Two decades of epidemiological surveillance of the pine wood nematode in France reveal its absence despite suitable conditions for its establishment

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Two decades of epidemiological surveillance of the pine wood nematode in France reveal its absence despite suitable conditions for its establishment

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Abstract

Key message This study takes stock of the first 20 years (2000–2019) of monitoring the pine wood nematode (PWN) in metropolitan France. While PWN was never found in the wild during this period, it was reported in some wood-based commodities entering or circulating on French territory. This stresses the importance of remaining extremely vigilant, as the conditions found in France, especially weather conditions, could be particularly suitable for the pest's establishment.

Context The pine wood nematode (PWN) *Bursaphelenchus xylophilus*, responsible for pine wilt disease (PWD), is one of the most important forest tree pests worldwide. It is thus the focus of many monitoring programmes. In the European Union, for example, it is categorised as a priority quarantine pest, so each member state is obliged to monitor the PWN on its territory.

Aims The first objective of this paper was to describe PWN monitoring in metropolitan France, namely how it is organised and whether it has led to the nematode's detection. Secondly, we wished to investigate what the levels of PWD expression for host pines infected by *B. xylophilus* would be in France. Thirdly, we wanted to find out whether other *Bursaphelenchus* species had been found on French territory during these two decades of PWN monitoring.

Methods We analysed data from samples collected in the framework of the monitoring programme between 2000 and 2019 to track the PWN in its host pines, its insect vector (*Monochamus* spp.) and in wood-based commodities imported into or circulating in metropolitan France. We also generated risk maps of PWD expression based on an evapo-transpiration model using climate data for the period 2000–2019.

Results This monitoring, which was regularly reinforced from 2000 to 2019, consisted of sampling and analysing around 18,000 wood samples and 66,000 insects over this period. Although the PWN was not detected in pine stands

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or within its insect vector, some wood-based commodities were found to be contaminated. Risk maps of PWD expression show that in the most recent years (2015–2019), the weather conditions in a large fraction of metropolitan France were suited to PWD expression, mostly with a delay (i.e., latency) between infection and observable wilt symptoms. PWN monitoring has also revealed the presence of other *Bursaphelenchus* species, most of which were discovered for the first time in metropolitan France and are described herein.

Conclusion While metropolitan France is still free of the PWN, this study emphasises the need to remain cautious as the French territory appears particularly suitable for this pest's establishment. Furthermore, our research has led us to propose some ideas on how to improve PWN monitoring.

Keywords *Bursaphelenchus xylophilus*, Priority quarantine pest, National monitoring programme, *Monochamus* spp., Forest

1 Introduction

It is undeniable that human beings have played, are playing and will continue to play a major role in global change. As numerous studies have pointed out, changes associated with anthropic activities induce selective pressures on both species and communities (Balmford et al. 2003; Crispo et al. 2010; DiBattista et al. 2011; Hendry et al. 2008; Parmesan 2006; Walther et al. 2002). Both trade and human travel lead to the introduction of exogenous species (McKinney 2006), and global change allows many species to expand their host ranges (Parmesan 2006; Thomas et al. 2001). One of the main drivers of species dispersal, however, is the intensification of trade, particularly when combined with the effects of global warming (Walther et al. 2009). Indeed, trade plays a critical role by fostering the dispersal of pests or species that can become invasive, leading to problems for the agricultural and forestry sectors. They must therefore be the subject of close epidemiological surveillance, which becomes the major means of forward planning through risk assessment.

Two main types of monitoring are available for outbreaks in the area of plant health. These are either specific monitoring or general surveillance (ISPM 6 (FAO) 1997). In France, the concept of biovigilance—which has been used to establish several baselines useful for general surveillance—has progressively broadened from a more experimental type of context to the concept of general surveillance. The actions initially carried out on both corn entomofauna and flora, and their successive stages illustrate this progression (Delos et al. 2007). One of the plant health approaches commonly used to understand outbreaks and manage diseases is the plant disease triangle (Brown et al. 1997). This concept, initially developed by Stevens (1960), states that the development of plant disease results from the interaction between three main factors over time, namely the host, the pathogen and the environment (Agrios 2005). This paradigm can be modulated or enhanced by adding new parameters corresponding to other factors such as climate change (Chappelka and Grulke 2015; Grulke 2011) or other ecological concepts (Liu et al. 2019).

