and in European Norm EN 16868:2019. The samplers of the network are placed on rooftops, at least 10–15 m high. We performed DNA extractions and qPCR targeting *C. corticale* (see following sections) to assess the detectability of *C. corticale* in aerobiological samples and to quantify the spores captured during a 24-h period.

We undertook two studies, at a regional and a continental scale, to evaluate the use of permanent aerobiological networks to assess *C. corticale* epidemiological surveillance. The regional study focused on French samplers, while the continental study covered locations in six European countries over a wide latitudinal and longitudinal range: Czechia, France, Italy, Portugal, Sweden and Switzerland.

Figure 1. 7-day volumetric air sampler (Burkard Manufacturing Co Ltd, Hertfordshire, UK) in Brno (Czechia) installed on the roof of the University hospital, 15 m above ground to ensure landscape-scale monitoring. Photo credit for Aneta Lukačevičová.

Regional study

We selected samplers to cover the SBD outbreak in north-eastern France in 2017 and 2018, following a two-year-spanned drought episode (from 2017 to 2018). We selected four samplers located in Mulhouse (the main focus of the outbreak), and in three locations at different distances from the main focus (with less records of the disease): Bart, Besançon and Strasbourg (Table 1, Fig. 2). We selected four additional locations in the south of France, which were available for the year 2018, and had lower historical records of the disease (Angoulême, Aurillac, Avignon and Gap; Table 1, Fig. 2). To determine the optimal sampling period, we analysed the detectability of *C. corticale* in a temporal series of aerobiological samples from Mulhouse every three days from the 1st of May to the 30th of September of 2018. The highest frequency of spores was detected in May and June. Accordingly, the sampling period and intensity for the regional study were fixed in May-June with a three-day frequency, i.e. 10 samples per location and year.

In order to align the aerobiological data with the presence of the disease, we used the disease records from the DSF database (as described above). From 1989 to 2021, 1708 health reports were done on maples, of which 1351 were on

Table 1. Selected French air samplers for the regional study with different SBD incidence.

City	Code	GPS Coordinates	Year of the first record at < 50 km	Year of the first record at < 100 km	Year of the first record at < 180 km
Mulhouse	MUL	47.7524, 7.3591	2010	2010	1992
Bart	BAR	47.4856, 6.7694	no records	2010	1992
Besançon	BES	47.2324, 6.0231	no records	2006	1992
Strasbourg	STR	48.5833, 7.7500	no records	2010	2010
Angoûleme	ANG	45.6494, 0.1645	no records	2016	1991
Aurillac	AUR	44.9258, 2.4341	no records	no records	2014
Avignon	AVI	43.9203, 4.8021	no records	no records	2002
Gap	GAP	44.5575, 6.0761	no records	no records	2002

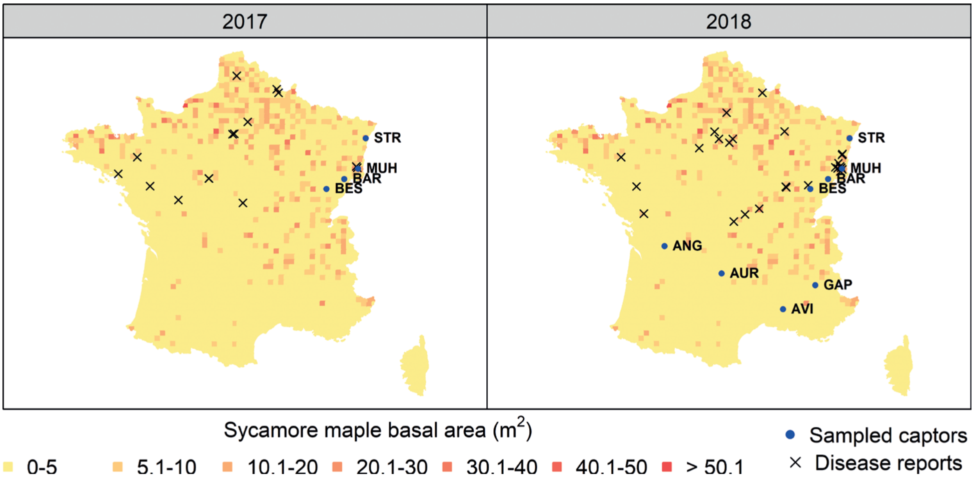

Figure 2. Selected air samplers, SBD records for years 2017 and 2018 considered for aerobiological sampling in 2017, and years 2018 and 2019 considered for 2018 sampling; and total sycamore maple basal area (m^2) in a 16×16 km grid.

A. pseudoplatanus and 172 corresponded to the SBD. We modelled the number of spores as a function of two variables: the distance to the disease and the maple basal area. We computed the distance to the closest disease record for each aerobiological sample (i.e. each captor) and year. We consider all the disease records taking place in both the year of the sampling and the following one, to capture the dispersion of the spores once the disease has been detected. Finally, we obtained host density data from the French National Forest Inventory (IFN, French acronym). We assigned to each sampler the sum of the total sycamore maple basal area in IFN plots in a radius of 50 km from each sampler, which is the reference area of influence of an aerobiological sampler (i.e. average distance at which the pollen is dispersed, Oteros et al. 2017), as a proxy for the host density. To test whether the radius at which we computed the maple basal area had an impact on its link to the number of spores, we tested a gradient of radius, from 40 to 130 km (by 10 km). Even though *C. corticale* can infect *Acer* spp. other than *A. pseudoplatanus*, such as *A. platanoides* and *A. campestre*, we only considered the latter in our study. Based on the French database, from all the SBD records from 1989 to 2021, *A. pseudoplatanus* is the main host (97.6% of cases).

Continental study

We selected a total of 12 air samplers across six European countries, spanning a large longitudinal and latitudinal range, in the axis north-south from Sweden to Portugal, and in the axis west-east from Portugal to Czechia (Table 2). The European samples were available every 12 days from the 1st of June to the 30th of September of 2018 (N = 10, per site), except for the French location of Gap, for which only June and July were available (N = 5).

Table 2. Locations of European air samplers for aerobiological samples analysed during the period from the 3rd of June to the 25th of September 2018, every 12 days (N = 10).

City	Code	GPS Coordinates	Country	Year first SBD record	Laboratory for DNA extraction
Brno	BRN	49.20374, 16.61800	Czechia	2005[1]	Mendel University (Czechia)
Gap	GAP	44.55750, 6.07610	France	1950[2]	INRAE Bordeaux (France)
Pontivy	PON	48.06670, -2.96830	France		INRAE Bordeaux (France)
Besançon	BES	47.23241, 6.02311	France		INRAE Bordeaux (France)
Bordeaux	BOR	44.80670, -0.58960	France		INRAE Bordeaux (France)
Bologna	BOL	44.49120, 11.36910	Italy	1952[3]	IPSP-CNR (Italy)
Perugia	PER	43.10091, 12.39593	Italy		IPSP-CNR (Italy)
Gävle	GÄV	60.67959, 17.14330	Sweden	Not reported	SLU (Sweden)
Visby	VIS	57.67336, 18.29269	Sweden		SLU (Sweden)
Lisbon	LIS	38.823718, -9.176685	Portugal	Not reported	SLU (Sweden)
Münsterlingen	MÜN	47.63040, 9.23679	Switzerland	1991[4]	WSL (Switzerland)
Payerne	PAY	46.81158, 6.94247	Switzerland		WSL (Switzerland)

[1] Koukol et al. 2015; [2] Cazaubon 2012; [3] Wilkins 1952; [4] Queloz et al. 2020.

Molecular detection of *C. corticale* in aerobiological samples

DNA extraction of aerobiological samples

Slides for the regional study were extracted in the laboratory of Forest Pathology at INRAE Nancy (France). For the continental study, the slides were extracted in different laboratories (Table 2), following the same procedure across studies and laboratories. Samples were processed according to the protocol by Aguayo et al. (2020). Briefly, mounted microscope slides were placed flat for 5–15 minutes on a constant heater set at 65 °C in order to unstick the glass cover slip. The sticky tape was recovered with laboratory forceps and cut into small pieces with sterile scissors. The tape pieces were then placed into tubes with screw caps containing one 3-mm sterile tungsten bead and 20 2-mm glass beads, and filled with 400 µl of AP1 buffer and 4 µl of RNase A (both from the DNeasy plant minikit Qiagen, Hilden, Germany). This mix was ground twice for 60 s (with a short cooling break) using a high-speed homogeniser, such as FastPrep 24 (MP Biomedicals) set at 6 m s^{-1} (INRAE Nancy, SLU Sweden and WSL Switzerland), Geno-Grinder (SPEX) with vertical shaking at 1500 rpm (INRAE Bordeaux), Mixer Mill MM400 (Retsch, Haan, Germany) set at 30Hz (Mendel University Czechia), and Mixer Mill 300 (Qiagen) (IPSP-CNR Italy). The genomic DNA from samples was then extracted with the Qiagen DNeasy plant minikit, following the manufacturers' specifications with a final DNA elution of 50 µl. Two types of negative controls were included in the extraction process. One control consisted of one vial left open while performing the slide preparation (one per day of extraction). Another one consisted of a negative control during the DNA extraction itself. The qPCR reactions (see next section) were performed at INRAE Nancy (France) for the regional study and at Mendel University (Czechia) for the continental study.

Development of a qPCR assay for *C. corticale*

The ITS region sequences with accurate identification were retrieved from GenBank for *C. corticale* and closely related species (*Biscogniauxia nummularia*, *B. mediterranea*, *B. latirima*, *B. philippinensis*, *Obolarina dryophila*, *Graphostroma platystoma*) to assure the specificity of the test. We also included, in the panel of species to be tested, species that are commonly found in *Acer* species, such as *Alternaria alternata*. Details of the included isolates are given in Suppl. material 2. We aligned the sequences using MUSCLE (Edgar 2004) implemented in Geneious V.R9 (https://www.geneious.com). The alignment was used to generate a series of couples of species-specific PCR primers and probes using Primer3 and Geneious. We evaluated melting temperatures and potential secondary structures in silico. Primer sequences were also checked for sequence homology with other DNA sequences by performing a BLAST search in GenBank. Further, we validated the specificity and inclusivity of the selected primers and probe by conventional PCR performed in a number of DNA extracts of *C. corticale* and non-target species (Suppl. material 2). The qPCR reactions were performed with a QuantStudio 6 (Applied Biosystems, Carlsbad, USA) in 20 µl volumes containing 10 µl 1X Brilliant II QPCR Master Mix (Agilent Technologies), 0.3 µM of each primer, 0.1 µM probe, 0.01 Uracil DNA Glycosylase (UDG) U/µl, 30 nM reference dye, 2 µl volume of DNA template, and PCR-grade water (up to 20 µl total volume). Thermal cycling conditions consisted of a UDG activation phase at 37 °C for 10 min, polymerase activation at 95 °C for 10 min, followed by 40 cycles of 10 s at 95 °C and 45 s at 62 °C. The limit of detection (LOD) was achieved by qPCR amplifications with ten-fold dilutions of DNA extracted from *C. corticale* mycelium following the same protocol like for other samples in this study. Extracted DNA was quantified with NanoDrop ONE (Ozyme). Serial dilutions from 1 ng/µl to 1 fg/µl of DNA per sample were tested indicating the lowest concentration as the LOD yielding systematic Cq values.

C. corticale detection in aerobiological samples

Samples were run in triplicate in the regional study and in duplicate in the continental study, and both a negative (no template DNA) and a positive control (*C. corticale* mycelium DNA extract) were included in all series of reactions. Previous experience using spore traps has shown that qPCR Cq values can be below the detection limit of the assays, which means that the pathogen is present in the samples, but not at quantifiable concentrations (cf. Grosdidier et al. 2017; Aguayo et al. 2018). In case of three replicates, a sample was considered positive when at least two out of the three replicates yielded a cycle threshold value (with no upper limit, cf. Grosdidier et al. 2017). In two-replicate runs, if one of the two replicates was negative, another two-replicate reaction was performed. If either the same result was achieved or the two replicates were positive, the sample was considered positive. Otherwise, the sample was considered negative.

To quantify the spores on each aerobiological sample, we prepared 5-fold serial dilutions of a spore solution obtained by adding purified water on the surface of a sporulating culture of a French *C. corticale* isolate, LSVM1510. Spore concentration was determined using a haemocytometer. We performed DNA extractions from of each of the five spore solutions spanning from 1144 to 2 spores/µl. We ran qPCR for the five DNA extracts in triplicate to obtain a standard curve. As both the initial volume and the final elution volume of the DNA extraction was 50 µl, to obtain the number of spores corresponding to each Cq, we multiplied the initial spore concentration per 2 µl used in the qPCR reaction. We then fitted a linear model with cycle threshold (Cq) as a function of the logarithm of the number of spores ($P < 0.0001$; $R^2 = 0.95$; Cq = 37.0–1.2 log(number of spores/µl)). The same DNA extractions for spore quantification were used to perform two different standard curves at the Forest Pathology laboratory at INRAE Nancy (France) for the regional study and at the Mendel University (Czechia) for the continental study, where the respective qPCR assays of the samples were performed.

Data analysis

Pathogen emergence study

We have fitted Bayesian models to test the different hypotheses as follows. To analyse the effect of the climatic conditions on the emergence of the SBD, we modelled the *SRR* as a function of $T_{X,summer}$, $P\text{-}ETP_{summer}$, and $P\text{-}ETP_{veg}$ that were calculated for one, two and three previous years (see section of data sources). We ran individual models due to the high collinearity between temperature and water balance. We then chose the model with lower deviance (comparing the 95% confidence interval of the deviance). The *SRR* followed a Poisson distribution (Eq. 2). We included a binomial process (Eq. 3) to account for zeros that arise in addition to those modelled by the Poisson process (i.e. failure to detect the disease in the field). Therefore, the model distinguished two potentially different processes that determine the occurrence of SBD: (1) the occurrence of conducive weather conditions so that the pathogen can develop and cause a number of disease cases, as a Poisson process, and (2) the detectability of the disease in the field which may depend on other factors such as the presence of inoculum (arrival of the exotic pathogen), as a binomial process. We compared models with and without the binomial process and chose the one with the lowest Deviance Information Criterion (DIC). Following Eq. 1 for the standardisation of the SBD records, and isolating the $NSBD_{ij}$, which is our response variable, we included the fraction $\frac{NRef}{NRef_{ij} \cdot NSBD}$, as an offset term in the deterministic equation of the model (Eq. 4).

$$\text{number of cases of SBD} \sim \text{Poisson}\ (\lambda_k * d_k) \qquad \text{Eq. 2}$$

where *k* is the observation at a given sampler and date, λ_k is the number of spores, and d_k is the detectability of the disease, which follows a Bernoulli distribution (Eq. 3).

$$d_k \sim \text{Bernoulli}\ (p) \qquad \text{Eq. 3}$$

$$\log(\lambda_k) = alpha_k + beta * predictor_i + \log\left(\frac{NRef}{NRef_{ij} \cdot NSBD}\right) \qquad \text{Eq. 4}$$

where *alpha* is the intercept which varies for each year, *j* is the year, *beta* is the parameter estimate for the *predictor*, which can be any of the variables (cf. to the section 'Time series data collection').

Aerobiological study

We modelled the number of spores detected per week as a function of the distance to the closer disease report (model distance) and as a function of the total sycamore maple basal area in a radius of 50 km from the sampler (model host). We did not include the distance to the disease report and the total sycamore maple basal area as predictors in the same model because their high collinearity prevented model convergence. The two models followed a Poisson distribution (Eq. 5), with lambda varying for each observation following a Gamma distribution to deal with overdispersion (Eq. 6–8). We included a binomial process (Eq. 9) to account for zeros that arise in addition to those modelled by the Poisson process (i.e. sampler's failure to capture spores even if they are present in the air). Therefore, the model distinguished two potentially different processes that determine the number of *C. corticale* spores in the air: (1) the sampler's efficacy to capture spores, as a binomial process, and (2) the number of spores, as a Poisson process. Finally, we compared models with and without the binomial process and chose the one with the lowest Deviance Information Criterion (DIC). The number of samples per week (from 1 to 4) was added as an offset of the Poisson model (Eq. 10). In both cases, the best models were the ones including the binomial process, hence our data was zero-inflated.

$$\text{number of spores} \sim \text{Poisson}\ (\lambda_k * e_k) \qquad \text{Eq. 5}$$

where *k* is the observation at a given sampler and date, λ_k is the number of spores, and e_k is the efficacy of the sampler (probability of capturing any spores), which follows a Bernoulli distribution (Eq. 6):

$$\lambda_k \sim \text{Gamma}\ (a_k, b_k) \qquad \text{Eq. 6}$$

where a_k and b_i are the shape and rate of the Gamma distribution, which relate to the mean number of spores and to the standard deviation (sigma) as follows (Eq. 4–5):

$$a_k = spores_k\text{^}2\ /\ sigma\text{^}2 \qquad \text{Eq. 7}$$

$$b_k = spores_k\ /\ sigma\text{^}2 \qquad \text{Eq. 8}$$

$$e_k \sim \text{Bernoulli}\ (p) \qquad \text{Eq. 9}$$

$$\log(spores_k) = alpha + beta * predictor_k + \log(Nsam_k) \qquad \text{Eq. 10}$$

where *alpha* is the intercept, *beta* is the parameter estimate for the *predictor*, which can be either the distance to the disease report (model distance) or the total sycamore maple basal area (model host), *Nsam* is the total number of samples analysed per week (offset term).

We modelled the probability of disease occurrence in a certain area of influence of the sampler (in a circumference of different radii, from 40 to 130 km of radius, by 10-km intervals) as a function of the number of detected spores. The two models followed a Bernoulli distribution (Eq. 10). The deterministic part of the model is shown in Eq. 11.

$$\text{Probability of disease occurrence in an area of 40 to 130 km radius} \sim \text{Bernoulli}\ (p_k) \qquad \text{Eq. 11}$$

where *k* is the observation at a given sampler and date, and p_i is the presence-absence of the disease at the given distance (40 to 130 km) from the sampler.

$$\log(p_k) = alpha + beta * spores_k \qquad \text{Eq. 12}$$

where *alpha* and *beta* are the parameters estimated by the model, and *spores* is the number of spores detected by the sampler.

To validate our models, we simulated data based on the likelihood of each model. We then compared the means, the coefficients of variation and the sums of squares of the residuals of the original dataset with each simulated dataset. The histogram of the differences for each statistic should be zero-centred, with the proportion of negative (or positive) differences being lower than 0.85 for the model to be accepted.

All Bayesian models were implemented using a Markov chain Monte Carlo (MCMC) sampler (JAGS, Just Another Gibbs Sampler; Plummer 2003) called from R (function jags.fit, package R2jags, Su and Yajima 2021). All models were fitted using three chains, 100 000 iterations with a 10 000 burn-in and noninformative priors. Chains were checked for convergence using the Gelman and Rubin diagnostic and the 95% credible intervals of the parameters and predictions were directly extracted from the estimated posterior distributions of the model (Rhat diagnostic). We simulated data for all the models following the corresponding distribution. We analysed the residuals of the simulated data and the predicted values of the model. The model was considered accurate if the residuals were zero-centred.

Results

qPCR assay for *C. corticale*

The selected primers and probe used in this study were ccITS2F (AGGTTGTGCTGTCCGGTG), reported in the study by Kelnarová et al. (2017), and the new reverse primer and probe developed here: SBD3R (AGCTCCTACCAACTACAGGGT) and SBD5P (FAM-ACCCTGTAGGAGGAGCTACCCTGTA-BHQ1), respectively. The LOD was fixed at 0.01 pg/µl (Cq 35.9 ± 0.2) in DNA extracts from mycelium samples (see Suppl. material 3). The detection of spores with our test ranged from 2 to 1144 spores/µl.

Pathogen emergence in France and Switzerland

The climatic variable best explaining the standardised SBD report rate was the water balance (*P-ETP*) in the vegetative season (April-August) of the year preceding the disease report (Table 3). Other models that yielded low deviance were the water balance in the summer of the year preceding disease, the mean number of days with temperature exceeding 25 °C of the two previous years of disease record, and the water balance in the spring of the year preceding disease (Table 3). We found that *SRR* was predicted to exceed 1 when at least 33 days per year (95% CI 29, 36) had a temperature higher than 25 °C during the two years preceding disease. The distribution of the residuals of the best model (water balance in the vegetative season) can be found in Suppl. material 4.

Table 3. Coefficient estimates for each climatic variable and their 95% credible intervals in brackets for models predicting the standardised SBD case rate per year. Estimates are generated from the posterior distributions of the variables in the Poisson model (Eq. 11). Each climatic variable is calculated for either the previous year (n-1), two (n-1 to n-2) or three (n-1 to n-3) previous years. Rhat is the potential scale reduction factor and indicates whether the model has converged. Successful convergence is reached when Rhat values are < 1.1. T_X: average daily maximal temperature; T_N: average daily minimal temperature; *P-ETP*: Water balance as the sum of the daily difference between rainfall and Penman-Monteith evapotranspiration; n25: number of days per year where the temperature exceeds 25 °C; summer: July-August; spring: April-June; winter: January-March; veg: April-August.