Among these additional factors is a vector of the considered pathogen, leading to a three-dimensional tetrahedron representation (Figure 6a in Appendix). Figure 6b in Appendix is its application to our study, where the pathogen is the pine wood nematode *Bursaphelenchus xylophilus* that causes pine wilt disease (PWD), and the vector is an insect belonging to the *Monochamus* genus. PWD is also driven by environmental conditions, particularly air temperature as it tends to occur in areas where the summer mean temperature remains above 20 °C (Rutherford and Webster 1987). This disease can have severe ecological and economic consequences for pine wood stands, as observed within Europe for Portugal (Sousa et al. 2011) but on other continents too, such as Asia (Zhao et al. 2020). The threat posed by *B. xylophilus* to European pine forests has notably led to the inclusion of this pest in the quarantine list defined in annex II, part B, of Commission Implementing Regulation (EU) 2019/2072 (Council directive 2019/2072/EU 2019) and its description as an EU quarantine priority pest according to the Commission Delegated Regulation (EU) 2019/1702 (Council directive 2019/1702/EU 2019).

General and specific surveillance concepts applied together to each component of the tetrahedron are crucial for implementing a relevant epidemiological survey of PWD. It is necessary to quickly detect the presence of plant or forest pests to have a better chance of avoiding any risk of dispersal, especially in the case of *B. xylophilus*, which is able to reproduce quickly (Zhao et al. 2008) and can be spread easily through its insect vector (David et al. 2014). Many destructive and non-destructive methods are available for the general or specific epidemiological surveillance of pests (Augustin et al. 2012). As early detection is one of the key tools for pest management, risk-mapping forecasts contribute to a better spatial and temporal understanding of potential colonisation, the goal being to adapt and improve risk assessment and management measures. In past decades, technological progress notably led to the development of non-invasive detection methods, including image processing, imaging- or spectroscopy-based approaches and the application of remote sensing technologies for disease detection (see the reviews of Ali et al. (2019) and Zhang et al. (2020)). These promising approaches may nevertheless be irrelevant for diseases whose symptoms are not clearly visible from the sky, especially in the case of remote sensing technologies used for crop and forest production surveillance (Zhang et al. 2020). This is particularly true in the case of PWD as contaminated pines do not always develop symptoms, especially in cool areas (Takeuchi and Futai 2007; Zhao et al. 2008). Whatever technical progress is made in the future, a confirmation/invalidation of diseases by laboratory analyses remains and will undoubtedly remain essential, as there are major, critical consequences in terms of forest management. As an illustration, the main management measure following the detection of the pine wood nematode consists in cutting down the contaminated tree (Council directive 2012/535/EU 2012). It is thus essential to establish the right diagnosis in the light of such consequences for pine forests.

Historically, the finding of *B. xylophilus* in Portugal—the first such discovery in Europe (Mota et al. 1999)—led to the design and implementation by the European Commission of emergency measures to prevent the entry into, and the spread within, the European Union (Council directive 2006/133/EC 2006). Following other findings, including the interception of *B. xylophilus* in wood packaging material from infested countries and trees in Spain (previously declared as hosting the disease but with few occurrences, EPPO (2022)), this regulation was updated (Council directive 2012/535/EU 2012). These emergency measures also enforce the establishment of annual monitoring, which consists in *(i)* looking for the nematode by implementing standardised analytical procedures in the laboratory on panels of samples collected from plants, wood or the bark of sensitive species, and *(ii)* looking for signs of the pine wood nematode inside its main insect vector, a Coleopteran belonging to the genus *Monochamus*. In addition to the benefits they bring by early detection of the PWN, monitoring programmes shed light on the diversity of nematodes associated with European pine forests, and especially other *Bursaphelenchus* species (Calin et al. 2015; d'Errico et al. 2015; Torrini et al. 2020). Information collected on such species, which share the same ecological niches as the PWN, can be particularly useful for both improving surveillance and evaluating the risk of the pest's spread (Jikumaru & Togashi 2004; Vincent et al. 2008a).