Variable	**Years**	**Coefficient estimate [95% CI]**	**Rhat**	**Deviance [95% CI]**
$T_{X,summer}$	n-1	1.19 [0.74, 1.68]	1.0012	209.1 [193.2, 228.3]
$T_{X,spring}$		1.21 [0.77, 1.70]	1.0009	199.1 [183.7, 217.9]
T_{Xveg}		1.15 [0.78, 1.53]	1.0009	201.0 [185.9, 218.8]
$T_{Nwinter}$		0.66 [0.43, 0.89]	1.0009	199.0 [183.9, 217.1]
$P\text{-}ETP_{summer}$		-1.08 [-1.38, -0.78]	1.0009	188.3 [172.7, 207.5]
$P\text{-}ETP_{veg}$		**-1.15 [-1.46, -0.85]**	**1.0009**	**183.6 [169.3, 200.5]**
$P\text{-}ETP_{spring}$		-1.35 [-1.85, -0.89]	1.0009	192.2 [178.1, 209.5]
n25		1.16 [0.78, 1.58]	1.0009	208.0 [193.2, 227.1]
$T_{X,summer}$	n-1 to n-2	1.37 [0.86, 1.98]	1.0009	197.9 [182.0, 217.9]
$T_{X,spring}$		1.23 [0.77, 1.71]	1.0013	203.0 [187.8, 221.5]
T_{Xveg}		1.25 [0.87, 1.66]	1.0009	196.1 [181.0, 214.3]
$T_{Nwinter}$		0.64 [0.41, 0.87]	1.0009	202.8 [187.9, 221.0]
$P\text{-}ETP_{summer}$		-1.08 [-1.50, -0.70]	1.0009	200.0 [185.8, 218.1]
$P\text{-}ETP_{veg}$		-1.07 [-1.50, -0.68]	1.0001	208.6 [193.7, 227.1]
$P\text{-}ETP_{spring}$		-0.74 [-1.26, -0.27]	1.0009	227.0 [212.3, 244.8]
n25		1.40 [0.94, 1.90]	1.0010	191.0 [175.5, 210.7]
$T_{X,summer}$	n-1 to n-3	0.83 [0.32, 1.43]	1.0009	222.2 [206.5, 241.2]
$T_{X,spring}$		1.21 [0.78, 1.65]	1.0009	201.0 [186.6, 218.9]
T_{Xveg}		1.09 [0.71, 1.49]	1.0009	205.8 [191.6, 222.8]
$T_{Nwinter}$		0.72 [0.49, 0.95]	1.0009	201.5 [187.1, 218.6]
$P\text{-}ETP_{summer}$		-0.61 [-1.10, -0.16]	1.0009	227.7 [212.5, 246.5]
$P\text{-}ETP_{veg}$		-0.50 [-0.85, -0.16]	1.0009	225.9 [211.7, 244.6]
$P\text{-}ETP_{spring}$		-0.42 [-0.80, -0.06]	1.0009	227.4 [214.2, 244.9]
n25		1.16 [0.57, 1.88]	1.0009	211.3 [194.8, 231.4]

The number of SBD cases increased exponentially with more negative water balance (Fig. 3a). On average, the model predicted a standardised SBD report rate higher than 1 (i.e. SBD occurrence higher than average) for total water balance in spring and summer lower than -132 mm (95% CI -170, -93, Fig. 3a), which qualifies a mild drought (extreme drought events taking place around -300 and -400 mm of *P-ETP*, Candel-Pérez et al. 2012). The number of SBD cases in France was on average higher than in Switzerland, corresponding to more negative accumulated water deficit during the vegetative season (Fig. 3b). SBD peaks in France paralleled those in Switzerland, with a marked increase from 2018 to 2021. Even though drought peaks in both countries did not appear to increase in magnitude across the years, they did

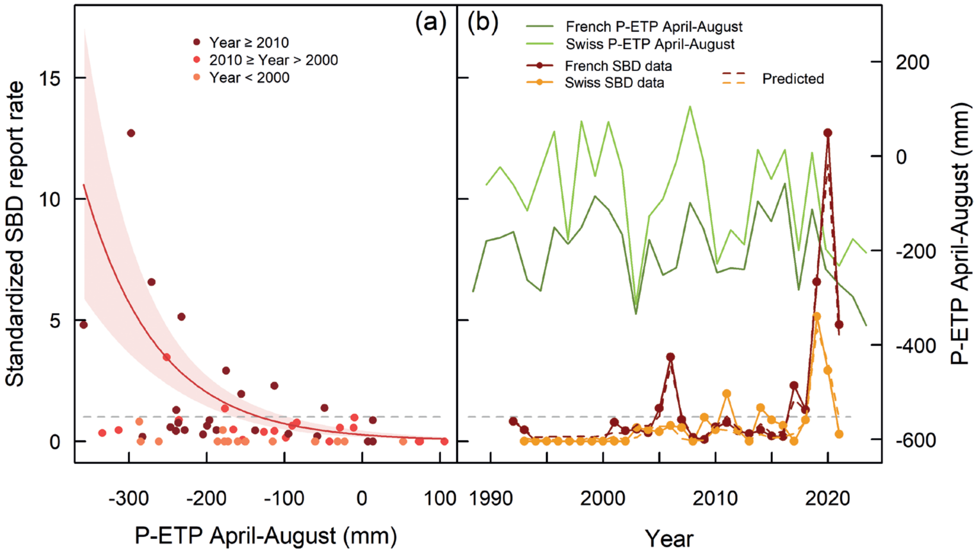

Figure 3. Model prediction of standardised SBD report rate as a function of water balance (measured as *P-ETP*) of the vegetative season (April-August) of the year previous to the disease report (**a**). Evolution of standardised SBD reports from 1990 to 2021 in France and Switzerland (**b**), and model predictions (Eq. 2). A dotted grey line indicates a standardised record rate that equals 1, above which the number of cases of the SBD is higher than average, and hence considered an outbreak of the disease.

tend to be more frequent after 2005 (Fig. 3b). No annual point earlier than 2005 exceeded the average report rate (*SRR* above 1, Fig. 3b). According to our model, the probability of disease absence not being linked to the water balance of the vegetative season (i.e. Bernoulli process, Eq. 3) was 0.05 (95% CI 0, 0.19). This zero-inflation, not explained by the water deficit, was mostly observed during the first decade of the time-series (Fig. 3).

Regional study

The number of spores detected per week was more abundant in samplers closer to disease reports (Fig. 4a). However, the magnitude of the increase was not large, and the coefficient estimate for the variable distance was not significant (i.e. the 95% CI contained the 0; Table 4). Our model predicted a detection of 27 spores per week (two sampling days per week) (95% CI 16, 40) at 10 km from the closest disease report, while 10 spores (95% CI 2, 25) were detected at a distance of 300 km (Fig. 4a). An increasing number of spores were detected in areas with a higher sycamore maple density (Fig. 4b). Even in areas with no sycamore maple in a radius of 50 km, the aerobiological samples presented *C. corticale* DNA, up to 20 spores. The coefficient estimate for the total sycamore maple area was positive on average, but not significant

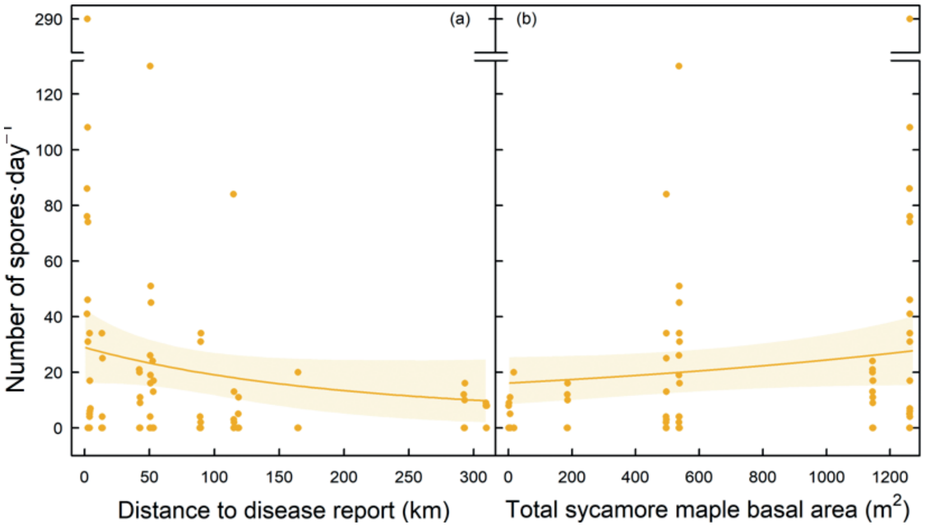

Figure 4. Number of spores per day as a function of the distance to the closest disease report (**a**), and as a function of the total maple basal area (m^2) in a radius of 50 km from the sampler (**b**).

(Table 4). The distribution of the residuals of the best models (distance to the closest disease report and total maple basal area in a 50-km radius from the sampler) can be found in Suppl. materials 4, 5, respectively. The other radius tested for the computation of the maple basal area showed the same deviance, hence no other radius value led to an estimation of maple basal area that better explained the presence of spores in the air (Suppl. material 7).

The two models (distance to the disease, and maple basal area) estimated a similar probability of spore capture (0.73 and 0.72, respectively, Bernoulli process in Eq. 8, Table 4). Therefore, 27% (or 28%) of the lack of spore detection (zero inflation) was due to a process other than the distance to the disease report (or the host density), and hence not explained by our deterministic model.

The probability of disease occurrence increased with the number of detected spores at a given distance (Fig. 5, Suppl. material 8). A 95% probability of disease in a radius of 60 km and 120 km corresponded to 41 and 127 spores detected, respectively. How-

Table 4. Parameter estimates and their 95% credible intervals in brackets for models describing spore detection as a function of distance to SBD reports and host density. Estimates are generated from the posterior distributions of the variables in the Poisson models (Eq. 6) with the variable response number of spores per week.

Response variable	Detected spores per week		
Parameter	**Intercept estimate**	**Coefficient estimate**	**Probability of spore capture**
Distance to disease report	2.64 [2.07, 3.07]	-0.41 [-0.97, 0.06]	0.73 [0.61, 0.85]
Total maple basal area	2.04 [1.41, 2.55]	0.04 [-0.01, 0.10]	0.72 [0.60, 0.85]

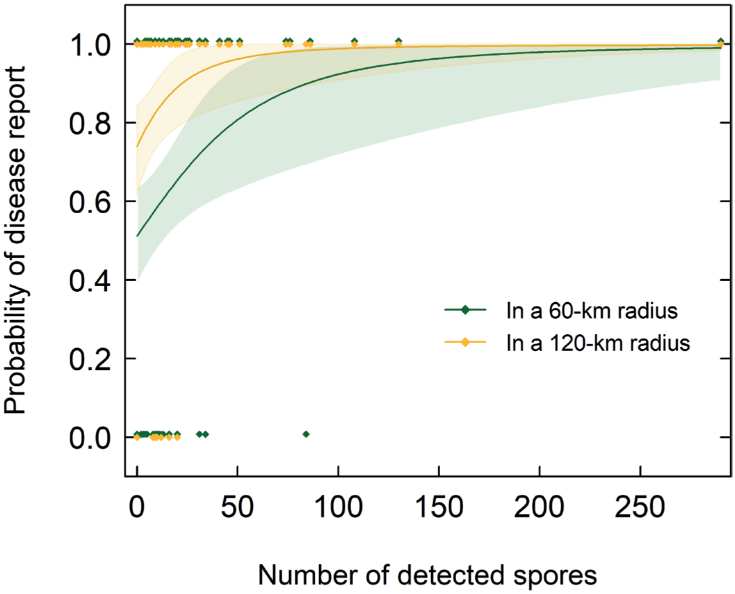

Figure 5. Probability of disease report in an area of 60-km and 120-km radius from the sampler as a function of the number of detected spores per day.

ever, the lack of spore detection did not correspond to the absence of disease reports. On the contrary, the disease could be detected in the field without being detected in aerobiological samples with two sampling days per week.

Continental study

The proportion of positive aerobiological samples based on the quantitative species-specific *C. corticale* PCR assay per year in Europe followed the reported presence of the disease in Europe (Fig. 6). Further, the regions that yielded more positive samples corresponded to those of the native range of sycamore maple, and hence with higher potential to maintain a sustained *C. corticale* population (samplers BES, PAY, MÜN and BRN, Fig. 6). Sweden and Portugal, the two countries where the disease had not been reported yet, did not present any positive samples (Figs 6, 7c, d). The highest proportions of positive samples were detected in France, Switzerland and Czechia (Figs 6, 7). Spores tended to be detected earlier in western than eastern locations. French samplers that produced positive samples, Bordeaux and Besançon, peaked spore detection in June and July, respectively (Fig. 7a). Payerne, in Switzerland, also presented a peak in July (Fig. 7b). Swiss location Münsterlingen and the Italian Bologna, presented higher *C. corticale* detection in August (Fig. 7b, e). Finally, the easternmost location in Czechia, Brno, peaked in September (Fig. 7f).

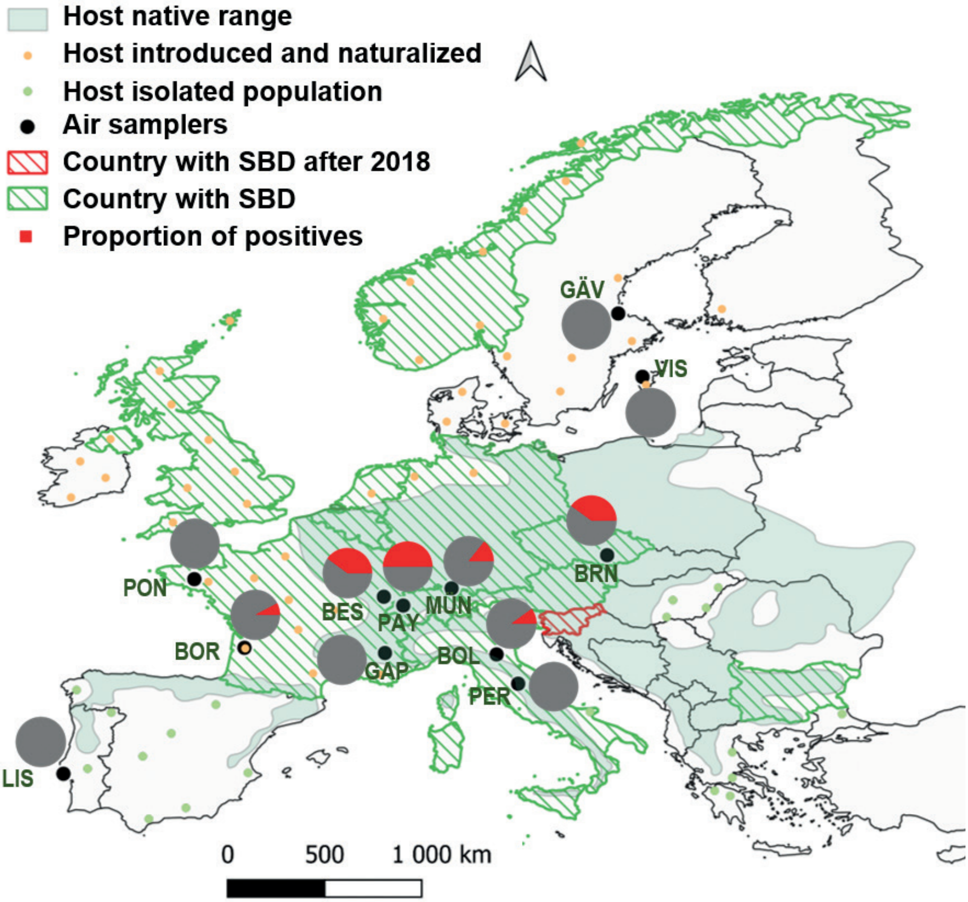

Figure 6. Proportion of aerobiological samples that tested positive from May to September of 2018 (n = 10, except for Gap where n = 5) in the different European samplers following the natural distribution of the host *Acer pseudoplatanus*.

Discussion

The present study aimed at analysing the emergence of SBD in Europe through the frequency of spore detection in aerobiological samples and time-series of disease records. Our results show that the SBD disease is at an exponentially increasing phase in France and Switzerland with an increase in the magnitude of the number of disease cases that peaks following a marked water deficit. Those episodic disease peaks do not show a deceleration, but they continue to increase in magnitude -the last peak is far higher than the precedent one (Fig. 3b)-, and they may continue to increase as drought events do. The spread of SBD has not occurred continuously, as in other invasive diseases like the ash dieback disease (Gross et al. 2014), due to the irregular, low-frequent nature of very hot and dry conditions needed for pathogen development (Ogris et al. 2021). Time-series data of SBD in France and Switzerland from 1990 to 2021 showed a series

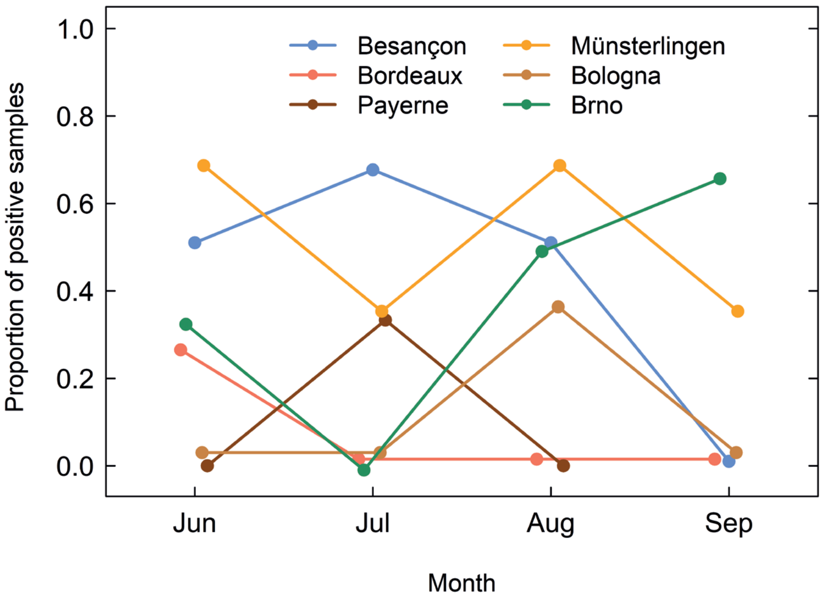

Figure 7. Phenology of spore emission of *Cryptostroma corticale* in the studied European countries showing the proportion of positive aerobiological samples per year. Jun: June; Jul: July; Aug: August; Sep: September.

of disease peaks coinciding with low spring and summer water balance and high temperatures. Our data show that drought favours pathogen development, as previously reported in experimental conditions (Ogris et al. 2021). However, as shown by our model using the number of days exceeding 25 °C, recorded disease outbreaks are also associated with high temperatures in the two previous years. Summer temperatures seem to control the internal spread of the fungus, with 25 °C as the optimal growth temperature in vitro (Abbey 1978; Ogris et al. 2021). We found this relationship in one of our best models. Outbreaks of the disease are associated with the occurrence of more than 33 days (95% CI 29, 36) with a temperature higher than 25 °C per year during the two years preceding the disease.

Temporal dynamic of the SBD

Disease peaks increased exponentially in magnitude with time. *C. corticale* is an invasive pathogen, reported for the first time in continental Europe in 1952, in France. In Switzerland, the first known report was in 1991. The highest peaks of the standardized number of disease records in 2020 in both countries suggest that the pathogen, after several outbreaks of the disease, might have colonized more forest plots, where the disease was eventually able to develop after conducive weather conditions. Two processes may have then taken place that explain the exponential increase in the last decades in France and Switzerland: (1) the dispersion and the establishment of the exotic pathogen, and (2) an increasing frequency of low water balance and high temperatures which are conducive conditions for the SBD disease. The relative contribution of the two processes in the SBD emergence cannot be fully disentangled from our model. However, they are likely to have occurred additively, as the main dispersion events of the pathogen are highly dependent on drought conditions. Hence, the increased frequency of drought events and heat peaks might have led to higher dispersion rates. The estimation of the zero-inflation at 5% in our climate model suggests that the disease is mainly climate-driven (i.e. only 5% of disease absence is not explained by high water deficit in the vegetative season). Before 2005, there was no *SRR* higher than one, which implies an outbreak of the SBD (higher recording of SBD compared to average). The lack of SBD reports in early years may be due to the absence of inoculum (early phases of the invasive process) and the less frequent conducive conditions to the SBD. However, the lack of awareness of the disease by surveillance agents and hence little attention to symptoms in the field might have also resulted in fewer reports. Although the disease is detectable some months before sporulating lesions develop on the trunk (sooty appearance), those early symptoms are not specific to the SBD: wilting of leaves, presence of stool shoots and branch dieback (Gregory and Waller 1951). This means the disease is usually reported at its later stage. Further, the apparent similarity of dark brown stroma of *C. corticale* to black stroma of the common saprophytic fungus *Eutypa maura* (Fr.) Sacc. (Saccardo 1882) may be another reason for misidentification. Finally, the SBD is commonly found in urban environments, which are recognized to be an entry pathway for exotic pathogens (Tubby and Webber 2010; Paap et al. 2017), and hence expected to be, in proportion, more frequently found in urban areas than natural ecosystems in the early phases of pathogen invasion. Urban environments are, in addition, known to be heat islands with warmer and drier conditions than forests. These two reasons could thus also explain why early cases of the disease, being more frequent in urban areas, were poorly reflected in our time-series data which mostly included forested areas. In any case, the SBD occurrence rate in this study focuses on its emergence in forests, giving a temporal frame of the development of an invasive disease linked to climate extremes.

Aerobiology of *C. corticale*

The presence of *C. corticale* in aerobiological samples paralleled the presence of the disease SBD in Europe. At the continental level, monitoring of aerobiological samples shows a great potential as a large-scale epidemio-surveillance method for the SBD in Europe. Especially, early aerial detection of *C. corticale* in disease-free countries, such as Portugal and Sweden, could help implement special measures for SBD detection and eradication in the field. The advantage of aerobiological monitoring is that the aerobiological networks are already established and samples can be potentially obtained periodically. This method has already been proved for other forest pathogens such as *Hymenoscyphus fraxineus*, *Heterobasidion annosum* s.l., *Erysiphe alphitoides*, and *Melampsora larici-populina* (Aguayo et al. 2020). The anamorphic *C. corticale* produces conidia emerging from conidiophores in the stroma formed between the inner and outer bark. Even though the release mechanism of conidia is still unknown, it has been suggested that, when the bark peels off, the spore mass of *C. corticale* is exposed and dispersed by wind (Gregory and Waller 1951; Bencheva 2014; Oliveira Longa et al. 2016). The number of spores has been reported to be approximately 30 to 179 million per square cm of black sooty layer (Abbey 1978). Our results suggest that the pathogen is effectively dispersed by wind. However, a few points should be considered if aerobiological surveillance is to be implemented for the SBD. First, our models estimated the detectability of the pathogen with a probability of 72%. This implies that in 28% of the cases, the pathogen's spores may be present in the air but either not captured by the air samplers, or not detected by qPCR. This explains why our model of the probability of disease presence predicts SBD occurrence with a probability of 50% in a 60-km-radius area even when no spores are detected by the samplers. Second, spore capture efficiency may be improved by increasing the spatial resolution of the selected air samplers or by considering the movement of air masses in the selection of locations. Third, the sampling period can impact the probability of detection. In the regional study, we sampled every three days in May and June. This period could be either intensified or prolonged during additional months. The results of the European sampling show that the sporulating period may be larger than we expected based on the Mulhouse captor we had analysed before this study (not shown). A whole-year period would also be informative, in future research, to evaluate fluctuations in spore release. Finally, our aerobiological data consists of two years in the regional study and one year in the continental study, in both cases with conducive climatic conditions. A longer time-series aerobiological data would allow for assessing whether inoculum production is detectable outside the period of outbreaks.

Distance tended to decrease the number of detected spores, but the magnitude of the effect was low and it was not significant (the confidence interval of parameter estimates contained 0). We detected *C. corticale* spores as far as 310 km from the closest disease report. This result suggests that the fungus can disperse long distances by wind. However, we cannot rule out the possibility of underreporting with unobserved SBD occurring closer to the samplers in urban settings. It is reasonable to assume that the main SBD foci in forests were registered in our database from 2017–2018 onwards, as the disease was well known at that time by the surveillance agents. But, outbreaks in parks or along roads may not have been as comprehensively included in our database. We did not sample locations at distances farther than 310 km. Therefore, we cannot establish the limit of aerial dispersal of the fungus. Other wind-borne pathogens have shorter dispersal distances, such as *H. fraxineus*, the causal agent of the ash dieback disease, whose spores can be detected up to 50–100 km from the disease front (Grosdidier et al. 2018). Our results support the idea that *C. corticale* is an efficiently dispersed pathogen. However, the high spore detection could have been remarkably favoured by the fact that the sampled years were affected by heat waves and the number of SBD cases was inherently high. Extended time-series aerobiological analyses are needed to further understand the epidemiology and dispersion pattern of the SBD.

The relatively abundant number of spores of *C. corticale* detected in the surveyed air samplers, placed in cities, reveals a potential risk to human health. The spores of *C. corticale* cause the MBD (Emanuel et al. 1962), currently called Maple Bark Stripper Lung (WHO 2022), a hypersensitivity pneumonitis, allergic asthma, flu-like infections and interstitial pneumonia (Braun et al. 2021). With drought predicted to increase in the Mediterranean basin and western and central Europe in the following decades (IPCC 2021), a further expansion and intensification of the SBD can be expected. Thus, the risks to human health and the environment are intertwined, as the One Health approach states. Surveillance of spore levels in the air is crucial to assess disease risk. We have presented here a suitable methodology, including the use of aerobiological samples to monitor the evolution of the SBD, that can also provide data to assess the potential risk of MBD to humans. Assessing SBD epidemiology at a continental scale implies access to harmonized databases of both pathogen occurrence and host density. We seemingly succeeded in our joint effort to homogenise molecular protocols to reduce the bias in molecular detection of *C. corticale* in aerobiological samples. However, there is a need to homogenise data from forest national inventories and disease reporting at the European level. That would allow models including larger geographical areas, which would provide a better understanding of disease dynamics.

Acknowledgements

This project has received funding from the European Union's Horizon 2020 Programme for Research & Innovation under grant agreement No 771271 (HOMED project, "HOlistic Management of Emergent forest pests and Diseases"), and from the research project SIAMOIS ("Smart and Innovative Monitoring Of airborne fungal Invaders by molecular methods") by the French laboratory of excellence LabEx ARBRE. We greatly appreciate the valuable assistance provided by Etienne Brejon-Lamartiniere, Laure Dubois, Julie Faivre d'Arcier, Manuela Branco Ferreira, Anaïs Gillet, Quirin Kupper, Aneta Lukačevičová, Miloslava Majerová, Sophie Strohecker, Fabrizio Cioldi. We thank the ARPAE Area Prevenzione Ambientale Metropolitana di Bologna, Italy, for providing part of the Italian samples analysed in this study. The mycology research unit of the ANSES Plant Health Laboratory is supported by a grant managed by the French National Research Agency as part of the French government's "Investing for the Future" (PIA) programme (ANR-11-LABX-0002-01, Laboratory of Excellence-ARBRE).

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Supplementary material 1

Records of SBD in France and Switzerland from 1990 to 2021 (red dots)

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Data type: figure (word document)

Link: https://doi.org/10.3897/neobiota.84.90549.suppl1

Supplementary material 2

Isolates which DNA was extracted and used to confirm the specificity of the primers ccITS2F and SBD3R and probe SBD5P

Authors: Elodie Muller, Miloň Dvořák, Benoit Marçais, Elsa Caeiro, Bernard Clot, Marie-Laure Desprez-Loustau, Björn Gedda, Karl Lunden, Duccio Migliorini, Gilles Oliver, Ana Paula Ramos, Daniel Rigling, Ondrej Rybnicek, Alberto Santini, Salome Schneider, Jan Stenlid, Emma Tedeschini, Jaime Aguayo, Mireia Gomez-Gallego

Data type: table (word document)

Link: https://doi.org/10.3897/neobiota.84.90549.suppl2

Supplementary material 3

Standard curve and its correlation coefficient to determine the limit of detection for the real-time PCR assay in ten-folded DNA solutions of C. corticale mycelium (a) and total number of spores in the qPCR reaction (b)

Authors: Elodie Muller, Miloň Dvořák, Benoit Marçais, Elsa Caeiro, Bernard Clot, Marie-Laure Desprez-Loustau, Björn Gedda, Karl Lunden, Duccio Migliorini, Gilles Oliver, Ana Paula Ramos, Daniel Rigling, Ondrej Rybnicek, Alberto Santini, Salome Schneider, Jan Stenlid, Emma Tedeschini, Jaime Aguayo, Mireia Gomez-Gallego

Data type: figures (word document)

Link: https://doi.org/10.3897/neobiota.84.90549.suppl3

Supplementary material 4

Zero-centred histogram of the residuals between simulated data and predictions of the model with the water balance (P-ETP) in the vegetative season (April-August) of the year preceding disease report as a predictor of the standardized record rate of the SBD

Authors: Elodie Muller, Miloň Dvořák, Benoit Marçais, Elsa Caeiro, Bernard Clot, Marie-Laure Desprez-Loustau, Björn Gedda, Karl Lunden, Duccio Migliorini, Gilles Oliver, Ana Paula Ramos, Daniel Rigling, Ondrej Rybnicek, Alberto Santini, Salome Schneider, Jan Stenlid, Emma Tedeschini, Jaime Aguayo, Mireia Gomez-Gallego

Data type: figure (word document)

Link: https://doi.org/10.3897/neobiota.84.90549.suppl4

Supplementary material 5

Zero-centred histogram of the residuals between simulated data and predictions of the model with the distance to the closest disease report as a predictor of the number of *Cryptostroma corticale* spores detected in aerobiological samples

Authors: Elodie Muller, Miloň Dvořák, Benoit Marçais, Elsa Caeiro, Bernard Clot, Marie-Laure Desprez-Loustau, Björn Gedda, Karl Lunden, Duccio Migliorini, Gilles Oliver, Ana Paula Ramos, Daniel Rigling, Ondrej Rybnicek, Alberto Santini, Salome Schneider, Jan Stenlid, Emma Tedeschini, Jaime Aguayo, Mireia Gomez-Gallego

Data type: figure (word document)

Link: https://doi.org/10.3897/neobiota.84.90549.suppl5

Supplementary material 6

Zero-centred histogram of the residuals between simulated data and predictions of the model with the total sycamore maple basal area in a radius of 50 km from the sampler as a predictor of the number of *Cryptostroma corticale* spores detected in aerobiological samples

Authors: Elodie Muller, Miloň Dvořák, Benoit Marçais, Elsa Caeiro, Bernard Clot, Marie-Laure Desprez-Loustau, Björn Gedda, Karl Lunden, Duccio Migliorini, Gilles Oliver, Ana Paula Ramos, Daniel Rigling, Ondrej Rybnicek, Alberto Santini, Salome Schneider, Jan Stenlid, Emma Tedeschini, Jaime Aguayo, Mireia Gomez-Gallego

Data type: figure (word document)

Link: https://doi.org/10.3897/neobiota.84.90549.suppl6

Supplementary material 7

Coefficient estimate for each variable of maple basal area computed for different radius and their 95% credible intervals in brackets for models predicting the number of spores detected per week

Authors: Elodie Muller, Miloň Dvořák, Benoit Marçais, Elsa Caeiro, Bernard Clot, Marie-Laure Desprez-Loustau, Björn Gedda, Karl Lunden, Duccio Migliorini, Gilles Oliver, Ana Paula Ramos, Daniel Rigling, Ondrej Rybnicek, Alberto Santini, Salome Schneider, Jan Stenlid, Emma Tedeschini, Jaime Aguayo, Mireia Gomez-Gallego

Data type: table (word document)

Link: https://doi.org/10.3897/neobiota.84.90549.suppl7

Supplementary material 8

Probability of disease report in an area of 40-km to 130-km radius from the sampler as a function of the number of detected spores per day

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Data type: figure (word document)

Link: https://doi.org/10.3897/neobiota.84.90549.suppl8

NeoBiota 84: 349–367 (2023)
doi: 10.3897/neobiota.84.86586
https://neobiota.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
NeoBiota
Advancing research on alien species and biological invasions

Tree species preference and impact on native species community by the bark beetle *Ips amitinus* in a recently invaded region

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Academic editor: Andrea Battisti | Received 15 May 2022 | Accepted 19 August 2022 | Published 18 May 2023

Citation: Cocos D, Klapwijk MJ, Schroeder M (2023) Tree species preference and impact on native species community by the bark beetle *Ips amitinus* in a recently invaded region. In: Jactel H, Orazio C, Robinet C, Douma JC, Santini A, Battisti A, Branco M, Seehausen L, Kenis M (Eds) Conceptual and technical innovations to better manage invasions of alien pests and pathogens in forests. NeoBiota 84: 349–367. https://doi.org/10.3897/neobiota.84.86586

Abstract

Non-native bark beetle species represent a major threat to forest ecosystems. The bark beetle *Ips amitinus* has recently expanded its range from Finland into northern Sweden. In the present study, we asked the following questions: (i) What is the distribution status in Sweden? (ii) Is there a difference in preference and reproductive success between Norway spruce and Scots pine? (iii) How common is the species after range expansion and does it influence the native community of bark- and wood-boring beetle species?

We established the presence of *I. amitinus* and co-existence with the native community through checks of logging residues at 382 localities in northern Sweden. In addition, attack densities and reproductive success were compared between spruce and pine through investigating field material and by a no-choice rearing experiment.

We found that *I. amitinus* is distributed over large parts of northern Sweden. Within its distribution area, it was found in 58% of all checked localities. It is one of the most common bark beetle species in logging residues and a higher proportion of Norway spruce objects compared to Scots pine were colonised. Attack density and reproductive success were higher in Norway spruce in field material and in the rearing experiment. There was no significant difference in the number of native bark- and wood-boring beetle taxa between localities where *I. amitinus* was present or absent.

Keywords

community ecology, ecological impact, invasion range, small spruce bark beetle, species interactions

Introduction

Non-native forest pests present a major threat to forest ecosystems globally (Brockerhoff et al. 2006; Aukema et al. 2011). In recent decades, establishments of alien species have steadily increased over time (Roques et al. 2009; Brockerhoff and Liebhold 2017; Seebens et al. 2017). Biological invasions can broadly be divided into two types: (1) human-mediated long-distance transportation of propagules into regions that could not be reached by natural dispersal (often inter-continental) and (2) range expansion caused by human-induced environmental changes allowing a species to disperse and establish in adjacent areas previously not colonised (Pyšek et al. 2012; Essl et al. 2019). In the latter case, species first establish in regions adjacent to the historic native range and subsequently colonise regions further away from the historic range as the expansion progresses (Essl et al. 2019). Additionally, in this case, human-mediated transportation may contribute to range expansion. Even though the second type of range expansions seem to have become more common (Lenoir and Svenning 2015), they have been given less attention in research than human-mediated long-distance establishments (Essl et al. 2019). However, there are important differences between these two modes of expansions. During long-distance human-mediated establishments, species encounter naïve hosts and very different ecological communities compared with those in their native areas. In the case of range expansions adjacent to native areas, species often encounter hosts and ecological communities that are similar to those in their native range.

One example of the second type of range expansion is the bark beetle *Ips amitinus* (Eichhoff, 1872). The distribution of *I. amitinus* ranges from the mountainous regions of central and south-eastern Europe to France, Belgium and The Netherlands to the West (Jurc and Bojović 2004; Holuša et al. 2012; Mazur and Kuźmiński 2013). In the last century, the species has expanded its range northwards. *Ips amitinus* was first observed in Estonia around the 1930s (Zolk 1932), in southern Finland in early 1950s (Koponen 1975) and is now established throughout Finland (Økland et al. 2019). In 2012, *I. amitinus* was recorded for the first time in Sweden, close to the Finish border in the most northern part of the country (Lindelöw 2013). However, the finding was a random encounter and not a result of a systematic search after the species then or previously. Thus, most probably the species was present in Sweden already before 2012.

In the present study, we assessed the distribution range of *I. amitinus* in Sweden, its performance in Norway spruce (*Picea abies* L. Karst) and Scots pine (*Pinus sylvestris* L.) and its influence on the native community of bark- and wood-boring beetles. *Ips amitinus* reproduces in both Norway spruce and Scots pine (Økland and Skarpaas 2008; EPPO 2022). Most studies of *I. amitinus* report attacks on spruce in Central Europe (Jakuš 1998; Witrylak 2008; Grodzki 2009; Holuša et al. 2012) and during the northern range expansion (Annila and Nuorteva 1976; Martikainen et al. 1996; Mandelshtam 1999) while a few studies from Russia report attacks on pine (Mandelshtam 1999; Kerchev and Krivets 2021). We are unaware of earlier studies comparing the preference and the reproductive success of *I. amitinus* between the two tree species. Understanding the connection between preference and reproduction success may improve predictions of extend and speed of further range expansion by *I. amitinus* in landscapes with different proportions of these two host trees.

Although range expansions have been documented for several bark beetle species (Dodds et al. 2018; Wermelinger et al. 2020), the effect on native communities of bark- and wood-boring beetles in the invaded regions has, to our knowledge, not yet received attention in scientific literature. We hypothesise that similarities between the communities associated with Norway spruce and Scots pine in the native and expanded range might result in a smooth invasion by *I. amitinus* without large consequences for the native community in the invaded range.

We asked the following questions for *I. amitinus*: (i) What is the current distribution in Sweden? (ii) Is there a difference in preference and reproductive success between Norway spruce and Scots pine? (iii) How does the presence of the invading species influence the native community of bark- and wood-boring beetle species?

Material and methods

Data collection

The study consists of: (1) a survey of *I. amitinus* in northern Sweden to assess its current distribution, commonness, host tree preferences and potential impact on native bark- and wood-boring beetle species, (2) investigation of naturally colonised material for assessing colonisation density and reproductive success and (3) a rearing experiment comparing *I. amitinus* reproductive success in Norway spruce and Scots pine (hereafter spruce and pine).

Surveys of occurrence, tree species and object type preferences

To assess the current distribution of *I. amitinus* in Sweden, we conducted two surveys in the counties of Norrbotten and Västerbotten in northern Sweden. Norrbotten is bordering Finland from where the species is expected to have spread. When the species were found in large parts of Norrbotten, we continued the survey further south in Västerbotten to find the southern range limit (Fig. 1). The first survey was carried out in the summers of 2016 and 2017. Five types of localities were inspected: clear-cuts, thinnings, cuttings along roads, cuttings under power lines and wind-felled trees in forest stands (Table 1). Five types of objects were checked: tops, branches, logs, cut small trees (trees with diameter up to 15 cm) and large trees (cut or wind-felled trees with diameter larger than 15 cm) (Table 2). All objects were cut or wind-felled during the previous winter. The presence of *I. amitinus* colonisation was determined by visual inspection after strips of bark were removed from the whole length of each object. If signs of beetle attack were present, more bark was removed to determine which species were present. The identification of *I. amitinus* was based on adults (when present) and/or the characteristics of the gallery system (Suppl. material 1: *Ips amitinus* description).

Table 1. Numbers of the five types of locations surveyed for presence of *Ips amitinus*. The first survey was conducted in 2016–2017 and the second in 2018–2019.

Year	Total no. localities	Location type				
		Clear-cut	Along Road	Stand	Thinning	Power lines
2016	12	–	10	–	1	1
2017	153	45	63	12	20	13
2018	118	100	–	–	18	–
2019	99	92	–	–	7	–

Table 2. Numbers of spruce and pine objects and of each of the five types of objects, surveyed in the first (2016–2017) and second (2018–2019) survey.

Year	Tree species		Type of objects					Total no. objects
	Spruce	Pine	Tops	Branches	Logs	Small trees	Large trees	
2016	11	10	–	–	2	5	14	**21**
2017	831	873	499	23	100	988	95	**1705**
2018	2255	1216	1470	31	114	1815	42	**3472**
2019	2981	2301	3407	–	–	1875	–	**5281**

In 2016, the survey focused on confirming the establishment of *I. amitinus* in Sweden. Trap logs of spruce and pine were cut close to the location of the first discovery in Sweden in 2012. Material such as wind-felled trees, found while driving between the trap log locations, was inspected as well. In 2017, the survey focused on establishing the presence of *I. amitinus* along the Finnish border, the assumed entry of the species to Sweden (Økland et al. 2019) and to determine how far south the species had spread in Sweden. In both years, inspection sites were located by driving along roads. In both 2016 and 2017, a maximum of 30 objects were checked in each locality when available. However, inspections ended when an *I. amitinus*-colonised object was found, even if it was the first checked object.

In the second survey (conducted in the summers of 2018–2019), we adjusted the survey methodology, based on experience gained in the first survey and only included clear-cuts and thinnings harvested during previous winter (between October and March) (Table 1). Spruce and pine forest stands were not surveyed (except recently thinned stands) because of a very low probability of finding suitable breeding material (i.e. dying or newly-dead conifers or parts of trees) inside stands that had not been thinned recently. We decided to focus on clear-cuts as these constituted most of all cuttings and information was available about their locations from the Swedish Forest Agency and forest companies. In addition, in the spring of 2019, clear-cuts from winter 2017–2018 were checked for colonisation by *I. amitinus* during the previous summer (2018), to increase the number of surveyed localities along the distribution limit (these clear-cuts were not inspected during 2018). In 2018, the same five types of objects were inspected as in the previous years. In 2019, we only inspected tops and small trees, as those objects had the highest probability of attack (see results). In 2018, at each location, 30 objects were randomly selected and inspected for the presence of *I. amitinus* (i.e. even if the species were found in the first object, all 30 objects were checked). In 2019, the protocol was adjusted to ensure a more accurate estimate of absence. Initially, 30 objects would be inspected. If *I. amitinus* were not detected in those 30 objects, we continued until 100 objects had been inspected. For each object, we recorded tree species (spruce or pine), type of object, diameter, length and the presence of *I. amitinus*.

For each inspected object during the second survey, we also recorded the presence of other (native) bark beetle species and other bark- and wood-boring beetles. Species identification was based on adults and gallery systems (Ehnström and Axelsson 2002). As there are two species of the bark beetle genus *Pityogenes* that commonly reproduce in logging residues of pine (*P. bidentatus* and *P. quadridens*) and because species identification is not possible, based on their gallery systems, their identification was only done to *genus* level (referred to as *Pityogenes* spp.). Only one *Pityogenes* species, (*P. chalcographus* Linnaeus, 1761*)*, is known to commonly colonise spruce in Sweden (Ehnström and Axelsson 2002; Jonsell et al. 2007). In Central Europe, *P. chalcographus* can often be found in pine as well (Foit 2012, 2015). However, that does not seem to be the case in Scandinavia (Ehnström and Axelsson 2002; Åke Lindelöw, personal communication, but see Lekander et al. 1977).

Performance in naturally colonised material

Field-collected colonised material was used to assess *I. amitinus* performance in spruce and pine. The material was collected between 21 and 26 May 2018, prior to emergence of offspring resulting from colonisation earlier in spring (no exit holes present), from seven clear-cuts harvested during the previous winter. Most of the collected material were spruce tops and small spruce trees (60 objects from 7 clear-cuts), but also some small pine trees were collected (11 objects from 2 clear-cuts) (Table 3).

The objects were grouped by tree species and location before being placed in separate emergence cages (seven cages with spruce and two cages with pine) in a climate chamber (20 °C, 20 hours day length). When adult emergence had ceased, we recorded for each object, diameter, length, *I. amitinus* attack density (number of male entrance holes and maternal galleries per mantel area) and number of maternal galleries per mating chamber. Emerging *I. amitinus* were collected daily from cages and stored in boxes with moist paper at 5 °C to be used later in the rearing experiment (see below).

Table 3. Number, mean length and diameter of naturally colonised objects used for evaluating the performance of *Ips amitinus* in Norway spruce and Scots pine.

Tree species	No. objects	Length (cm) Mean ± SE	Range	Diameter (cm) Mean ± SE	Range
Norway spruce	60	61.67 ± 0.51	48–70	8.9 ± 0.4	4–15.5
Scots pine	11	56.54 ± 1.95	41–61	12.4 ± 0.41	11–15.5

Performance in rearing experiment

We used the *I. amitinus* adults reared from the naturally colonised spruce and pine for a no-choice experiment with stem sections of spruce and pine. From seven spruce and five pine trees, a 60 cm long stem section was cut (at 3 m from the base of the trees) between 9 and 10 July near Uppsala. The diameter of the stem sections was 17.6 ± 0.4 cm (mean ± SE) and they were stored at room temperature for 48 hours after which cut surfaces were waxed to prevent desiccation. Subsequently, the stem sections were stored at room temperature for another 24 hours before being moved to a climate chamber (20 °C, 20 hours day length). Each section was placed standing in a separate cage (70 × 53 × 50 cm). The following day, 90 *I. amitinus* adults were released into each cage. All reared beetles were mixed together prior to release. As colonisation success was deemed insufficient (based on amount of boring dust), an additional 70 adults were released into each cage after three days. Low vitality adults (slow in movement) were discarded prior to release.

All dead beetles were collected from each cage prior to the emergence of the new generation. These dead beetles were parent beetles that left the stem section after mating and egg laying, in addition to beetles that never entered the logs. The emerging offspring were collected daily and colour-classified during the first weeks to ensure that they were not parent beetles (darker). After emergence ended, density and length of *I. amitinus* maternal galleries, number of maternal galleries per mating chamber and number of male entrance holes were recorded. We also noted the success or failure of maternal galleries, based on presence (success) and absence (failure) of larval galleries.

Statistical analyses

All statistical analyses were performed in R, version 2021.09.2 (R Core Team 2019).

Surveys of occurrence

All data collected during the surveys from 2016 to 2019 were used to calculate the distribution area. The speed of range expansion was calculated using the latitudinal difference between the most southern record for each survey year, from 2017 to 2019. During the 2018 and 2019 survey, we checked 13.5% and 13%, respectively, of all fresh clear-cuts along the yearly southern distribution limit (on an approximate 66 × 10 and 65 × 12 km area).

Tree species and object type preference

For analysis of observational data from the second survey, we used generalised linear mixed models with a binomial error distribution (glmer, lme4 package; Bates et al. 2015), to estimate the relationship between colonisation (i.e. the presence/absence of *I. amitinus*), using the explanatory variables tree species (two levels: spruce and pine), object type (two levels: tops and small trees), diameter and total number of other bark beetle taxa as covariates. Interactions between explanatory variables tree species and object type and between object type and the number of other species were also included in the model. Object diameter was included to control for potential effects on the response variable. Year and clear-cut identity were included as random factors. We encountered some problems with model convergence. Hence, we set adaptive Gauss-Hermite quadrature points (nAGQ) to zero (Olver et al. 2010), to calculate the log-likelihood, even though the accuracy of the method in parameter estimation decreased.

Performance in naturally colonised material

To assess *I. amitinus* performance in the field-collected material, we used attack density per m^2 of bark (two measures: male entrance holes and maternal galleries), reproductive success (number of daughters per maternal gallery) and the number of offspring produced per m^2 bark area as the response variables. Cage was the level of replication for the reproductive success. The attack densities were recorded for each object (level of replication). We used a linear model with the explanatory variable tree species (two levels: spruce or pine). We used Levene's test to check for homogeneity of variance in the residuals (LeveneTest; car-package). When the model assumption of homogeneity of variance was violated, the response variable was log-transformed (natural logarithm), to comply with model assumptions.

Performance in rearing experiment

We performed the same analyses for the no-choice rearing experiment. One spruce log was excluded from the analysis because of lack of reproductive activity. We used the same response variables, which we calculated in the same way, as for the field-collected material.

Co-occurrence with native species

To assess the co-occurrence of *I. amitinus* with the native species, we used manyglm model (mvabund package; Wang et al. 2012), fitted with a negative binomial distribution, with the number of other bark-and wood-boring beetle species present in each locality as the multivariate response variable and presence/absence of *I. amitinus* as the explanatory variable. The data were pooled by locality and split by tree species; the model was run separately for spruce and pine. We also performed a series of Non-Metric Multidimensional Scaling (NMDS), using metaMDS function (Vegan package; Oksanen et al. 2013) to allow for better visualisation of our data. NMDS is a method that allows for a better visualisation of patterns in community abundance, in a reduced number of dimensions (Dexter et al. 2018). We looked at the presence/

absence of other species versus presence/absence of *I. amitinus* at locality level in both spruce and pine. We set the distance metric to "bray", number of dimensions (K) set to 3, max. number of iterations set to 999 and the number of random starts was set at 300. For both the manyglm model and for the NMDS analysis, only bark- and wood-boring beetle species known to attack each one of the tree species were included in the analyses. Only localities colonised by at least one species were included in the model.

Results

Surveys of occurrence

Ips amitinus was found in 184 out of 382 inspected localities in the years 2016–2019. North of its southern distribution limit, the species was recorded in 57.9% of all checked localities during the four-year study. Our observations show that the species is presently distributed from the border of Finland in the north-eastern part of the Province of Norrbotten (67°29.915'N, 23°17.330'E) and south to the northern part of the Province of Västerbotten (65°11.628'N, 20°46.218'E) (Fig. 1A).

Based on the yearly surveys, we found that the estimated speed of expansion varied yearly from 17 km from 2017 to 2018 and 11 km from 2018 to 2019, suggesting an average range expansion of 14 km per year (Fig. 1B).

The average proportion of objects colonised by *I. amitinus* per occupied locality was 16.6 ± 1.9% in 2018, ranging from 3% to 57% and 11.6 ± 1.2% in 2019, ranging from 3% to 37%. *Ips amitinus* was not detected in any of the 64 localities checked south of the 2019 distribution limit. No overwintering adults were found under bark in colonised substrates in any of the 33 clear-cuts (79 attacked objects) that were colonised in 2018 and checked in early spring 2019.

Tree species and object type preference

Ips amitinus was present in 23.4% (376 colonised objects) of sampled spruce tops and small trees in 2018 and 16.2% (236 colonised objects) in 2019, within its distribution limit. The colonisation rate in pine was lower in both 2018 (3.0%, 25 colonised objects) and 2019 (0.2%, two colonised objects). The interaction between tree species and object type indicated that, for pine, *I. amitinus* prefers small trees compared to tops, whereas for spruce, no clear difference was detected (Fig. 2, Table 4).

The interaction between object type and the number of other bark beetle taxa present indicates that, in small trees, the presence of more species is associated with a higher probability of colonisation by *I. amitinus*. We found a positive significant relationship with object diameter independent of object type (0.31 ± 0.03; $X^2 = 127.39$, $p < 0.0001$) and this relationship is also independent from other explanatory variables (Table 4).

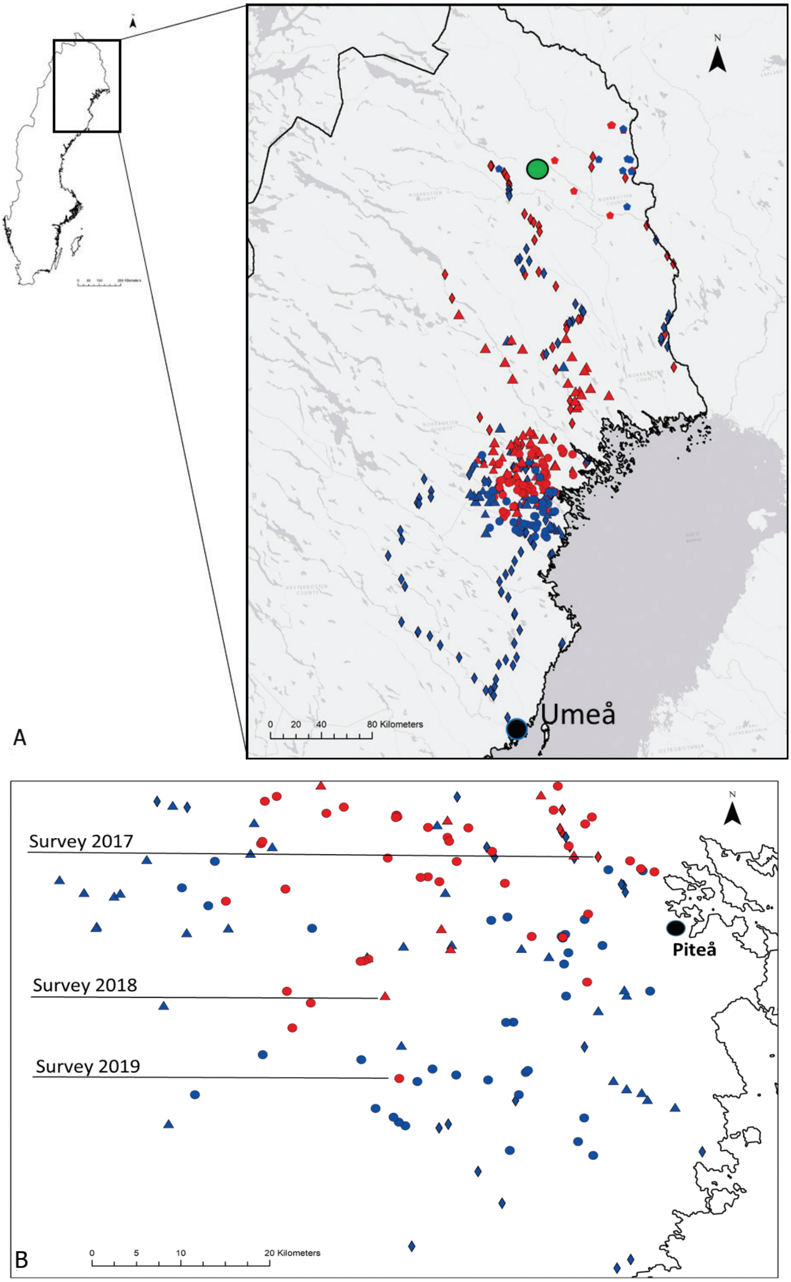

Figure 1. A locations checked for presence of *Ips amitinus* in northern Sweden in 2016 – 2019. Red symbols = presence and blue symbols = absence. Green symbol = first record in 2012. 2016 = pentagon, 2017 = diamond, 2018 = triangle and 2019 = circle. Umeå (63°49.877'N, 20°15.651'E) **B** *Ips amitinus* southern distribution limit in 2017, 2018 and 2019 in northern Sweden. Localities are with (red symbols) and without (blue symbols) findings of *I. amitinus* in the surveys. Diamond symbols represent the 2017 survey, triangle – 2018 and circle - 2019 survey. Black horizontal lines denote the most southern location with *I. amitinus* for each year. The distance from the first find in 2012 (green circle in Fig. 1A) to the distribution limit in 2017 is 250 km.

Figure 2. The probability of attack between the two different tree species and object types, based on the results from the 2018 and 2019 survey. The central lines of the box plot represent the median, the box indicates lower and upper quartiles and the whiskers represent the largest and smallest observations that fall within 1.5 times the box size from the nearest quartile. Black circles represent outliers.

Table 4. Anova (type III test; Anova; car-package) and estimates for the generalised linear mixed effects model testing the effect of tree species, object type, number of other bark beetle taxa and diameter on *I. amitinus* colonisation probability. The Table shows the final model with the chi-squared value, degrees of freedom (df), estimates and the standard error of the mean (SE). The standard deviation for intercept (for the random effects) is given as well. The variables printed in bold are significant at $p < 0.05$. The intercept represents the overall mean. Values were obtained by using sum contrasts, as we wanted to compare the intercept to the overall mean.

Response	Explanatory	X^2	df	Est	SE	P	Random effects: Site, Year	
							Std. dev.	Intercept
							Site	Year
I. amitinus colonisation	Intercept	**312.88**	**1**	**-6.20**	**0.35**	**<0.0001**	0.96	0.10
	Tree species (pine)	**164.45**	**1**	**-1.74**	**0.14**	**<0.0001**		
	Object type (small tree)	0.12	1	0.06	0.18	–		
	Other bark beetle taxa	0.76	1	-0.10	0.12	–		
	Diameter	**127.39**	**1**	**0.31**	**0.03**	**<0.0001**		
	Tree species (pine) × Object type (small tree)	**25.02**	**1**	**0.66**	**0.13**	**0.0001**		
	Object type (small tree) × Other bark beetles	**10.21**	**1**	**0.36**	**0.11**	**0.001**		

Performance in naturally colonised material

Attack density, expressed as density of male entrance holes per m^2 of bark ($F_{1,69} = 8.261$, $p = 0.005$; Fig. 3A), as well as the density of maternal galleries ($F_{1,69} = 8.238$, $p = 0.005$; Fig. 3B) were significantly higher in spruce objects compared to pine, independent of object type.

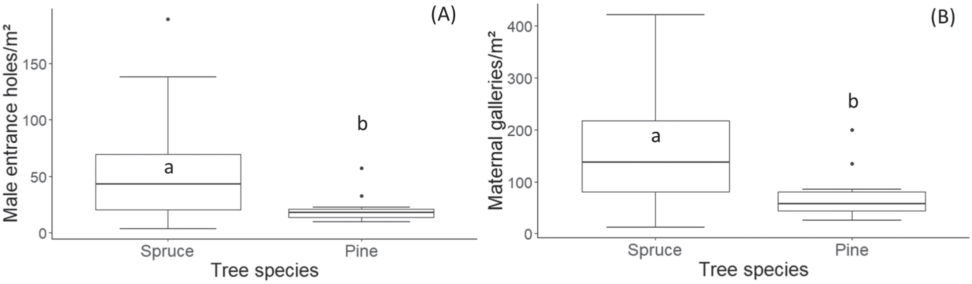

Figure 3. Male attack and female gallery density in host material naturally colonised by *Ips amitinus*. (**A**) Male entrance holes per m^2 bark and (**B**) maternal galleries per m^2 bark.

However, there was no significant difference in the number of maternal galleries per mating chamber between spruce (3.08 ± 0.08) and pine (3.27 ± 0.26; $F_{1,69} = 0.267$, p = 0.61). Reproductive success, expressed as number of daughters per mother (i.e. per maternal gallery), was significantly higher in spruce (4.53 ± 0.70), compared to pine (1.90 ± 0.06; $F_{1,7} = 5.369$, p = 0.05). In addition, the number of offspring produced per m^2 was significantly higher in spruce (1447 ± 469) compared to pine (328 ± 96), ($F_{1,7} = 5.056$, p = 0.05). Maternal gallery length was significantly longer in pine (11.97 ± 0.78 cm) compared to spruce (8.92 ± 0.28 cm; $F_{1,69} = 16.88$, p = 0.0001). The density of *I. amitinus* male entrance holes did not differ between the pine objects with and without the bark beetle *Tomicus piniperda* (L.) ($F_{1,9} = 3.006$, p = 0.11). However, the density of *I. amitinus* maternal galleries per m^2 was more than two-fold higher within pine when *T. piniperda* was absent (108 ± 27), compared to when both species were present in the same object (47 ± 7.6; $F_{1,9} = 5.379$, p = 0.04). No living adult *I. amitinus* beetles were found under the bark at the end of the rearing of naturally colonised material though a few dead individuals were found.

Performance in rearing experiment

The density of male entrance holes per m^2 of bark was significantly higher in spruce compared to pine ($F_{1,9} = 5.155$, p = 0.05; Fig. 4A). The density of maternal galleries, on the other hand, only showed an indication of being higher in spruce ($F_{1,9} = 3.849$, p = 0.08; Fig. 4B). The reproductive success was significantly higher in spruce compared to pine ($F_{1,9} =5.407$, p = 0.05; Fig. 4C). In addition, the number of offspring produced per m^2 was significantly higher in spruce compared to pine ($F_{1,9} = 19.1$, p = 0.002; Fig. 4D).

Maternal gallery length did not differ between the pine and spruce (13.8 ± 5.6 cm for spruce and 10.6 ± 4.7 cm for pine, $F_{1,9} = 1.93$, p = 0.19). We did not find a difference in the number of maternal galleries per mating chamber, between spruce and pine (2.4 ± 0.2 and 2.1 ± 0.3 respectively, $F_{1,9} = 1.291$, p = 0.28). The emergence time of the new generation did not differ between the two tree species. No adult beetles remained under the bark at the end of the experiment.

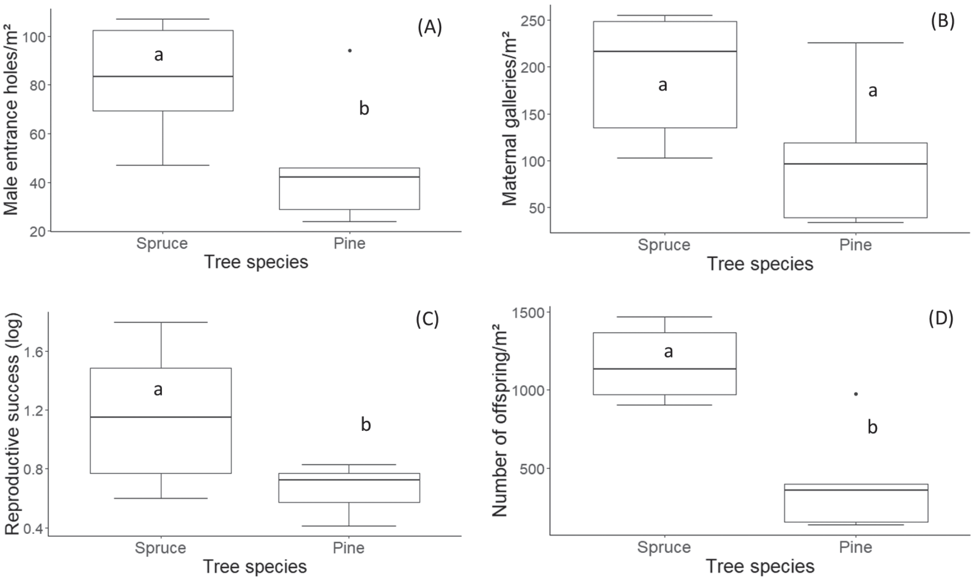

Figure 4. The results from the no-choice rearing experiment with *Ips amitinus*. Male entrance holes per m^2 bark (**A**), maternal galleries per m^2 bark (**B**), reproductive success (log transformed), (**C**) and number of offspring produced per m^2 bark (**D**). Means with different lowercase letters are significantly different at $p < 0.05$. There were six replicates for Norway spruce and five for Scots pine.

Co-occurrence with native species

A total of 16 native bark beetle, weevil and long horn beetle species/taxa were recorded in the study (Suppl. material 1: table S1). The results from manyglm ANOVA show that, for spruce, there was no significant difference in the number of native bark- and wood-boring beetle species between localities where *I. amitinus* was present or absent (LRT = 14, p = 0.09). The result of our NMDS analysis shows also a similar pattern in the number of native bark- and wood-boring beetle species between localities when *I. amitinus* is present or absent (Fig. 5A). For the pine, we did find a significant difference between the total number of other bark- and wood-boring beetle species and locations with and without *I. amitinus* (LRT = 20, p = 0.002). Amongst the species, a significant negative relationship with the presence of *I. amitinus* was found for *T. piniperda* (LTR = 5.7, p = 0.04). This is also confirmed by the reduced overlap in the NMDS analysis between the area when *I. amitinus* is present and absent (Fig 5B).

Ips amitinus was the third most common species colonising the inspected wood objects within its distribution limit. When looking at only spruce objects, *I. amitinus* was the second most common species and, on only pine objects, the fourth most common species (Suppl. material 1: table S1.) Spruce accounted for 57.5% of all objects inspected within the distribution area for *I. amitinus*, the remaining 42.5% being pine. On average 5.2% of all the objects checked during the four years were not colonised by any species. From all the colonised objects, only 0.07% were colonised by *I. amitinus*

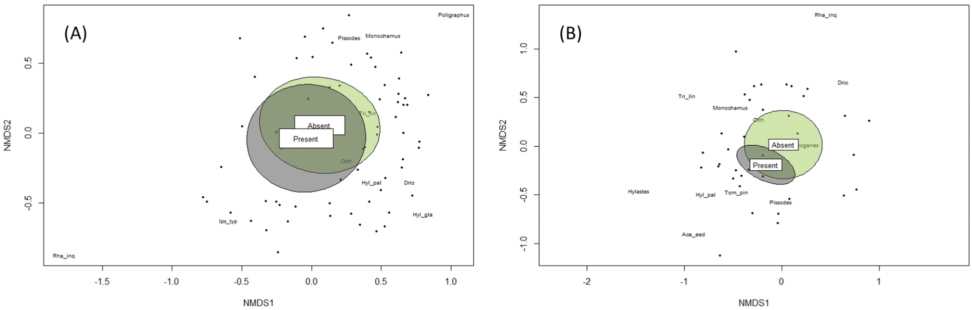

Figure 5. NMDS visualisation of the relationship between the other species found in spruce (**A**) and pine (**B**) in relation to the presence/absence of *I. amitinus*. Stress: 0.1868 (spruce) and 0.1173 (pine).

alone. There was no marked difference in the average number of other bark beetle taxa that colonised either spruce or pine north of the distribution limit compared to south (1.2 – 1.1 for spruce and 1.0 – 1.1 for pine). Only objects colonised by at least one species were included in this calculation.

Discussion

In this study, we set out to establish the range distribution of *I. amitinus* in Sweden and the preferred tree species and host material used in the new range. We found that *I. amitinus* has expanded its range south approximately 200 km over land from the Finnish border (Fig. 1A). In addition, we observed a preference for spruce as a host on which the species also achieves the highest reproduction success. We did not find an indication that the arrival of *I. amitinus* has a transformative effect on the native bark beetle community despite being one of the most common species.

Due to the lack of *I. amitinus* records around Umeå (Fig. 1A), we argue that our observations support the conclusion that this species spread over land into Sweden from Finland. The continuous presence of *I. amitinus* from the northern Finnish border with Sweden to the southern limit of observations in Sweden is additional support for range expansion over land. The first observation of *I. amitinus* in Sweden in 2012 was a chance observation; it is unclear what the distribution limit of the species was at that time. When the targeted surveys started in 2017, our observations indicate that, from the southern range limit observed in 2017 to the range limit observed in 2019, *I. amitinus* expanded south with an estimated average speed of 14 km per year, which is similar to the average rate of spread through Finland observed in previous studies (Økland et al. 2019; Fig. 1B).

In earlier studies, spruce has been recorded as breeding material more often than pine (Annila and Nuorteva 1976; Witrylak 2008; Grodzki 2009; Holuša et al. 2012; Mazur and Kuźmiński 2013), but without quantitative comparisons. Our study shows that the brood of this species is most frequently found in spruce and male attack density is higher in spruce compared to pine in both field and laboratory studies, indicating a male preference for spruce. Competition with *T. piniperda* in pine (which colonises hosts earlier in spring than *I. amitinus*) might be one factor that has contributed to higher preference and higher male and female attack density in spruce. The higher reproductive success in spruce, for both the field and experimental material, could be another contributing factor for the preference for spruce (Fig. 4). Neither of the rearing experiments showed a significant difference between the number of maternal galleries per nuptial chamber between the tree species, which indicates that once males manage to colonise an object, they are equally attractive to females regardless of the tree species they chose.

To our knowledge, this is the first study to show that both *I. amitinus* preference and performance are highest in spruce within its invaded range. In the native range, Stauffer and Zuber (1998) compared *I. amitinus* performance between Norway spruce and the Swiss stone pine (*Pinus cembra*, Linnaeus). They showed that *I. amitinus* can breed successfully in both tree species, both in a dual choice (where spruce was preferred) and a no-choice experiment. However, the offspring production was lower in stone pine. Apart from the above-mentioned study, most other studies on *I. amitinus* performance (such as abundance, attack and emergence rates) only considered spruce (Annila and Nuorteva 1976; Witrylak 2008; Grodzki 2009; Holuša et al. 2012; Mazur and Kuźmiński 2013).

Our surveys show that *I. amitinus* is one of the most common bark- and wood-boring species in fresh logging residues, even though the species has most probably only been present for a rather short time (See Økland et al. 2019; Fig. 1A; Suppl. material 1: table S1). *Ips amitinus* was the second most common bark beetle taxon in spruce and the fourth in pine logging residues. In studies conducted in Central Europe, *I. amitinus* is also reported to be one of the most common bark beetle species, together with *P. chalcographus* and *I. typographus*, although quantitative comparisons are lacking (Grodzki 1997, 2009; Witrylak 2008; Holuša et al. 2012; Mazur and Kuźmiński 2013). This begs the question, how did *I. amitinus* become so abundant in northern Sweden in a relatively short time? One explanation might be that the host tree species are the same and the community of bark- and wood-living beetles is similar to the native range. An additional explanation may be the species' ability to successfully reproduce in logging residues. At the landscape level, harvesting of forest stands are conducted each year in many locations, thereby creating a relatively continuous supply of breeding material. The use of logging residuals by *I. amitinus* was also observed in Finland, a country with similar forestry practices as Sweden (Annila and Nuorteva 1976; Martikainen et al. 1996) and where the rate of spread was similar to our results.

We find that the community of bark boring insects in spruce is not different when *I. amitinus* is present or absent (Fig. 5A), indicating that the species found in the spruce manage to attack and reproduce regardless of *I. amitinus* presence. In other words, our results for spruce could indicate a vacant niche for *I. amitinus* in the invaded community. However, the results for pine (Fig. 5B) show a smaller overlap, which could indicate that more species are associated with objects not colonised by *I. amitinus*, compared to objects colonised by *I. amitinus*, with the strongest negative relationship between *T. piniperda* presence and *I. amitinus* presence. Based on these results, we propose that the invasion of *I. amitinus* did not have strong negative consequences for the native community associated with spruce in the invaded areas.

The significant difference observed for *T. piniperda*, in relation to the presence of *I. amitinus*, might be explained by the earlier flight period for *T. piniperda* than for *I. amitinus*. The earlier flight period will give *T. piniperda* the opportunity to colonise breeding material before *I. amitinus*, potentially leading to competitive exclusion of *I. amitinus*.

As our study merely scratches the surface of potential ecological effects of *I. amitinus* invasion, we see a need for more detailed studies into the effects on reproductive success and enemy pressure on *I. amitinus* and its community. We expect that the space available for brood production on logging residues by native species have been reduced to some extent, especially since only a small proportion of the inspected objects were not colonised and some of these may have been too dry or in some other way unsuitable for native bark beetle colonisation. Our observations strongly suggest that *I. amitinus* will continue expanding its range south in Sweden.

In conclusion, the range expansion of *I. amitinus* in Sweden does not appear to markedly affect the native community of bark- and wood-boring insects. In areas where pine is dominating, the invasion success of *I. amitinus* might be slowed down because of its lower reproduction success and stronger competition with *T. piniperda*. The low impact of *I. amitinus* in its invaded range might be related to the similarities with the community in its native range. Future studies of and comparisons with other species expanding into a naïve range and potential host switching will be needed to understand the importance of this similarity.

Acknowledgements

The forest company SCA provided information about clear-cuts and thinnings for the survey. Jan ten Hoopen helped with fieldwork, Åke Lindelöw confirmed the correct identification of *I. amitinus* adults. Andrew (Sandy) Liebhold improved earlier drafts of the manuscript by providing useful discussion and comments.

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Supplementary material 1

***Ips amitinus* description, table S1**

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Data type: pdf file

Explanation note: *Ips amitinus* adults were collected for identification in the laboratory when possible. *Ips amitinus* differ from other *Ips* species present in Sweden by a shiny declivity at the back of the elytra (Knižek 2001; Nierhaus-Wunderwald and Forster 2004; Åke Lindelöw, pers. communication). The gallery system is highly characteristic for the species. It has a very large nuptial chamber in the late stages, as the male usually attracts between two and seven females. The mother galleries often start away from the direction of the wood fibre, then turn after about one centimetre and continue along the wood fibre in a rather windy way. The gallery system could be confused to other bark beetles in the early stages (like Orthotomicus spp.), but it is rather specific in the later stages (for a visual comparison, see Knižek 2001); **table S1.** Percentages of localities and wood objects colonised by bark- and wood-boring beetle taxa north and south of the *Ips amitinus* 2019 southern distribution limit. The number of colonised localities and objects is given within parenthesis. Species were ordered, based on the percentage (and number) of colonised localities north of *Ips amitinus* distribution limit. Taxonomic group: B = bark beetle; L = longhorn beetle; W = weevil (except bark beetles). Host tree: S = Norway spruce; P = Scots pine. The percentage of colonised objects is calculated, based on the number of objects from their host tree species.

Link: https://doi.org/10.3897/neobiota.84.86586.suppl1

NeoBiota 84: 369–396 (2023)
doi: 10.3897/neobiota.84.91540
https://neobiota.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
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Modelling the invasion dynamics of the African citrus psyllid: The role of human-mediated dispersal and urban and peri-urban citrus trees

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Academic editor: Andrea Battisti | Received 11 August 2022 | Accepted 29 September 2022 | Published 18 May 2023

Citation: Nunes P, Robinet C, Branco M, Franco JC (2023) Modelling the invasion dynamics of the African citrus psyllid: The role of human-mediated dispersal and urban and peri-urban citrus trees. In: Jactel H, Orazio C, Robinet C, Douma JC, Santini A, Battisti A, Branco M, Seehausen L, Kenis M (Eds) Conceptual and technical innovations to better manage invasions of alien pests and pathogens in forests. NeoBiota 84: 369–396. https://doi.org/10.3897/neobiota.84.91540

Abstract

The African citrus psyllid, *Trioza erytreae* (Del Guercio) (Hemiptera, Triozidae), is native to tropical Africa and invasive species in North America and Europe. The main host plants are citrus, displaying a preference for lemon trees. This psyllid was recently detected in the northwest region of the Iberian Peninsula, both in Spain and Portugal. Here, we used a model combining a reaction-diffusion model to a stochastic long-distance dispersal model to simulate the invasion dynamics of *T. erytreae* in Portugal. The psyllid spread in Portugal was simulated between 2015 and 2021 for different combinations of model parameters: two fecundity levels; spread with and without stochastic long-distance dispersal; single or two introductions of *T. erytreae*; and considering or not the urban and peri-urban citrus trees, besides citrus orchards, estimated using Google Street view imagery. The incorporation of long-distance human mediated dispersal significantly improved the F1-score in the model validation using the official reports as the observed data. Concomitantly, the dispersal rate of *T. erytreae* in Portugal was on average about 66 km/year, whereas removing long-distance dispersal events, the observed mean was 7.8 ± 0.3 km/year. The dispersal was mainly towards the south along the coastline, where human population is concentrated. The inclusion of the estimated citrus trees outside orchards areas significantly increased the F1-score in the model validation, revealing the importance these isolated host plants hold as stepping stones for the species current invasion and possibly for other species alike.

Keywords

insect vectors, invasive, isolated trees, models, non-native species, psyllids, spread, *Trioza erytreae*

Introduction

Pest species introductions outside the native range have significantly increased in the last decades worldwide (Walther et al. 2009; Seebens et al. 2017; Turner et al. 2021). This is mainly attributed to the intensification of global trade and human travel (Roques 2010; Brockerhoff and Liebhold 2017). Some of these introduced species are invasive, causing high ecological and/or economic impact in agricultural or forest ecosystems (Pimentel et al. 2005; Kenis et al. 2009; Zenni et al. 2021).

The African citrus psyllid, *Trioza erytreae* (Del Guercio) (Hemiptera, Triozidae), is a small sap-sucking insect, native to tropical Africa (Moran and Blowers 1967). The main host plants of *T. erytreae* are Rutaceae, such as citrus, displaying a preference for lemon trees (*Citrus limon*) over sweet orange trees (*Citrus sinensis*) (Aubert 1987). The psyllid was recently introduced in Continental Europe. It was detected in the northwest region of the Iberian Peninsula, both in Spain in 2014 and in Portugal in 2015 (Monzó et al. 2015; Pérez-Otero et al. 2015; DGAV 2021). The introduction of this invasive species has drawn major attention, as it is a vector of the huanglongbing (HLB), also known as the greening disease (McClean and Oberholzer 1965), considered the most damaging citrus disease in the world. The disease is caused by the *Candidatus liberibacter* spp. bacteria (Bové 2006; Gottwald 2010), which is not yet present in Europe. Illustratively, since HLB was introduced in Florida, orange production dropped by 74%, between 2005 and 2019 (Singerman and Rogers 2020). In Europe, *T. erytreae* and HLB are classified as A2 (Annex II B) and A1 quarantine pests (Annex II A), respectively (EU 2019; EPPO 2022a; 2022b).

Since its detection, *T. erytreae* has been expanding southwards along the Portuguese coastal area, despite the phytosanitary measures that were implemented by the Ministry of Agriculture to contain its spread (DGAV 2021). It has a high invasive potential due to its high fecundity (between 327 and 827 eggs per female), no diapause and multivoltine biological cycle (up to 8 generations per year) (Moran and Blowers 1967; Catling 1969, 1972; Tamesse and Messi 2004). However, the number of yearly generations can be reduced to three in hot and dry summer conditions or other unfavourable conditions for the host leaf flushing, as *T. erytreae* reproduction requires the availability of young leaf shoots (Catling 1972; Tamesse and Messi 2004; Cocuzza et al. 2017). Adults of *T. erytreae* were estimated to fly up to 1.5 km, especially if forced by external factors, such as lack of leaf flushes (Samways and Manicom 1983; Van den Berg and Deacon 1988). Its dispersal may be further aided by wind currents, as was shown in the case of the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Aubert and Hua 1990; Antolínez et al. 2022. Human activities, such as the transportation of fruit or plant material, may also be involved in the long-range dispersal of the species (Aubert and Hua 1990; Antolínez et al. 2022. This has been shown for other agricultural and forest pests (Shigesada and Kawasaki 1997; Tobin and Blackburn 2008; Robinet et al. 2009), including the Asian citrus psyllid (Halbert et al. 2010).

Citrus orchards in Portugal represent around 19,000 ha, mostly concentrated in the south of the country, the major production region (EU 2021). However, besides citrus orchards, it is common to find citrus plants in urban and peri-urban landscapes, all over the country, including urban trees in villages and cities, mostly in central and southern Portugal, but also trees in home gardens and backyards (Duarte 2012). These citrus trees are not included in the official statistics and may represent a significant area within the global spatial distribution range of citrus plants in Portugal, which has not been estimated. They may have an important role in the dispersal of *T. erytreae*. Indeed, scattered host plants could play an important role in the spread of invasive species, in general, as was clearly demonstrated for the pine processionary moth, *Thaumetopoea pityocampa* Denis & Schiffermüller (Rossi et al. 2016). For *T. erytreae*, isolated citrus trees may be a reservoir of the psyllid (Van den Berg et al. 1991) and they are expected to influence the connectivity between the fragmented citrus producing lands.

A few studies used the bioclimatic suitability of the different geographic regions of Portugal and Spain to predict the potential spread of *T. erytreae* (Benhadi-Marín et al. 2020, 2022; Paiva et al. 2020). Paiva et al. (2020) used the water vapour pressure deficit (based on the results of Green and Catling 1971) to predict climate suitability for the psyllid, in Portugal, based on data collected from 18 weather stations, distributed throughout the country. Benhadi-Marín et al. (2020) carried out a pest risk analysis modelling approach to predict the expected spread of *T. erytreae* in the Iberian Peninsula. They compared three models: (1) a radial range expansion model, (2) a hybrid model of logistic growth and radial rate and (3) the deterministic version of the dispersal kernel model. The kernel model with two hypothetical entry points (Vila Nova de Arousa, in Spain and Porto, in Portugal) showed to accurately predict the distribution of the psyllid with respect to latitude, five years after its detection. More recently, the same research team refined the approach used previously (Benhadi-Marín et al. 2022) by: improving the spatial data resolution (1 km); including a physical barrier (altitude of 400 m) and long-distance dispersal events (cells up to 500 km apart were allowed to be colonised) for modelling purposes; extending the prediction to 30 years after the introduction of *T. erytreae* in the Iberian Peninsula; simulating different scenarios (very low, low, medium and high spread). Using this approach, Benhadi-Marín et al. (2022) identified three key risk areas, one in Portugal, the citrus growing areas of Setúbal and two in Spain, Huelva and the potential citrus corridor that connects Guipúzcoa, claiming that these areas should have special attention for the monitoring of *T. erytreae*.

To explore the role of human activities in the spread of *T. erytreae* in continental Portugal, we used a model that combined a reaction-diffusion model to simulate the natural spread of the species and a stochastic long-distance dispersal model to simulate human-mediated dispersal of the species. Reaction-diffusion models have been commonly used for simulating the spatial spread of invasive species as they describe both population growth and population dispersal in a spatially explicit way to provide an estimate of population density over time and space (e.g. Shigesada and Kawasaki 1997). These models describe diffusive dispersal (e.g. dispersal into adjacent habitats), but cannot describe jumps at long distances. To model explicitly a stratified dispersal (allowing both diffusive dispersal and long-distance jumps), we thus combined a reaction-diffusion model to a stochastic long distance dispersal model. This approach has been previously used to explore the role of human mediated dispersal in expanding insect populations (e.g. Robinet et al. 2019). To our knowledge, this is the first time this modelling approach is used for *T. erytreae*. Our specific objectives were: (i) to understand the role that human-mediated spread has played in the current invasion of the psyllid in Portugal; (ii) to highlight the importance of trees outside citrus orchards in the psyllid's spread and the general importance that isolated trees data can have for large-scale pest species modelling. With this aim, we provided an important innovation in the utilisation of Google Street view imagery to estimate citrus trees density in urban and peri-urban areas. Finally, we tested the hypothesis of multiple introductions of *T. erytreae*, as suggested by Ruíz-Rivero et al. (2021).

Materials and methods

Model development

A reaction-diffusion model was developed to simulate the local spread of *T. erytreae* since its arrival in Portugal for the whole continental area of the country. This type of modelling is commonly used for describing the spatial spread of invasive species (e.g. Shigesada and Kawasaki 1997). The model which incorporates the dispersal of the species and the population's growth can be expressed using the following Fisher equation:

$$\frac{\partial N}{\partial t} = D\left(\frac{\partial^2 N}{\partial x^2} + \frac{\partial^2 N}{\partial y^2}\right) + rN\left(1 - \frac{N}{K}\right) \qquad \text{eq.1}$$

where N is the population density of *T. erytreae* (km^{-2}), dependent on time t and spatial location (x,y); D is the coefficient of diffusion (km^2 /year); r is the population growth rate (year^{-1}); and K is the carrying capacity (km^{-2}). The model exhibits a travelling wave with a constant spread rate (C; km/ year) defined by:

$$C = 2\sqrt{rD} \qquad \text{eq.2}$$

where C, K and r were all estimated beforehand (see text below), while D was assumed to be homogeneous across Portugal, based on eq. 2 (similarly to Robinet et al. 2017), considering C and r of the area infested by *T. erytreae* at the end of 2015.

The model described was applied over a grid of 5 km × 5 km resolution, from 2015 to 2021 with a yearly time step.

The population dynamics and spread parameters were obtained, based on previous research studies on the biology and ecology of the psyllid, so that model simulation could be then validated using the independent presence/absence of observed data.

Spread rate and carrying capacity

The estimate of the local spread rate C (6 km/year) was based on the maximum flight capacity of *T. erytreae* determined by Van den Berg and Deacon (1988), i.e. 1.5 km, multiplied by 4, the estimated number of yearly generations that *T. erytreae* can have in Porto, where *T. erytreae* was first detected.

The carrying capacity K was estimated for each cell in the model, as the product of the average maximum capacity of *T. erytreae* per host tree, k_{tree} and the estimated number of citrus trees in the cell. k_{tree} was determined using data from Catling (1972), corresponding to the mean number of individuals per citrus tree (adults, nymphs and eggs), observed in a 3-year study, under favourable conditions, using the following formula:

$$k_{tree} = \text{Adults} + (0.289 \times \text{Nymphs} + (0.289 \times 0.95 \times \text{Eggs}) \qquad \text{eq. 3}$$

where 0.289 is the nymphal survival rate under natural conditions (Caitling 1970) and 0.95 is the egg viability in optimum environmental conditions (Catling 1972; Van den Berg et al. 1991) to obtain k_{tree} = 2719 adults of *T. erytreae* per tree.

To estimate the spatial density of citrus trees in orchards throughout the country, we used the data from the 2019 agricultural census (INE 2021). This dataset does not include citrus plants in urban and peri-urban areas, as well as isolated citrus trees in rural landscapes. The dataset from INE (2021) provides the area of citrus orchards per county. The density of citrus trees in orchards was assumed, for simplicity, to be the same all over the territory, i.e. 400 citrus trees/ha, considering 5 m × 5 m per tree. Although citrus-tree spacing may vary between 5 m × 4 m or lower and 7 m × 5 m (Cavaco and Calouro 2005; Vacante and Gerson 2012), we considered for simplicity a median value of 5 m × 5 m. The citrus tree density for each 25 km^2 grid cell of the model was estimated, based on spatial data from the Land Use and Occupancy Mapping - COS2018, (available at https://www.dgterritorio.gov.pt/) and the data from the 2019 agriculture census (INE 2021), about the area of citrus orchards.

As data on the density of citrus trees in urban and peri-urban areas were not available from the 2019 agriculture census (INE 2021) and considering its possible influence on the dispersal of *T. erytreae*, we developed an innovative approach to estimate it. We used the spatial data of the COS2018 dataset, to classify the urban and peri-urban areas. Then, we divided these areas into three different classes: Vertical urban areas; Horizontal urban areas; and Discontinuous urban areas (see S1). For each class, the mean density of citrus trees was estimated using Google Street view imagery to survey the number of visible trees in randomly selected polygon areas extracted from the COS2018 spatial dataset throughout the country (Rousselet et al. 2013; Berland and Lange 2017). The estimations were made using the survey counts of citrus trees in each area, weighted against the sample area sizes. Only areas with good image quality were used. We surveyed at least 250 ha for each of the three urban classes considered to provide a confident estimation of citrus tree density. All the surveys were conducted by the first author.

Growth rate

To calculate the growth rate, r, for *T. erytreae* in Portugal, we used climatic modelled data collected for 30 years (Palma 2017). These data were collected for each centroid of a grid of 25 km × 25 km covering Portugal. The climatic variables considered were the daily mean temperature, daily maximum temperature and the daily minimum relative air humidity. The average daily climatic data were grouped into three periods per month. Each period had 10 days, except the last third of each month, which varied from 8 to 11 days, depending on the month. These periods are henceforth called as "10-day periods", needed to calculate the *T. erytreae* survival rate, using the method developed by Catling (1969).

For each 10-day periods, the number of "viable days" (i.e. the number of days above the lower temperature threshold for development) was calculated. Temperatures were estimated, based on the average of the 30 years of the climatic data. A lower temperature threshold of 12.0 °C was considered, based on citrus tree growth not occurring below these temperature values (Webber and Batchelor 1943; Kumar 1977), as well as the inability of *T. erytreae* larva growth (Catling 1973). An upper temperature threshold was not used since we took into consideration the effect of high temperature and low humidity in the variable weather survival.

Weather Survival ($WS\%$) was calculated for each 10-day period, using the mean Vapour Pressure Deficit (VPD) of the three days with the highest daily values of maximum temperature, using Saturated Vapour Pressure (SVP) (Murray 1967; Green and Catling 1971):

$$VPD = ((100 - RH)/100) \times SVP \qquad \text{eq. 4}$$

$$SVP = 610.7 \times 10^{7.5\,Tmax/(237.3+Tmax)} \qquad \text{eq. 5}$$

$$\text{Weather Survival } (WS\%) = 0.0308\,X_3^2 - 4.1825\,X_3 + 137.7709 \qquad \text{eq. 6}$$

where X_3 is the mean value of the VPD in millibars, of the three days with the highest maximum temperatures during the 10-day period.

For the model calculations, we used Weather Mortality ($WM\%$):

$$WM = 1\text{-}(WS/100) \qquad \text{eq. 7}$$

For each area, the number of possible yearly generations was then estimated, as the sum of life cycle progress rounded down from each yearly 10-day period from February until the end of September, the most important period of leaf flushing for citrus trees in Portugal (Paiva et al. 2020). Life Cycle Progress was calculated by dividing the average viable days and the estimated total life cycle duration in days for each 10-day period, calculated using the average temperature of the viable days (VD) and the life cycle duration (G), that is the expected total number of days to successfully complete the insect life cycle from egg to adult.

$$\text{Life Cycle Progress} = VD\,/\,G \qquad \text{eq. 8}$$

The life cycle duration G, in days, was calculated, based on the number of days needed to complete egg incubation (*Idays*) plus the number of days needed to complete nymphal growth (*Ndays*), based on the equations proposed by Catling (1969). To this period, we added 5 days of the pre-oviposition period, being the mean of the pre-oviposition time of the species (Van der Merwe 1923; Catling 1973; Van den Berg 1990) and another 10 days to reach the peak of oviposition (Catling 1973).

$$Idays = 4.9763 + 3.3443 \times 0.8452^{\mathrm{Tmed}-20} \qquad \text{eq. 9}$$

$$Ndays = 16.7974 + 5.2726 \times 0.7843^{\mathrm{Tmed}-20} \qquad \text{eq. 10}$$

$$G = Idays + Ndays + 15 \qquad \text{eq. 11}$$

The growth rate at a given time period t in the year, R_t was calculated as:

$$R_t = f x\ 0.5 \times (1\text{-}WM) \times (1\text{-}\ 0.8\ WM) \times (1\text{-}\ 0.3\ WM) \times \\ (1\text{-}\ 0.15\ WM) \times (1\text{-}\ 0.075\ WM) \times 0.289 \qquad \text{eq. 12}$$

where f is the female fecundity, estimated by the average number of eggs per female. Due to the different fecundity estimates reported in literature, we tested two different values, i.e. 827 and 327 eggs per female (Moran and Blowers 1967; Catling 1969) in the model. The mean fecundity value was multiplied by egg viability rate, estimated as 0.95 in optimal environmental conditions (Catling 1972). We assumed a sex ratio of 1:1 (Van den Berg 1990). *WM*, 0.8 *WM*, 0.3 *WM*, 0.15 *WM*, 0.075 *WM* correspond to the weather mortality for the 1st, 2nd, 3rd, 4th and 5th instars, respectively, since larval stages increasingly become more resilient to the adverse environmental conditions (Catling 1972). Finally, 0.289 is the natural survival rate from egg to adult in “perfect conditions”, with the presence of natural enemies, according to Catling (1970).

For the remaining generations, we considered a geometric growth, $N_t = N_{t-1}R_t$

The resulting R^G=, $\prod R_t$ where G is the maximum potential generations in each cell per year, will reflect the total growth potential of the species in the area for one year (Pointeau et al. 2021). The intrinsic yearly growth rate of the cell is then calculated as follows:

$$r = \ln(R^G)$$

We repeated this procedure for each cell of the model.

A cold limitation factor was further generated considering the reproduction of this species is limited by extremely cold temperatures. Cold and long winter periods can hinder population reproduction and limit the species’ capacity to stay in the region throughout the entire year. Thus, we consider that regions having on average less than 2 days of viable days (i.e. with a mean daily temperature above 12.0 °C), for the three consecutive winter months (December, January and February), were not suitable for *T. erytreae* to establish. The model found that, in these areas, the number of *T. erytreae* was always 0. The period of three months is based on the maximum longevity of 82 days recorded for the species during winter (Catling 1969).

Long distance dispersal

Modelling long-distance dispersal is always very challenging since it is based on stochastic and relatively rare dispersal events. To model human-mediated long-distance dispersal, we calculated the number of long-distance dispersal events (*NB*) that occur in each year, which was randomly chosen using the following equation:

$$NB = 1 + e \qquad \text{eq. 13}$$

where *e* denotes an independent and identically distributed random variable with a Poisson distribution with mean $\lambda = (\ln((P/2642)+1/\ln(2)) \times 5)$, with *P* being the number of cells estimated to be infested at a given time *t* and 2642 is the total number of cells that can be infested in the model.

We used the Poisson distribution as it is a simple discrete distribution that is often used to model jump processes (Hooten and Wikle 2008). The mean λ was defined as a concave increasing function of *P*, with a minimum value of 2.8 when $P = 0$ and a maximum value of 6.0 when $P = 2642$. In this way, the number of simulated long-distance jumps increases with the area infested, taking relatively realistic values compared to the spread pattern observed.

For parameter testing, we made simulations using low-frequency jumps, with low $\lambda = (\text{mean } \lambda)/2$ and high frequency jumps with high $\lambda = (\text{mean } \lambda) \times 2$.

For each long-distance dispersal event, the model randomly chooses a cell that is not yet infested ($N < 1$), with a growth rate $r > 1$ and a suitable human population density. The minimum human density threshold (*H*) allowing the arrival of a long-distance jump was set to 125 habitats/km^2, using the same threshold considered for the spread of the yellow-legged wasp in France (Robinet et al. 2017), which also delimits urban areas relatively well in Portugal, being mostly in the coastal areas of the country, represented as dark red areas in Fig. 1. A recent study showed that both the distance to roads and urbanisation intensity play an important role in spatial and temporal dynamics of the dispersal of the Asian citrus psyllid, *D. citri* in California (Bayles et al. 2022). We did not use road network data nor urbanisation intensity due to lack of reliable complete data available for Portugal. Instead, we used human population density (data from 2017 available at https://www.ine.pt/), as it was demonstrated to being a suitable proxy for road traffic in Portugal (Barata 2012).

Cells infested by such long-distance jumps received an arbitrary set of 50 individuals.

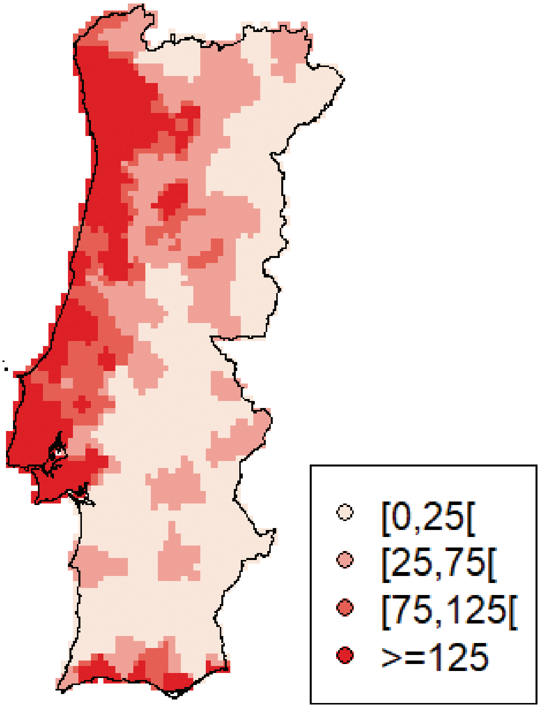

Figure 1. Distribution of the human population density in Portugal (number of inhabitants/km^2) in 2017. The human population threshold of 125 habitants/km^2 was used to characterise locations where long-range distance dispersal could occur in the model.

Multiple introductions

A molecular study on the genetic diversity of *T. erytreae* populations in the Iberian Peninsula suggested the possibility of multiple introductions of the psyllid (Ruíz-Rivero et al. 2021). Two main genetic clusters were observed along the Portuguese coast. Based on these results, we defined two possible scenarios in the model: 1) one single introduction of *T. erytreae* in the north (Porto in 2014); 2) two introductions, the first in the north (Porto in 2014), and a second in the region of Lisbon (Lisbon in 2017), which has an international port and airport. For the second introduction scenario, we added 1000 individuals to a specific cell in the Lisbon area, where *T. erytreae* was first detected in the region (data provided by the Portuguese National Plant Protection Authority reports).

Model running and validation

We combined estimates of local spread and growth with estimates of long-distance dispersal. We applied these models on a grid that covers Portugal with a spatial resolution of 5 km × 5 km. The simulation began in 2014 in one cell in the Porto region with 1000 individuals for the initial condition of the invasion, which was later discovered already spreading, in 2015.

Since the first detection of *T. erytreae* in Porto, in 2015, the Portuguese National Plant Protection Authority, has been monitoring the species spread at the parish level, with the deployment of a trapping protocol surrounding the infested areas within a 3 km buffer range, with yellow-sticky traps and additional, monitoring within 10 km range. In addition, they also included reports from citizens and stakeholders, after confirming their authenticity. Reports are publicly available whenever there are newly-infested areas (available at: https://www.dgav.pt/plantas/conteudo/sanidade-vegetal/inspecao-fitossanitaria/informacao-fitossanitaria/trioza-erytreae/). This complete dataset was provided to us by the Portuguese National Plant Protection Authority (DGAV). We compiled it yearly and used it to validate our model, as well as determining the dispersal capacity, from 2015 until the end of 2021. All the parameters used for the model were estimated independently from this presence/absence observed data, allowing for independent validation.

To validate the model and identify the dispersal scenarios that best fit the observed data, the species' spread was simulated between 2014 and 2021 for different combinations of model parameters (Table 1).

For the simulations considering long-distance dispersal (stochastic sub-model), we ran 300 replicate simulations using randomly generated long-distance events.

We consider that a cell is infested when N ≥ 1 individual.

For the replication of simulations considering long-distance dispersal, we calculated the percentage of simulations that classified each cell as infested. For each cell, if 50% or more simulations predicted the infestation, then the model classifies those cells as infested. Thereafter, for each model, using the simulation data from 2021, we calculated the *F*1-Score performance criteria (Chinchor and Sundheim 1993), using the following equation:

$$F1-\text{Score}=\frac{2x(\text{ precision } x \text{ recall })}{\text{precision } + \text{ recall}}=\left(\frac{TP}{TP+\frac{1}{2}(FP+FN)}\right) \qquad \text{eq.14}$$

where *TP* is the sum of true positives, *FP* is the sum of false positives and *FN* is the sum of false negatives. *F*1-score is the harmonic average of precision and recall.

We compared models with different parameters using the model's performance criteria, *F*1-Score. We also calculated standard errors for each model's *F*1-Score, using 2000 bootstrap simulations taken from the 300 original simulations of each stochastic model. These were used to obtain the *p*-values for testing the equality of the *F*1-Score for pairwise comparisons between two models. *P*-value was calculated by the inversion of the bootstrap normal confidence interval for the difference in means (Thulin 2021) using the following equation:

$$P\text{-value } = 2\left(1-2\left(1-\Phi^{-1}(F1a-F1b)/(\sqrt{SEa+SEb}\right.\right. \qquad \text{eq.15}$$

where Φ^{-1} denotes the inverse cumulative distribution function of the standardised normal distribution. $F1a$ and $F1b$ are the $F1$-score of model a and model b, respectively and *SEa* and *SEb* are the Standard error values of model a and model b, respectively.

We also calculated the Area Under Cover (AUC) of the receiver operating characteristics plots (Fielding and Bell 1997) and the Youden index of each model (Youden 1950), but they led to the same conclusions as *F*1-Score.

The tested parameters were the two different female fecundity rate values (Low or High), the inclusion or not of long-distance dispersal events (Yes or No), the frequency of the long-distance dispersal events (Low, Medium, High), the inclusion of the estimated residential urban citrus trees (Yes or No) and the occurrence of a second introduction of *T. erytreae* in Lisbon (Yes or No) (Table 1).

We compared the estimated local spread rate value against the observed short-range dispersal, based on presence-absence data reports from DGAV. For each year, we calculated the mean least distance between all newly-infested parishes centroids and past infested parish centroids (*DP*). Infested parishes attributed to long-distance dispersal were removed from the short-range dispersal rate calculation. We identified such parishes, when their *DP* was higher than 30 km or was higher than the distance between its centroid and a long-distance dispersal parish centroid. Thirty km is an arbitrary distance value that is significantly higher than the yearly estimated flight capacity of *T. erytreae* and three times the 10 km radius used by the Portuguese Plant Protection Authority for the species monitoring. Finally, with the average value of *DP* from each year, we calculated the average short-range dispersal rate of *T. erytreae* in Portugal for each year and in total using all *DP* values independently of the year of infestation. Furthermore, to calculate the total dispersal capacity to the east and to the south of the country, the main directions the species could spread in Portugal, we used the distance between the infestation origin and the furthest parish towards the east and the south,

Table 1. Parameters and scenarios tested in the modelling.

Model parameters	Scenarios tested	Details
Long-distance dispersal (LDD)	No, Low, Medium, High	No = No LDD Low = λ = (log((*P*/2642)+1/log(2)) × 5) / 2 Medium = λ = (log((*P*/2642)+1/log(2)) × 5) High = λ = (log((*P*/2642)+1/log(2)) × 5) × 2
Fecundity (Fecund)	Low, High	Low = 327 eggs/female High = 827 eggs/female
Number of introductions of *T. erytreae* (LIS)	True False	True = Two introductions; in Porto 2014 and Lisbon 2017 False = One introduction in Porto
Host trees available (Urb)	True False	True = Trees from orchards, plus trees from urban and peri-urban areas False = Trees from orchards only

as was done for the spread pattern of *V. velutina* (Verdasca et al. 2021). Additionally, we calculated the total infestation area of the infested parishes along the invasion years.

Model simulation running, validation and all statistical analysis were done with the statistical language R version 4.2.0 (R Core Team 2022). Modelling Data and R code are available at https://zenodo.org/record/7096566.

Data resources

Portuguese National Plant Protection Authority (DGAV) *Trioza eytreae* Reports – Available at https://www.dgav.pt/plantas/conteudo/sanidade-vegetal/inspecao-fitossanitaria/informacao-fitossanitaria/trioza-erytreae/. Cos2018, Land Use and Occupancy Map 2018 – Available at https://www.dgterritorio.gov.pt/. Agricultural census of 2019 (INE 2021) - Available at https://www.ine.pt/xurl/pub/437178558. Human population density in Portugal from 2017 – Available at https://www.ine.pt/. The R script and the data needed to run the model - Available at https://zenodo.org/record/7096566.

Results

Spread rate

The reports of the Portuguese Plant Protection Authority denote a fast dispersal of *T. erytreae* in Portugal (Fig. 2). Between 2015 and 2021, the African citrus psyllid was able to spread mostly southwards, along the coastal area of Portugal, covering a maximum distance of about 461 km, between Porto and western Algarve and a cumulative area of about 14,239 km^2 (Fig. 2). This corresponds to an average of about 65.9 km/year and 2034 km^2/year. The dispersal towards the east was only 100 km (14 km/year). However, removing long-distance dispersal events, the observed mean dispersal rate of *T. erytreae* in Portugal was 7.8 ± 0.3 km/year (Table 2). The estimated short-range dispersal capacity used in our model simulation was 6 km/year, which turned out to be very close to the observed data (ranging from 5.6 to 10.4 km/year) (Table 2).

Growth rate

Our estimates of the number of yearly *T. erytreae* generations in Portugal varied from 3 to 4 generations per year. The estimated grow rate (*r*) of *T. erytreae* in the Portuguese territory was found to be higher along the coast area (Fig. 3). A different female fecundity rate had a major impact on the growth rates estimated, especially in the interior central and southern regions (Fig. 3). The model included a cold limiting factor, portraying areas whose winter was deemed as too extreme for *T. erytreae* survival, where the growth rate was 0. The cold limited areas are all located in the northern interior part of the country (Fig. 3), where most areas are mountainous and the climate is colder, especially in the winter.

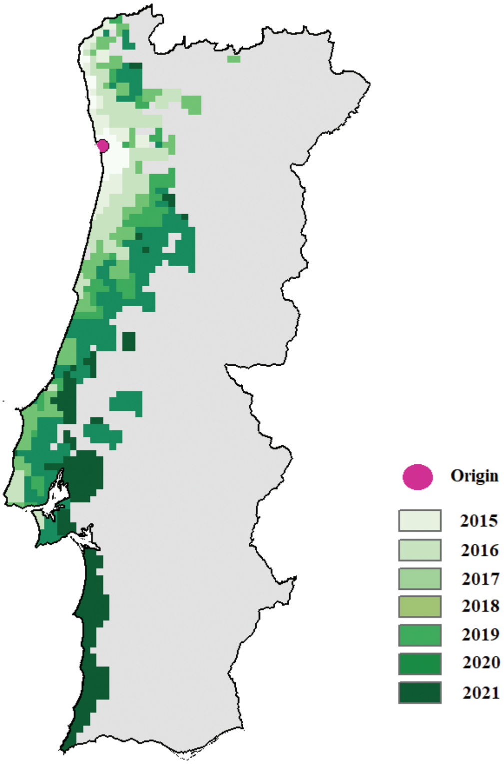

Figure 2. Spatio-temporal representation of Portugal's invasion by *Trioza erytreae*, between 2015 and 2021. Elaborated, based on data from the published by the Portuguese Plant Protection Authority.

Table 2. Short-range yearly mean dispersal distance (± SE) and area of *Trioza erytreae* in Portugal, between 2015 and 2021, based on the reports published by the Portuguese Plant Protection Authority.

Short-range dispersal rate	2015	2016	2017	2018	2019	2020	2021	Total
Mean dispersal distance (km)	10.4 ± 1.3	7.5 ± 2.8	8.5 ± 0.8	5.6 ± 0.6	7.9 ± 0.7	8.1 ± 0.6	5.6 ± 0.6	7.8 ± 0.3
Dispersal area (km^2)	384.7	698.4	1604.5	1612.0	1075.2	4401.7	4462.0	2034.1

Host availability

According to the most recent agriculture census of Portugal, there is a total surface of 21,681 ha of citrus orchards in continental Portugal, 74% of which located in the south, in the Algarve Region (INE 2021). We estimated a total of 11,993,645 citrus trees in orchards and 7,427 trees in urban and peri-urban areas (Fig. 4). The estimated citrus-trees density in Vertical, Horizontal and Discontinuous urban areas were 0.37, 3.2 and 5.14 trees per hectare, respectively (see Suppl. material 1: table S1).

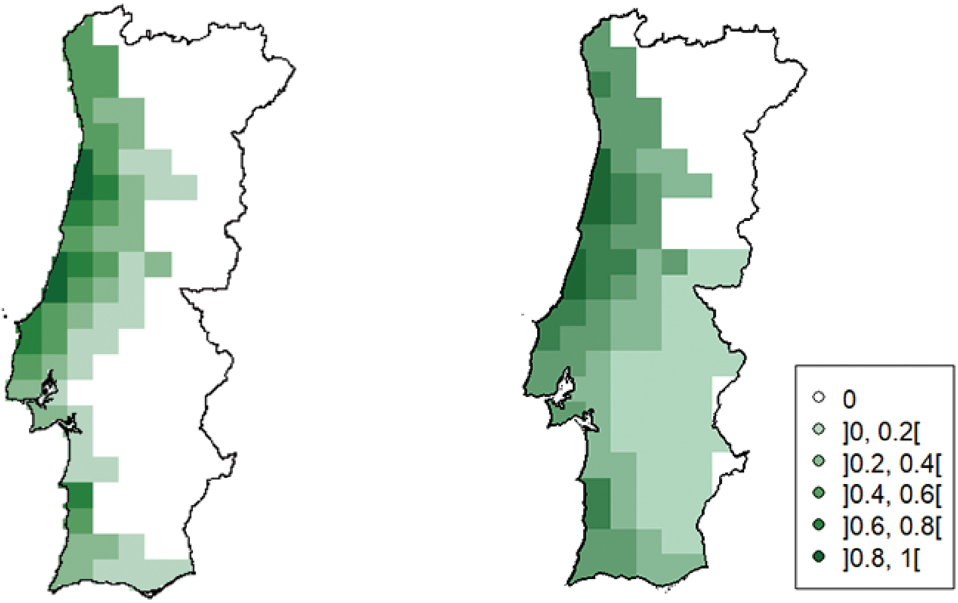

Figure 3. The estimated growth index of *Trioza erytreae* in Portugal, considering two fecundity levels: 327 eggs per female (left); 827 eggs per female (right).

Model validation

The model simulations that included long-distance dispersal fit the observed data better than those that did not (Table 3; see Suppl. material 1: tables S2 for *p*-values). This is shown by the significantly higher *F*1-Score between every model with the same combination of parameters besides the long-distance dispersal, independently of the frequency considered, i.e. low, medium or high (e.g. model 6 *F*1-Score = 0.733 vs. model 30 *F*1-Score = 0.803, *p*-value < 0.001; Table 3, see Suppl. material 1: table S2.1). The difference is even greater if the second introduction scenario is not considered (e.g. model 5 *F*1-Score = 0.583 vs. model 29 *F*1-Score = 0.801, *p*-value < 0.001; Table 3, see Suppl. material 1: table S2.1).

Different frequencies of long-distance dispersal events (low, medium and high) were not consistent in model improvement in all parameter combinations (Table 3, see Suppl. material 1: table S2.2).

The inclusion of the estimated urban and peri-urban citrus trees significantly increased the model performance, with significantly higher *F*1-score values in every model combination (e.g. model 30 *F*1-Score = 0.803 vs. model 32 *F*1-Score = 0.686, *p*-value < 0.001; Table 3, see Suppl. material 1: table S2.3).

The scenario of considering a second introduction was beneficial only for the simulations not using long-distance dispersal, when compared with similar models (e.g. model 6 *F*1-Score = 0.733 vs. model 5 *F*1-Score 0.583, *p*-value < 0.001; Table 3,

Table 3. The 32 different model simulations covering all parameter combinations and the corresponding *F*1-Scores for the model validation against the 2021 observed data.

Simulations	Parameters				Statistics	
	LDD	**Fecundity**	**Urban trees**	**Second introduction**	***F1*-Score**	**SE**
1	No	low	Yes	No	0.530	0.0
2	No	low	Yes	Yes	0.669	0.0
3	No	low	No	No	0.421	0.0
4	No	low	No	Yes	0.546	0.0
5	No	High	Yes	No	0.583	0.0
6	No	High	Yes	Yes	0.733	0.0
7	No	High	No	No	0.463	0.0
8	No	High	No	Yes	0.596	0.0
9	low	low	Yes	No	0.791	0.0035
10	low	low	Yes	Yes	0.790	0.0029
11	low	low	No	No	0.640	0.0055
12	low	low	No	Yes	0.640	0.0060
13	low	High	Yes	No	0.804	0.0023
14	low	High	Yes	Yes	0.795	0.0023
15	low	High	No	No	0.689	0.0026
16	low	High	No	Yes	0.688	0.0024
17	medium	low	Yes	No	0.786	0.0043
18	medium	low	Yes	Yes	0.794	0.0036
19	medium	low	No	No	0.622	0.0065
20	medium	low	No	Yes	0.643	0.0053
21	medium	High	Yes	No	0.800	0.0024
22	medium	High	Yes	Yes	0.800	0.0024
23	medium	High	No	No	0.687	0.0021
24	medium	High	No	Yes	0.684	0.0021
25	High	low	Yes	No	0.789	0.0037
26	High	low	Yes	Yes	0.794	0.0027
27	High	low	No	No	0.615	0.0090
28	High	low	No	Yes	0.637	0.0058
29	High	High	Yes	No	0.801	0.0024
30	High	High	Yes	Yes	0.803	0.0023
31	High	High	No	No	0.683	0.0026
32	High	High	No	Yes	0.686	0.0018

see Suppl. material 1: table S2.4). The same was not true for model simulations considering long-distance dispersal. The parameter was sometimes not significant (e.g. model 9 *F*1-Score = 0.791 vs. model 10 *F*1-Score = 0.790, *p*-value = 0.97), sometimes beneficial (e.g. model 19 *F*1-Score = 0.622 vs. model 20 *F*1-Score = 0.643, p-value = 0.011) and sometimes negative towards model performance (e.g. model 13 *F*1-Score = 0.804 vs. model 14 F1-Score = 0.795, *p*-value = 0.006; Table 3, see Suppl. material 1: table S2.4).

Finally, model simulations that considered high fecundity (827 eggs per female) performed better those with low fecundity (327 eggs per female) in 6 out of 8 parameter combinations. In two cases, changing fecundity did not significantly affect model performance (e.g. model 22 *F*1-Score = 0.800 vs. model 18 *F*1-Score = 0.794, *p*-value = 0.126; Table 3, see Suppl. material 1: table S2.5).

Overall, the model simulations with the highest performance were 13, 21, 22 and 30, showing no significant differences (see Suppl. material 1: table S2.6). All these models used long-distance dispersal, high female fecundity and urban citrus trees, but differed in the long-distance dispersal frequency and in considering the second introduction of *T. erytreae*.

Altogether and considering the temporal evolution between 2015 and 2021, our best model simulations showed a high concordance between the observed and predicted distribution of *T. erytreae* over the seven years after its detection in Portugal (Fig. 5).

Discussion

The major result of this study is that human-mediated dispersal and citrus trees outside orchards play an important role in the spread of *T. erytreae* in Portugal. Hereafter, we discuss in more detail these results as well as other findings.

Role of human-mediated dispersal

Human-mediated dispersal is a well-known documented phenomenon, recognised as a key issue in invasion science (Ricciardi et al. 2017; Bullock et al. 2018; Gippet et al. 2019). Human activities leading to insect dispersal can be divided into three pathways: Contamination, hitchhiking and harvesting (Pergl et al. 2017; Gippet et al. 2019). For the spread of *T. erytreae*, we believe the major pathways behind human-mediated dispersal of the species would be hitchhiking, as suggested for the invasion of *D. citri* in southern California (Bayles et al. 2017) and the invasion of the yellow-legged wasp, *Vespa velutina* in Portugal (Verdasca et al. 2021). In this pathway, adults' psyllids would be accidentally attached to a vehicle vector, from where they may be transported further away from their flight capacity, increasing the potential dispersal capacity of the species. This dispersal pattern coincides with higher dispersal along the coastline, where the human population is denser. It also reflects the distribution of north-south highways along the coast. Additionally, the movement of infested citrus plants is another possible pathway for the dispersal of *T. erytreae* in Portugal.

Since its detection, in 2015, the African citrus psyllid was able to spread mostly southwards, along the coastal area of Portugal, covering a maximum distance of about 461 km, between Porto and western Algarve, in seven years, corresponding to an average of about 66 km/year. This dispersal rate is about 4 to 8 times higher than the values reported for other Hemiptera, such as the hemlock woolly adelgid, *Adelges tsugae* Annand (Adelgidae) (8–13 km/year) and the beech scale, *Cryptococcus fagisuga* Lindinger (Eriococcidae) (14–15 km/year) (Liebhold and Tobin 2008). However, without considering long-distance dispersion events, the observed mean dispersal rate of *T. erytreae* in Portugal was 7.8 km/year, similar to the spread rate of the other insect cases (Liebhold and Tobin 2008).

These results highlight that the spread of *T. erytreae* corresponds to a combination of short-range and occasional long-range dispersal events. This dispersal pattern, called "stratified dispersal" is commonly observed in the spread of invasive insect species (Liebhold and Tobin 2008). For *T. erytreae*, the inclusion of long-distance dispersal events greatly improved model performance in predicting the observed data (Table 3). In the current study, the large difference between the mean global dispersal rate (66 km/year) and the mean diffusion dispersal rate, estimated excluding the long-distance dispersal events (7.8 km/year), greatly highlights the importance of long-distance dispersal events for the species spread, which often are anthropogenic (Liebhold and Tobin 2008).

Likewise, the predicted and observed spread of *T. erytreae* along the coastal area of Portugal is also related with the high population density in the area, mostly between Porto and Setubal regions (Fig. 1), where long-distance dispersal events were concentrated. The role of human mediated dispersal was also reported in the yellow-legged hornet's rapid expansion along the coast of Portugal, attributed to the density of motorways (Verdasca et al. 2021). Nevertheless, motorways density and vehicle traffic are correlated with population density (Barata 2012). Although human-mediated movement of insect life stages is usually the dominant modality of long-distance dispersal, other mechanisms may also be involved, such as the wind, which has not been considered here. For example, Antolínez et al. (2022) showed that the dispersal of the Asian citrus psyllid, *D. citri* may be influenced by wind speed. On the contrary, wind direction was not found to be a significant factor in an experimental trial on *D. citri* dispersal conducted by Lewis-Rosenblumet et al. (2015). Nevertheless, Bayles et al. (2017) suggested that the observed spread pattern of the psyllid in California could be related with the prevailing wind direction, but without supporting a definitive conclusion. Future studies should investigate the possibility of assisted dispersal of psyllids in the upper wind, as an additional long-distance dispersal mechanism.

Our model provided contrasting results regarding the hypothesis of additional introductions of *T. erytreae* in Portugal during its invasion, as suggested by Ruíz-Rivero et al. (2021), based on the genetic diversity of *T. erytreae* populations. When not using long-distance dispersal in the model, including a second introduction, improved model performance towards predicting *T. erytreae* spread in Portugal (Table 3). Yet, when coupled with the long-distance dispersal parameter, the effect of a second introduction on model performance was inconsistent. This was shown by the best model simulations (13, 20, 21 and 30), that either used or did not use the second introduction parameter. This is likely due to long-distance dispersal diluting the importance of the introduction in the model since both have a similar impact on the model. In fact, the approach, which was used to include in the model an additional introduction of *T. erytreae*, was basically based on an input of individuals (1,000) in a defined cell of Lisbon area, which is not much different from a non-random long-distance dispersal event. This outcome further reveals that secondary introductions of invasive species might be frequently misled with long-distance dispersals. Nevertheless, multiple introductions are more than just an addition of individuals to the founding invasive population. They may have an important role in incrementing genetic diversity of the invasive population, compensating the low genetic variability associated with founder effects (Handley et al. 2011). If this increment of genetic diversity is related with new adaptive traits, such as higher fecundity and/or survival rates, then additional introductions are expected to influence the dispersal dynamics of the invasive population. This scenario could be considered in the model, by changing the parameters fecundity and survival. In this respect, it is interesting to note that the higher fecundity value tested was associated with a higher performance of the model.

Role of urban and peri-urban citrus trees

Urban areas often facilitate the introduction, establishment and spread of non-native species, in biological invasions (Cadotte et al. 2017; Gaertner et al. 2017; Hui et al. 2017; Padayachee et al. 2017). Their green areas, including ornamental trees, public gardens, parks and backyard gardens, may function as stepping stones for non-natives species to disperse and invade agroecosystems (Hui et al. 2017). In Portugal, citrus trees are one of the most common plant species present in urban and peri-urban landscapes, used as ornamental plants in street trees, public gardens, parks, as well as food plants in backyard gardens. Using an innovative method, based on Google Street View imagery, we estimated the spatial distribution of those citrus trees outside orchards and its density according to Urban Areas typology (see Suppl. material 1: table S1). Our results showed that these citrus trees played a very important role in the dispersal of *T. erytreae* throughout the country (Table 3, see Suppl. material 1: table S2.3). For large areas of the observed distribution of the psyllid in 2021, where citrus orchards are almost non-existent (Fig. 2), the major source of host plants are the citrus trees in the urban and peri-urban areas (Fig. 4). This corroborates the previous claim that ornamental host species can contribute to connecting fragmented citrus-producing lands, as well as act as reservoir areas for the psyllid, especially in the framework of management actions in citrus-producing lands (Van den Berg et al. 1991).

Similarly, a recent study in California (Bayles et al. 2022), where citrus trees are also common ornamental and food plants in urban and peri-urban areas, also pointed out the importance that these trees played in the invasion dynamics of the Asian citrus psyllid, including its spill-over between urban and agricultural habitats (commercial citrus orchards).

Biological factors

We found no recent information in literature regarding female fecundity and no data available from Portugal on this parameter. For the modelling scenarios, we used two values provided in old literature, one considering a high fecundity of 827 eggs/female (Catling 1973) and an alternative one estimating a lower fecundity of 327 eggs/female (Moran and Blowers 1967) (Table 3, see Suppl. material 1: table S2.5). Our modelling results

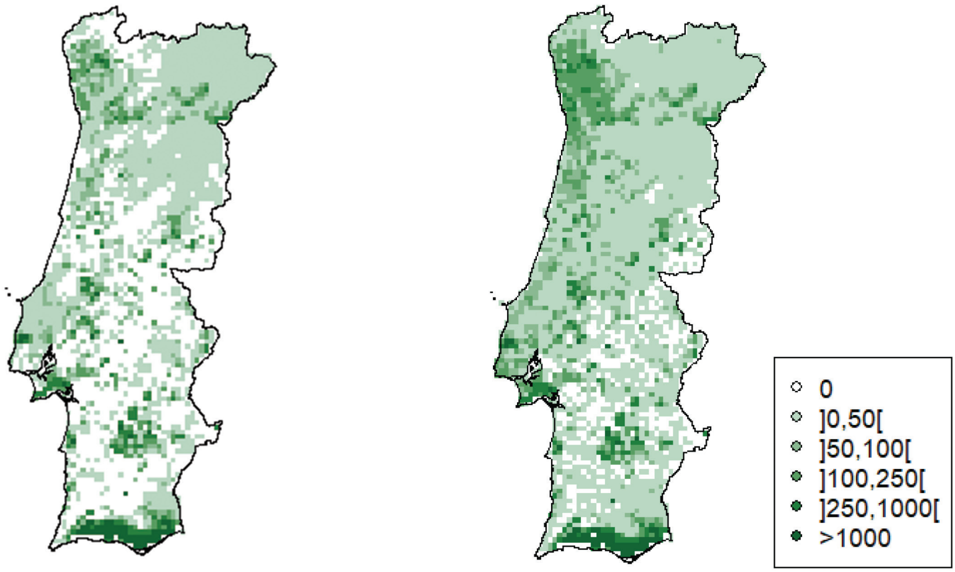

Figure 4. The spatial representations of the estimated citrus trees density (number of trees/km^2) in Portugal. The left map represents the estimation of citrus trees density in Portugal, based on the area of citrus orchards reported in the last agricultural census (INE 2021), while the right map was obtained using both the data from the agricultural census (INE 2021) and our estimates of the number of citrus trees in urban and peri-urban areas, based on Google Street imagery.

showed significant differences according to this parameter, with the simulations using the higher fecundity performing significantly better than those with the lower fecundity. This outcome evidences the relevance to retrieve this type of basic biological data for the understanding of population invasion dynamics. Regrettably, these biological data are sometimes scarce and with low sampling power. Additionally, biological information may change with populations and differ in the invaded range. As these data are essential for modelling potential spread over several generations, we recommend that efforts should be spent on collecting such biological information on the invaded range of the species.

Model performance

Globally, our model was able to predict most of the spatio-temporal dynamics of *T. erytreae* spread quite well, except the recent invasion of the south-western area in 2021, in the coast of Alentejo and west coast of Algarve, for which the model predicted a low colonisation probability of 17% by the psyllid (Fig. 5). This low probability associated to a long-distance dispersal event, into the referred region, is explained by the low human population density in the region. However, a high seasonal touristic flow from the north occurring in this coastal area during Spring and Summer periods was not considered in our model. This large movement of people, including many residents from *T. erytreae*

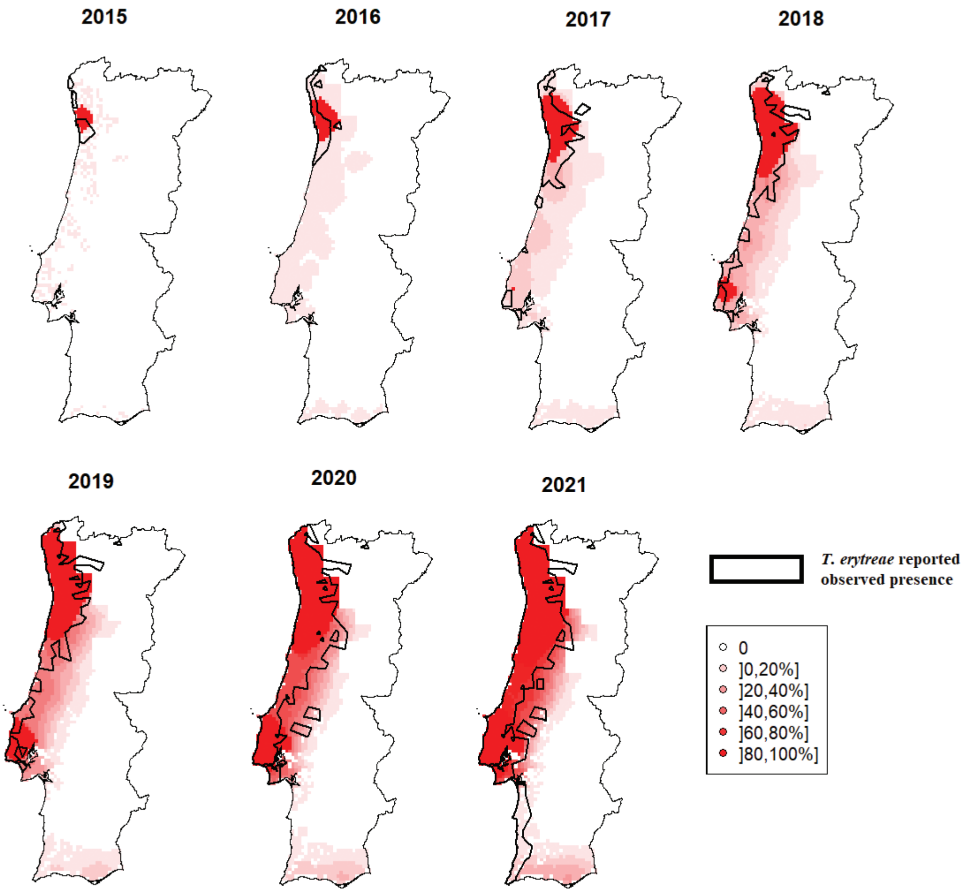

Figure 5. Spatio-temporal dynamics of *Trioza erytreae* spread in Portugal from 2015 up to 2021, predicted using 300 replicate simulations of model simulation #30. The colour gradient represents the probability of each grid cell to be infested according to the model, calculated as the relative number of simulations that predicted the area's infestation. The border of the observed distribution area of *T. erytreae* is represented by a black line (elaborated, based on data obtained from DGAV reports) to allow visual assessment of model performance.

infested areas, may favour hitchhiking mechanisms of dispersal (Gippet et al. 2019). Nevertheless, the observed presence of the psyllid in the area consisted mainly of small colonies or damage in isolated trees or small groups of trees in backyards and gardens (Amílcar Duarte, University of Algarve and Celestino Soares, DRAPALG pers. com., 2021). Furthermore, even if the model predicts low probability of invasion there, the probability is above 0, so it does not predict absence (Fig. 5). This infestation results from relatively rare and stochastic events, which are difficult to predict with a high probability.

The fast spread of *T. erytreae* in Portugal occurred despite the efforts carried out by the Portuguese Plant Protection Authority to contain its dispersal and eradicate it. The measures implemented included the delimitation of demarcated areas, being the infested areas plus a buffer zone surrounding the infested areas with trap placement and active monitoring, along with various other control measures within the infested areas. Such control measures have not been accounted for in our spread model simulations and this may explain the reason why some areas in southern and inner Portugal, with relatively moderate predicted infestation probabilities, were not invaded by *T. erytreae* (Fig. 5). The apparent failure of the model in some parts of the country may result from a certain level of success of the implemented control measures.

Conclusions

Our model showed to be a good tool for simulating the invasion dynamics of *T. erytreae*. It was able to predict the observed spread of *T. erytreae* in Portugal from 2015 to 2021, when considering long-distance human-mediated dispersal and urban and peri-urban citrus trees. Our results support the hypothesis of human-mediated spread being a key-factor in the fast invasion of *T. erytreae* in Portuguese territory. This was highlighted by the fast spread pattern favouring the southern axis, mostly along the coastal area, where there is higher human population density, which was considered for the long-distance dispersal events in the model. Other factors possibly involved, such as the wind, should be considered in future studies. Our results did not support the hypothesis of a second invasion event of *T. erytreae* in Portugal. However, this hypothesis cannot be excluded, based on our results, since our model was not primarily designed to test the hypothesis.

Additionally, our work showed that citrus trees from urban and peri-urban environments had a very important role in the spread of *T. erytreae* in Portugal. This is highlighted by the major impact that they had on model performance, considering their very low relative number in comparison with the estimated orchard trees. Our results contribute to highlighting the importance that isolated host trees can have for species invasive dynamics. These trees are generally disregarded due to lack of statistical data. Finally, we showed that Google Street view imagery can be an efficient tool to estimate the density of urban and peri-urban trees.

Acknowledgements

Thanks are due to the Portuguese National Plant Protection Authority (DGAV) for sending us the reports about the invasion of *T. erytreae*. We also thank Amílcar Duarte (University of Algarve) and Celestino Soares (Direção Regional de Agricultura e Pescas do Algarve) for providing information on the invasion of the coastal area of Alentejo and Algarve by *T. erytreae* and Luis Catela Nunes for advice in the statistical analysis. Thanks are also due to the editor and reviewers for their comments and suggestions, which allowed us to improve an earlier version of the manuscript.

This study was granted by HOMED project, which received funding from the European Union's Horizon 2020 Research and Innovation Programme under grant agreement no. 771271. Pedro Nunes received support from the PESSOA project (TC-05/10) and a SUSFOR (PD/00157/2012) doctoral grant, funded by the Foundation for Science and Technology (FCT), Portugal (PD/BD/142960/2018). Forest Research Centre (CEF) (UIDB/00239/2020) and the Laboratory for Sustainable Land Use and Ecosystem Services – TERRA (LA/P/0092/2020) are research units funded by FCT, Portugal.

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Supplementary material 1

Supplementary tables

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Data type: Docx file.

Explanation note: **table S1.** Estimated citrus tree density of each urban area class, the sampled area used for their estimation in hectares and the description of the urban and peri-urban area classes; **table S2.1.** *p*-values of the *F*1-score pair-comparisons between simulated models not using Long-distance dispersal (LDD) and model simulations using LDD with frequency ranging from low, medium and high; **table S2.2.** *p*-values of the *F*1-score pair-comparisons between simulated models using different Long-distance dispersal frequencies, low, medium and high; **table S2.3**. *p*-values of the *F*1-score pair-comparisons between simulated models using and not using the estimated urban citrus trees in the model; **table S2.4.** *p*-values of the *F*1-score pair-comparisons between simulated models with or without a second introduction of *Trioza erytreae* in 2017 in the model; **table S2.5.** *p*-values of the *F*1-score pair-comparisons between simulated models using *Trioza erytreae* higher or low female fecundity estimates (827 vs 327 eggs/female) for the model; **table S2.6.** *p*-values of the *F*1-score pair-comparisons between the best performing models, using *F*1-score as defining criteria.

Link: https://doi.org/10.3897/neobiota.84.91540.suppl1

NeoBiota 84: 397–413 (2023)
doi: 10.3897/neobiota.84.94109
https://neobiota.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
NeoBiota
Advancing research on alien species and biological invasions

Tree diversity reduces co-infestation of Douglas fir by two exotic pests and pathogens

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Academic editor: M. Branco | Received 26 August 2022 | Accepted 11 January 2023 | Published 18 May 2023

Citation: Stemmelen A, Castagneyrol B, Ponette Q, Prospero S, San Martin G, Schneider S, Jactel H (2023) Tree diversity reduces co-infestation of Douglas fir by two exotic pests and pathogens. In: Jactel H, Orazio C, Robinet C, Douma JC, Santini A, Battisti A, Branco M, Seehausen L, Kenis M (Eds) Conceptual and technical innovations to better manage invasions of alien pests and pathogens in forests. NeoBiota 84: 397–413. https://doi.org/10.3897/neobiota.84.94109

Abstract

The number of non-native invasive pests and pathogens has increased dramatically in recent years, with disastrous consequences for the health of forests worldwide. Multiple studies have shown that mixed forests may suffer less damage from insect pests than single species forest. This "associational resistance" can be notably explained by the fact that heterospecific neighbours make it more difficult for herbivores to locate and then exploit their host tree. However, the validity of these findings in the case of non-native, invasive pests and pathogens remains to be demonstrated. In this study, we monitored over two hundred Douglas firs in pure and mixed plots of a tree diversity experiment to assess the damage from the non-native gall midge *Contarinia pseudotsugae* and the non-native needle cast *Nothophaeocryptopus gaeumannii*. The probability of Swiss needle-cast infection was lower in Douglas fir trees surrounded by heterospecific neighbours. Gall midge damage was lower on Douglas firs surrounded by taller neighbours, consistent with the hypothesis of reduced host Apparency. Douglas fir trees that were more damaged by *C. pseudotsugae* were also more often infected by *N. gaeumannii*. Our study thus provides partial support of the associational resistance hypothesis of mixed forests against exotic pests and pathogens. Promoting forest species diversity at the stand level could, therefore, offer interesting prospects for reducing the impact of biological invasions, especially those involving both pests and pathogens.

Keywords

Douglas fir, exotic pathogen, exotic pest, resource concentration, tree apparency, tree diversity

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Introduction

In the last decades, climate change and increasing global trade and travel have led to a dramatic increase in invasion by non-native forest insects and pathogens (Parmesan 2006; Jactel et al. 2020). Many of those invasive alien species, such as the emerald ash borer (Poland and McCullough 2006), Asian longhorn beetle (Haack et al. 2010), pinewood nematode (Kim et al. 2020) or Swiss needle cast (Hansen et al. 2000) are often responsible for tremendous damage in forests around the world. Invasive pests and pathogens are of great concern not only because of the severe economic damage they generate (Aukema et al. 2011; Haight et al. 2011; Zenni et al. 2021), but also because they threaten the integrity and vitality of forests, thereby limiting the provision of important ecosystem services, such as climate change mitigation, habitat for forest species, pollination or biomass production (Brockerhoff et al. 2017; Griscom et al. 2017; Mori et al. 2017). Hence, while there is a need to reduce the rate of introduction of non-native species, it is also essential to improve our knowledge of the mechanisms that can help mitigate their damage in forests.

Studies from agricultural systems have shown that plant diversity can lead to a decrease in insect damage (Barbosa et al. 2009). This phenomenon, called “associational resistance”, has also received consistent support in forests, where trees are less prone to herbivory when growing in mixtures compared to monocultures (Guyot et al. 2019; Jactel et al. 2021; Ward et al. 2022). Two non-exclusive hypotheses have been proposed to explain associational resistance (Jactel et al. 2021): the natural enemies’ hypothesis (Root 1973; Stemmelen et al. 2022) and the resource concentration hypothesis (Hambäck and Englund 2005). The natural enemies’ hypothesis states that richer plant communities can favour herbivore’s natural enemies by increasing the availability of suitable microhabitats and the amount of complementary prey or alternative resources, such as pollen or nectar and will result in a better control of herbivores. On the other hand, the resource concentration hypothesis states that herbivores will be more likely to immigrate to and less likely to emigrate from monospecific forest patches due to higher resource availability. Another consequence of growing trees in monoculture is that host trees are more accessible, i.e. more easily located by their herbivores. Following the “host apparency” theory (Castagneyrol et al. 2013), host trees surrounded by non-host trees that are taller or emit repellent volatile organic compounds are less likely to be colonised by specialist herbivores (Castagneyrol et al. 2013; Haase et al. 2015).

The mechanisms driving associational resistance effects are further complicated by overlooked horizontal interactions between herbivores and between herbivores and plant pathogens. For example, a meta-analysis by Fernandez-Conradi et al. (2018a) reports that, on average, plant infestation with pathogenic fungi alters host foraging behaviour and reduces insect herbivore performance. Yet, pathogenic fungi are also influenced by tree diversity, which has been well documented in the case of the interaction between oaks and powdery mildew (Field et al. 2020). It follows that any effect of tree diversity on fungal pathogens likely alters the strength and direction of associational effects on insect herbivores (Field et al. 2020). However, since many plant pathogens are vectored by insects or simply benefit from mechanical injuries made by insect mouthparts to plants, the inverse relationship may also be true. It is unsure whether the same mechanisms that are effective against native herbivores are symmetrically efficient against introduced pests. On the contrary, the fact that introduced species have not co-evolved with trees, herbivores and enemies in their introduced range likely alter the effect of tree diversity (Brockerhoff and Liebhold 2017; Nunez-Mir et al. 2017). For example, biological control can be expected to be enhanced in mixed forests only if native predators or parasitoids are generalist enough to switch to exotic preys (Fernandez-Conradi et al. 2018b). If not, then even an increase in the diversity of herbivores' enemies in mixed stands may not be sufficient to increase biological control of exotic pests. On the other hand, the dilution or diversion effect of host trees by non-host trees in mixed species forests can be expected to be more powerful against exotic herbivores that have not co-evolved with these native tree species.

Contarinia pseudotsugae Condrashoff (Cecidomyiidae) is an exotic invasive forest pest in western Europe, originating from North America where it causes damage on Douglas fir (*Pseudotsuga menziesii* Franco), a coniferous tree species also originating from North America (Condrashoff 1961; Roques et al. 2019). It is part of a complex of three species of needle midge, the others being *Contarinia constricta* Condrashoff and *Contarinia cuniculator* Condrashoff (henceforth often referred to as *Contarinia* spp.). In Europe, it was first reported in Belgium and the Netherlands in 2015, from where it spread to France and Germany in 2016 (EPPO 2019; Wilson et al. 2020). Adults lay eggs within opening buds. Few days later, eggs hatch and larvae enter the needles, inducing the formation of galls. Damaged needles tend to bend, gradually change colour and to fall prematurely. Although it is not considered a mortality-inducing factor on its own, heavy infestation by *C. pseudotsugae* can lead to severe defoliation, in association with other pests or pathogens and could threaten the health of Douglas fir trees or impair their growth (EPPO 2019; Ligot et al. 2020; Wilson et al. 2020).

One of the main pathogens frequently co-occurring with *C. pseudotsugae* on Douglas fir is the fungus *Nothophaeocryptopus gaeumannii* (T. Rohde) Videira, C. Nakash., which causes a foliar disease called Swiss needle cast. Although discovered in Switzerland in 1925, this pathogen is native to North America – hence its misleading common name – and is nowadays present in Europe and Australasia (Hood and Kimberley 2005; Kimberley et al. 2011). Symptoms develop slowly, but lead to chlorosis, necrosis and premature fall of the infected needles (Black et al. 2010; Wilson et al. 2020). It is well known that insects can act as vectors for fungal pathogens, either directly or indirectly by wounding the plants and promoting the infection (Almeida and Purcell 2005). Larvae of *C. pseudotsugae* induce the production of new plant tissue during the cecidogenous process and might alter the ability of fungi to invade needle tissues. However and despite the fact that *C. pseudotsugae* and *N. gaeumannii* symptoms often co-occur on Douglas fir, no study so far has investigated the potential interaction dynamics between the pest and the pathogen.

In this study, we assessed the effect of forest mixtures on damage caused to Douglas fir by the exotic pest *C. pseudotsugae* and the exotic pathogen *N. gaeumannii*, as well as the potential effect of *C. pseudotsugae* damage on the co-occurring infection by *N. gaeumannii*. We used a long-term tree diversity experiment to sample 207 Douglas firs of various height, located in plots of increasing tree diversity from monoculture to four species mixtures. We measured needle damage by the Douglas fir needle midge and infection by the Swiss needle cast to test the following hypotheses: (1) Needle damage by *C. pseudotsugae* and *N. gaeumannii* increases with increasing proportion of Douglas fir in forest plot (host concentration hypothesis); (2) Douglas firs taller than neighbouring trees suffer more needle damage than smaller ones (host Apparency hypothesis); (3) Damage by *C. pseudotsugae* increases the probability of infection by *N. gaeumannii*. In doing so, our study aimed at a better understanding of the ecological factors driving primary and subsequent invasion by exotic pests and pathogens in pure vs. mixed forests.

Materials and methods

Study site and tree selection

The study was conducted in Belgium, six years after the first detection of *C. pseudotsugae* in the country, in the tree diversity experiment FORBIO (Verheyen et al. 2013), belonging to the larger network TreeDivNet (Paquette et al. 2018). Specifically, the experiment took place on the site of Gedinne, located in the Ardennes (Belgium, 49°59'N, 4°58'E) and consisting of two sub-sites ca. 2 km apart (Gribelle and Gouverneurs). At each sub-site, the same pool of five species was planted: sycamore maple (*Acer pseudoplatanus* L.), European beech (*Fagus silvatica* L.), hybrid larch (*Larix x eurolepis*), sessile oak (*Quercus petraea* Liebl.) and Douglas fir. Each sub-site is composed of 42 (Gribelle) to 44 (Gouverneurs) plots of increasing tree species richness, ranging from 1–4 tree species (Fig. 1), with a total of twenty different compositions: all five monocultures, all five possible four-species combinations and a random selection of five two- and five three-species combinations (For more details on plot composition, see Verheyen et al. (2013)).

Tree species were planted at an equivalent proportion in mixtures (e.g. 50:50 in two-species mixtures). Even in mixture, trees were planted in monospecific patches of 3 × 3 trees, with patches arranged in a checkerboard pattern in the two-species mixtures and randomly distributed in the three- and four-species mixtures. Therefore, individual trees were surrounded by a varying number of conspecific neighbours, even in mixed plots in which the central tree of each monospecific patch was always surrounded by conspecific neighbours. Finally, four subplots were delimited in each plot and consisted in a 4 × 4 tree patches, where tree height and circumference are measured yearly.

In each of the 20 plots containing Douglas fir (10 plots in each sub-site), we randomly selected three of the four sub-plots in which tree height and circumference had been measured in the current year (2021). We sampled every Douglas fir present

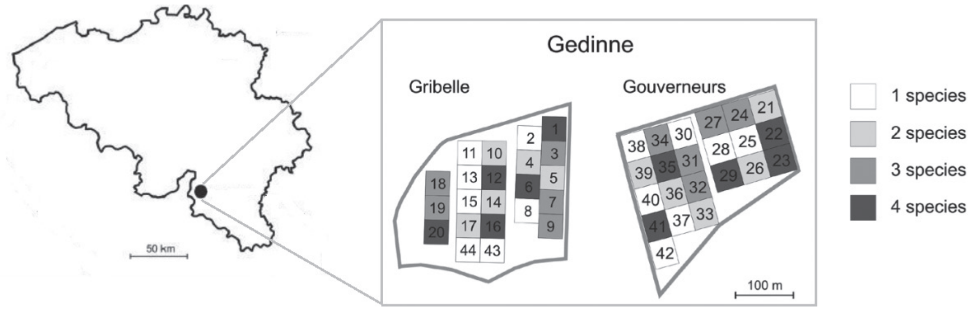

Figure 1. Map of Belgium with the location and experimental set-up of the FORBIO Gedinne sub-sites. The tree species diversity per plot ranged from one species (white) to four species (dark grey).

in those subplots, for a total of 207 trees. Finally, we calculated the mean height difference between each selected Douglas fir and their direct neighbours, regardless of whether they were other Douglas firs or trees of the other three species (henceforth called "Apparency"), setting tree height to zero when neighbours of sampled Douglas fir were missing (dead).

Needle damage by *Contarinia pseudotsugae*

On each sampled Douglas fir, we selected a branch, at mid-height of the tree crown and collected five current-year shoots, starting with the terminal shoot, then picking up every second lateral shoot down to the base of the branch. Shoots were then placed in paper bags, sealed and sent to Bordeaux (France) for damage assessment by a single observer (AS), blind to treatment (plot) identity.

We estimated "needle damage" as the percentage of needles on a shoot that turned dark-brown because of the presence of *Contarinia pseudotsugae* galls, following the methodology developed by the Walloon Forest Health Observatory (OWSF). We used an ordinal scale of six percentage classes of damage: 0%; 1 – 10%; 11 – 20%; 21 – 40%; 41 – 60%; 61 – 90%; 91 – 100%, assigning each of the five shoots to one damage class. Finally, we estimated needle damage at the level of individual tree by averaging the median values of defoliation class across the five shoots. Five needles per trees were collected and used to detect the presence or absence of *N. gaeumannii*.

Infection by *Nothophaeocryptopus gaeumannii*

For DNA extraction, the collected needles were placed in 2ml Eppendorf Tubes containing a sterile metal bead (4mm diameter). Tubes were closed with an AirPore tape, stored at -20 °C and lyophilised in an Alpha 2-4 LD plus freeze dryer (Christ, Osterode am Harz, Germany) for 1.5 days. After lyophilisation, the needles were crushed in a MM400 Retsch Mill (Retsch, Haan, Germany) at 30 Hz for 2 min. Further steps in DNA extraction were done using the Sbeadex Plant kit (LGC Genomics GmbH, Berlin, Germany). Lysis was performed by adding 300 µl Lysis Buffer PVP

(LGC Genomics GmbH) containing 40 µl/ml Debris capture beads (LGC Genomics GmbH), 0.3 µg/µl Proteinase K (LGC Genomics GmbH) and 1.3% 1-thioglycol (Sigma-Aldrich, Buchs, Switzerland) to each sample and subsequent mixing until the samples were well suspended. After incubation at 60 °C for 1 hr, samples were centrifuged at 5,700 g for 5 min and 200 µl of lysate added to a 96-well deep-well plate (Thermo Fisher Scientific, Waltham, USA) containing 420 µl Binding Buffer PN (LGC Genomics GmbH) and 10 µl Sbeadex Particles (LGC Genomics GmbH). The following steps were conducted using the automated KingFisherTM Flex Purification 96 System (Thermo Fisher Scientific). After mixing for 30 min, the Sbeadex Particles with the DNA attached were collected and transferred to 400 µl Wash Buffer PN1 (LGC Genomics GmbH) and mixed for 6 min. This step was repeated before the particles were transferred into 400 µl Wash Buffer PN2 (LGC Genomics GmbH). After mixing for 6 min, the particles were transferred to 50 µl Elution Buffer AMP (LGC Genomics GmbH) and mixed for 7 min to elute the DNA attached to the particles. All DNA purification steps were performed at room temperature. The DNA extracts were stored at -20 °C before dilution and further analyses.

To detect *N. gaeumannii*, we performed a multiplex quantitative real-time PCR (qPCR) with a specific primer pair targeting the beta tubulin gene and the corresponding dual-labelled probe (Winton et al. 2002). An universal 18S primer pair with the corresponding dual-labelled probe 18S uni-P (Ioos et al. 2010) was also used, targeting a highly conserved region of the 18S ribosomal rDNA, to assess DNA quality. The concentrations of the universal primers and probes were reduced in the multiplex reaction mixture to avoid any competition for reagents with the other primers and probes. Quantitative PCR conditions established by Winton et al. (2002) were adapted for high-throughput analyses using the qPCR Takyon core kit NO ROX (Eurogentec, Seraing, Belgium). Reaction volumes of 20 µl contained 5 µl of the 1:10 diluted needle DNA extracts, 1× reaction buffer (Eurogentec), 5.5 mM $MgCl_2$ (Eurogentec), 0.4 mM dNTPs (Eurogentec), 0.1 × ROX reference dye (Invitrogen, Waltham, USA), 0.5 µM of the specific forward and reverse primer, 0.15 µM each of the universal primers, 0.3 µM of the specific probe, 0.05 µM of the universal probe, 0.5 U Takyon enzyme (Eurogentec) and LiChrosolv Water (Merck, Darmstadt, Germany). Cycling conditions were 3 min of initial denaturation at 95 °C, followed by 40 cycles of 10 s of denaturation at 95 °C and 60 s of annealing and extension at 61 °C. All qPCRs were performed in a QuantStudio 5 Real-Time PCR System (Applied Biosystems, Rotkreuz, Switzerland). As a standard in the qPCR assay, a plasmid construct (pUC57 derivate, Eurogentec) containing the sequence obtained by the PGBT primers, was used in a 10-fold serial dilution from 5×10^7 to 5 copies per µl. Five µl of the serial dilution were used for each dilution step and standard curve reactions were performed in triplicate.

Explanatory variables

To test the hypothesis that taller trees are more damaged by *C. pseudotsugae* or more exposed to infection by *N. gaeumannii*, we used Douglas fir absolute (Height) and relative (Apparency) height. We also included the density of Douglas fir in the plot and in the direct neighbourhood – number of Douglas fir trees present amongst the eight nearest neighbors – of a focal Douglas fir tree to investigate the potential validity of the resource concentration hypothesis. Density of Douglas fir corresponded to the number of Douglas fir either in the plot or in the direct neighbourhood of a focal Douglas fir. Finally, as damage by *C. pseudotsugae* could alter the biology of the needle or weaken Douglas fir prior an infection by the Swiss needle cast, we included *C. pseudotsugae* damage as an additional explanatory variable only in the model built to explain the probability of infection by *N. gaeumannii*.

Statistical analyses

We built two different models to test for the effect of explanatory variables on needle damage by *C. pseudotsugae* and infection by *P. gaeumannii*.

First, we used a linear mixed model to test the effect of Douglas fir absolute (Height) and relative (Apparency) height and Douglas fir density in the plot (Density plot) and in the direct neighbourhood (Density neigh.) as well as the interaction between height and Apparency on mean *C. pseudotsugae* damage per tree. Subplots (Subplot_ID), nested within Plot (Plot_ID), were included as random factors to account for spatial autocorrelation. Response variable was square-rooted to satisfy model assumptions of normality and homogeneity of residuals and predictors were scaled. Variance inflation factors were checked for every explanatory variables used in the model and were never > 5, the usual cut-off values used to check for multicollinearity issues (Miles 2014). As the interaction between height and Apparency had no significant effect, we removed it from the final model during the model simplification process.

Second, we used a generalised linear model with a binomial error distribution family to analyse sources of variation in the probability of Douglas fir infection by the Swiss needle cast. We used the same model structure as in Eq. 1, adding *C. pseudotsugae*. damage as an additional covariate. Again, the interaction between height and apparency had no significant effect and we removed it from the final model during the model simplification process. Statistical analyses were performed using R software version 4.1.0 (R Core Team 2022), with the package lme4 (Bates et al. 2015).

Results

The height of the focal Douglas fir studied ranged from 175 to 954 cm, with a mean (± SE) of 559.6 ± 13.0 cm. Mean height (± SE) of European beech, sycamore maple, sessile oak and hybrid larch were 357.8 ± 9.2, 364.0 ± 16.3, 407.6 ± 14.2 and 948.8 ± 13.6 cm, respectively (Supplementary material). Height difference between a Douglas fir and its closest neighbours was on average (± SE) -14.2 ± 13.8 cm, i.e. Douglas firs were on average shorter than their neighbouring trees. Maximum differences were recorded in plots 20 and 22 (Species composition: Beech, maple, larch and Douglas fir, in the Gribelle and Gouverneurs blocks, respectively), where Douglas fir was 400 cm shorter and 520 cm taller than their neighbours, respectively.

Mean needle damage by *C. pseudotsugae* was on average 16.25 ± 1.25% needles attacked, ranging from no damage at all on 12 Douglas firs to 91.5% of damage on the most impacted Douglas fir. Infection by the Swiss needle cast was confirmed on 118 (57%) of the 207 sampled Douglas firs.

Taller Douglas firs (absolute height) suffered significantly more damage by *C. pseudotsugae* than smaller ones (average standardised model coefficient parameter estimate ± standard error: 1.10 ± 0.24, n = 192, Fig. 2, Table 1, model 1). Mean needle damage by *C. pseudotsugae* also significantly increased with Douglas fir Apparency. Douglas firs that were taller than their neighbours suffered from higher damage than Douglas fir that were smaller than their neighbours (0.59 ± 0.22, n = 192, Fig. 3, Table 1, model 1), but the coefficient parameter estimate for this effect was almost twice lower than the one of tree absolute height. Lower Douglas fir Apparency seems to occur more frequently in mixed species plots including *Larix x eurolepis*, the tallest species in the experiment (Supplementary material). None of the other predictors had a significant effect on mean needle damage by *C. pseudotsugae*. Fixed predictors explained 12.1% of the variance in mean needle damage. Fixed plus random predictors explained 19.7% of the variability in mean needle damage by *C. pseudotsugae*.

The probability of presence of *N. gaeumannii* increased significantly with the density of Douglas firs at the neighbouring scale (0.70 ± 0.22, n = 192, Fig. 4B, Table 1, model 2), but not at the plot scale. Additionally, the probability of a Douglas fir being infected by the Swiss needle cast pathogen increased with increasing mean needle damage by *Contarinia* (0.65 ± 0.23, n = 192, Fig. 4A, Table 1, model 2). In this model, fixed predictors and fixed plus random predictors explained 19.6% and 51.8% of the variability in the probability of infection by *N. gaeumannii*, respectively.

Table 1. Summary of model coefficients for the two models presented in this study. Bold characters indicate that parameters are significant. R^2m and R^2c represent R^2 of fixed and fixed plus random factors, respectively.

Models / Parameter	Estimate	Standard error	95% Cl	P value	R^2m (R^2c)
Model 1 – Mean needle damage					**0.121 (0.197)**
(Intercept)	**3.47**	**0.19**	**(3.10; 3.83)**	**< 0.001**	
Apparency	**0.59**	**0.22**	**(0.16; 1.04)**	**0.008**	
Height	**1.10**	**0.24**	**(0.63; 1.60)**	**< 0.001**	
Density neigh.	-0.01	0.15	(-0.31; 0.29)	0.919	
Density plot	0.08	0.19	(-0.29 0.45)	0.668	
Model 2 – Infection rate					**0.196 (0.518)**
(Intercept)	0.29	0.36	(-0.48; 1.09)	0.420	
Apparency	-0.12	0.34	(-0.83; 0.53)	0.711	
Height	-0.54	0.42	(-1.46; 0.26)	0.201	
Density neigh.	**0.70**	**0.22**	**(0.27; 1.17)**	**0.002**	
Density plot	0.61	0.37	(-0.15; 1.45)	0.105	
Contarinia damage	**0.65**	**0.23**	**(0.21; 1.16)**	**0.006**	

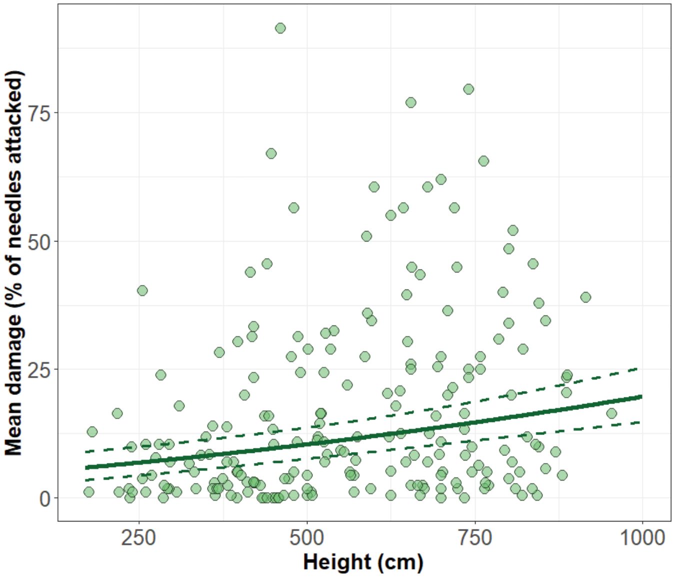

Figure 2. Effect of tree height of a focal Douglas fir on mean damage by *C. pseudotsugae.* Green dots represent individual trees. Solid and dashed lines represent prediction and adjusted standard error of model 1, with values of other model variables set at average (Table 1).

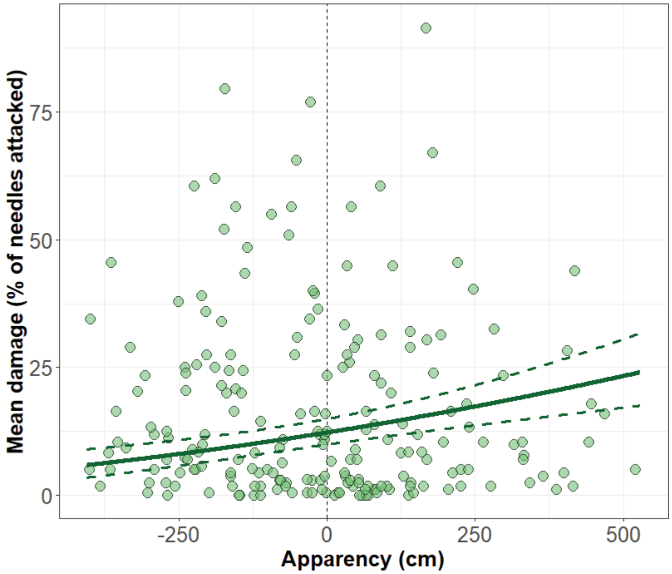

Figure 3. Effect of Douglas fir apparency on the mean damage by *C. pseudotsugae.* Apparency is the mean height difference between a focal Douglas fir and its direct neighbours. Dots at the right and at the left of the vertical dashed line are Douglas fir trees that are taller and smaller than their neighbours, respectively. Solid and dashed lines represent prediction and adjusted standard error of model 1, with values of other model parameters set at average (Table 1).

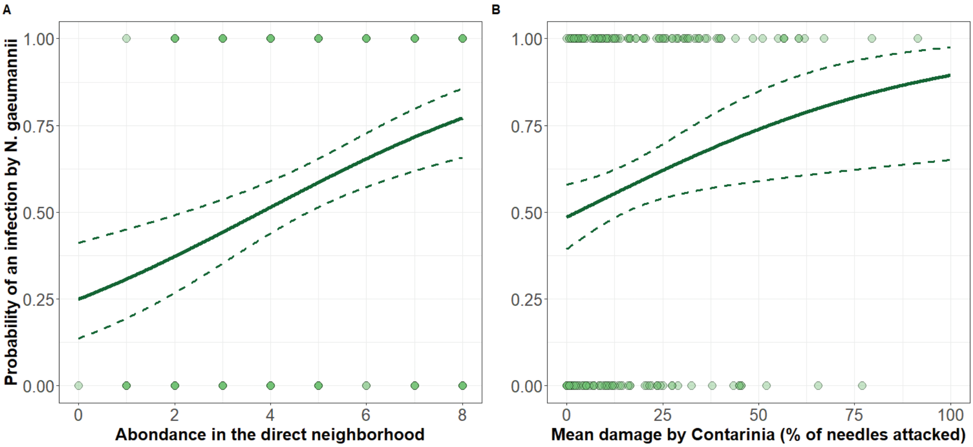

Figure 4. Effects of (**A**) the density of Douglas fir in the direct neighbourhood of a focal Douglas fir and (**B**) of mean damage by *C. pseudotsugae* on the probability of a Douglas fir being infected by *Nothophaeocryptopus gaeumannii*, the causal agent of Swiss needle cast. Solid and dashed lines represent prediction and adjusted standard error of model 2 (Table 1). Dots at probability 0 and 1 represent Douglas fir that were (1) or were not (0) infected.

Discussion

Our study shows that tree height and Apparency were two main drivers of needle midge *C. pseudotsugae* on Douglas fir. Douglas firs taller than their neighbours exhibited more needle damage. We found no effect of Douglas fir concentration, nor at the plot level, nor at the direct neighbours' scale, on damage by *C. pseudotsugae*. However, there was a significant, positive effect of Douglas concentration at the local scale on the probability of infection by the Swiss needle cast fungus *N. gaeumannii*. Douglas fir suffering more needle damage by *C. pseudotsugae* also had higher probability of being infected by the fungal pathogen.

Tree Apparency is a component of tree diversity. The concept was initially coined to describe the probability of a plant being identified by its herbivores (Feeny 1970; Endara and Coley 2011; Strauss et al. 2015). This definition was then refined to include not only the plant's own characteristics (size, colour and odour), but also those of its neighbours, ultimately determining how much a plant is prone to be found by herbivores (Castagneyrol et al. 2013). In this study, we found that more apparent Douglas firs suffered from more needle damage by the Douglas fir needle midge *C. pseudotsugae* than less apparent trees. Taller, non-host neighbouring trees are known to contribute to the disruption of visual (Dulaurent et al. 2012) or olfactory cues (Jactel et al. 2011) used by herbivorous insect. Although no studies so far have been done to identify the mechanisms used by *C. pseudotsugae* to locate Douglas fir trees, it has been shown that midge of the same *Cecidomyiidae* family are using both visual and olfactory cues to find potential hosts (Harris and Rose 1990; Sharma and Franzmann 2001). It could be then possible that taller neighbours would have made Douglas fir trees more difficult to locate by the Douglas fir needle midge, thus leading to a lower level of attacks. Those findings would be consistent with other studies having reported lower herbivore damage on trees concealed by non-host neighbours (Floater and Zalucki 2000; Hughes 2012). The Apparency of Douglas fir trees was calculated, based on the height of their neighbours, regardless of their species. It should be noted, however, that the reduction in this Apparency was more often achieved in the presence of larch trees next to Douglas-firs, as *Larix x eurolepis* grows in height faster than Douglas fir at the juvenile stage (Supplementary material). This suggests that mixed stands of Douglas fir and larch might be less attacked by the midge, although not explicitly tested in this study and will be consistent with the associational resistance hypothesis. Conversely, Douglas fir was consistently more apparent in mixtures with slow-growing oaks and could have resulted in more gall midge damage, i.e. associative susceptibility. This leads us to suggest that it might not be species richness per se that confers greater resistance to mixed-species forests, but the identity and functional traits of the associated species included in the mixture (Jactel et al. 2021).

In addition to the effect of tree Apparency, we also found a positive effect of absolute tree height, with taller Douglas firs suffering from more needle damage than smaller ones. This result confirms the outcome of a study conducted by Castagneyrol et al. (2013) in a similar tree diversity experiment in France, showing that both tree Apparency and absolute tree height were positively correlated with damage by herbivorous insects on oaks. A simple explanation of this finding could be that taller trees are more likely to intercept insects moving in the environment than smaller trees. This effect will add to the increase Apparency of taller Douglas firs giving more visual cues than smaller ones. Another, although more speculative explanation of this pattern is related to the growth-differentiation balance hypothesis (Herms and Mattson 1992; Glynn et al. 2007), which suggests a physiological trade-off between growth and production of secondary metabolites in trees. Secondary metabolites in plants are often involved in defence mechanisms and, as such, fast growing trees are expected to produce fewer secondary metabolites, henceforth having lower defence and exhibiting higher level of herbivory (Lerdau et al. 1994; Massad 2013). Since all trees on the FORBIO tree diversity have the same age, it could be then possible that taller, fast-growing Douglas firs, had a lower amount of chemical defences and, thus, represented more suitable hosts for *C. pseudotsugae*. On the other hand, the Douglas fir midge belongs to the guild of gall makers that are known to manipulate the flow of photosynthetic products in the tree for their own benefit. Thus, the largest and, therefore, most vigorous Douglas-fir trees may be the best hosts for *C. pseudotsugae* as a primary pest.

Unexpectedly, we did not find any effect of Douglas fir density on mean damage by *C. pseudotsugae*. Plots with more abundant resource for the invasive pest were not more infested than plots with a lower concentration of host trees, contradicting the resource concentration hypothesis. This might be due to the small size of the plots in the FORBIO experiment (42 m × 42 m) (Bommarco and Banks 2003), the low level of active dispersion behaviour of the Douglas fir needle midge, or the overriding effect of Douglas fir Apparency that depends more on the species composition of the plots than on Douglas-fir density.

By contrast, the probability of a Douglas fir to be infected by *N. gaeumannii* was positively correlated with the density of Douglas fir in its direct neighbourhood. This result is consistent with recent studies that linked host tree proportion and pathogen transmission amongst neighbouring individuals (Pautasso et al. 2005; Field et al. 2020). Increased resource availability has been theorised to be a key factor in the augmentation of infection by fungal pathogens amongst co-occurring, neighbouring, host trees (García-Guzmán and Dirzo 2004; Keesing et al. 2006, but see Hantsch et al. (2014)). For example, Hantsch et al. (2013) found in a tree diversity experiment in Germany that pathogen loads of several biotrophic fungi of European beech were higher when host trees were surrounded by conspecific neighbours. Ascospores of the Swiss needle cast are wind and water-splash dispersed (Ritóková et al. 2016). As such, it is likely that the transmission of the pathogen was facilitated by the shorter distances between infected and recipient neighbouring Douglas firs in pure plots.

Finally, we found that damage by *C. pseudotsugae* increased the probability of an infection by *N. gaeumannii*. Interactions between two or more invasive species in an area can result in invasion meltdown (Simberloff and Von Holle 1999; O'Loughlin and Green 2017), where a non-native species may facilitate the arrival or establishment of a subsequent invader. It is possible that the presence of *C. pseudotsugae* had indirect effects on the probability of infection by the fungal pathogen, by making Douglas fir more susceptible to colonisation. For example, Meyer et al. (2015) showed that abandoned galls of the chestnut gall wasp *Dryocosmus kuriphilus* could be colonised by the virulent form of the chestnut blight fungus *Cryphonectria parasitica*, which could lead to increased load of virulent inoculum in forests. Gossner et al. (2021) also showed that feeding damage by the weevil *Orchestes fagi* led to higher infection rates of beech leaves by the pathogenic fungus *Petrakia liobae*, by providing entry ports for fungal colonisation. In line with these studies, Douglas firs with a higher amount of galls could be more prone to a subsequent infection by the Swiss needle cast fungus *N. gaeumannii*. However, the opposite may also occur, with the Douglas fir needle midge causing more damage on Douglas firs infected by *N. gaeumannii*. Indeed, numerous studies have already shown that infections by pathogens can lead, directly or indirectly, to a greater susceptibility of trees to insect damage (Stout et al. 2006; Fernandez-Conradi, Jactel et al. 2018b; Moreira et al. 2018). Furthermore, we cannot rule out the possibility that both damage by the Douglas fir needle midge and infection by the pathogen responded to common tree traits that were not measured in this study, but could explain the co-linearity between the two variables. Finally, since the experimental design of this study did not allow us to determine precisely which of the pest or the pathogen initially affect focal Douglas fir trees, it would be interesting to better investigate the temporal dynamic behind the invasion by those two pests and pathogens and the effect that tree diversity could have on the mitigation of invasion meltdown.

Conclusions

Our study provides indirect support of the associational resistance hypothesis of mixed forests against exotic pests and pathogens. In the case of Douglas-fir Swiss needle cast, the effect was mediated by host dilution amongst heterospecific, non-host tree neighbours. In the case of Douglas-fir midge, the resistance effect was due to a reduction in host tree Apparency by taller neighbours. It should be noted, however, that the reduction of infestations by these exotic organisms was not general in mixed stands and might mainly depend on the species composition of the mixtures. In particular, the need to incorporate tree species growing faster than Douglas-fir, such as hybrid larch, could be investigated as a mean of reducing pest damage. We also demonstrated for the first time a positive interaction between Swiss needle cast and Douglas fir needle midge. This underlines the necessity to develop a holistic approach to the problem of biological invasions in forests and, in particular, to look for generic prevention methods, amongst which the increase of forest diversity appears to be promising.

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