The introduction of the PWN would be particularly problematic for a country like France, which has a large surface area covered by pine species susceptible to the PWN, especially in the Landes de Gascogne forest in South-West France which mainly consists of the maritime pine *P. pinaster* (Salas-González et al. 2001) (Figure 7 in Appendix). Moreover, the weather conditions found in France could be suitable to PWD expression, especially in the south, as predicted by an evapotranspiration model (Gruffudd et al. 2016). These predictions were nevertheless based on meteorological data collected from 2009 to 2011, and the symptomatic areas may have expanded northward due to the increase in temperatures observed since this period (IPCC 2022). Furthermore, it could be advantageous to know in which areas there is expected to be some latency in symptoms following the tree's infection by the PWN. Indeed, it can be difficult to detect *B. xylophylus* infestation in time for successful eradication in such areas because apparently healthy trees can harbour the nematode (EPPO 2018). Basically, epidemiological surveillance in Europe (and thus in France) combines two main tools—annual monitoring, and the triggering of an emergency plan if the nematode is found in a host tree. These strategies should be constantly reviewed so as to develop them further, tailoring and updating them so they remain geared to the different forms that drivers of dissemination and the introduction of invasive species can take. This is very important because of the exponential increase in international trade (also related to the origin of plant materials introduced, which is probably the most important driver of spread in Europe, Levine and D'Antonio (2003)) and global change (Grulke 2011). The potential introduction of new pathogen species through new plant essences or geographical and political changes in European borders may also play a key role.

This paper describes how PWN monitoring is organised in metropolitan France and takes stock of the first 20 years of its application (2000–2019). It also predicts the potential distribution areas of PWD in this country regarding the expression of wilt symptoms and investigates the diversity of *Bursaphelenchus* spp. present on the territory. We have thus addressed three research questions, namely (i) How many samples were collected and analysed in the framework of PWN monitoring? (and Did this monitoring reveal the presence of the PWN?), (ii) What is the distribution of symptomatic (with or without latency) and asymptomatic PWD areas in metropolitan France according to their respective weather conditions?, and (iii) What is the diversity of *Bursaphelenchus* species revealed by the PWN monitoring conducted in France?

2 Material and methods

2.1 Organisation of PWN surveillance in France

Initiated in 2000, the PWN monitoring applied throughout metropolitan France has been regularly updated in keeping with changing European regulations and new scientific findings. It can be divided into three parts (Figure 8 in Appendix). The first part concerns the inspection of wood-based commodities entering or circulating in France for the presence of the PWN or its vector, *Monochamus*. The second part of the plan focuses on the monitoring of host trees i.e. checking whether the nematode is present in coniferous stands. Thirdly, the PWN is also monitored by looking for its vector, *Monochamus sp.*, using traps set up throughout France. Many actors are involved in French PWN monitoring, including in the coordination, inspections, sampling and the analytical process (Figure 8a in Appendix). All the laboratories involved in sample analysis (i.e. the National Reference Laboratory (NRL) and official laboratories) are accredited to ISO standard 17025 (ISO/IEC 2017), guaranteeing their technical competencies and the reliability of the results produced. As *B. xylophilus* has been regulated as a priority quarantine pest in the European Union since 2019, member states are required to draft a national emergency plan. The French contingency plan was designed to prepare official services for the implementation of sanitary measures. The plan, which would be activated if a host tree is found positive to the PWN by an official analysis (Figure 8b in Appendix), specifies the limits of the infested area and a buffer zone around it. It also explains the inspections that must be implemented as part of the contingency measures to eradicate the pathogen or restrict its spread.

2.2 Sampling of the PWN

2.2.1 Monitoring of wood-based commodities entering or circulating within France

Inspections concern primarily wood packaging material (such as crates, pallets or dunnage), logs (mainly with bark), sawn timber and large areas of bark. Checks are carried out at border control points by the border inspection services and on high-risk sites by official samplers. High-risk sites are those targeted by regional risk analysis for their high probability of introducing or spreading the nematode; they include ports, logistic hubs or motorway service areas. Particular attention is paid to wood-based commodities from countries known to be infested by the pine wood nematode. The country of origin of these commodities is either known through the ISPM15 stamp, certifying their phytosanitary treatment (ISPM 15 (FAO) 2018) or their European phytosanitary passport (in the case of wood from the European Union). Checks consist of visual inspections of the wood to detect any signs that may be related to the presence of the PWN, such as emergence holes made by *Monochamus* beetles or the presence of fungi. Indeed, because the PWN can have two kinds of diet—phytophagous and mycophagous—fungi are a source of food allowing the nematode to survive (Karmezi et al. 2022). If such signs are observed or, to a lesser extent, even in the case of asymptomatic wood without any signs, this visual inspection is followed by sampling. For this purpose, different boards making up the wood packaging materials are sampled at different points by drilling holes either with a large wood bit to obtain chips or with a hole saw to obtain circular samples known as wood pieces (Fig. 1). These wood pieces must not be bigger than 3 cm per side to ensure the absence of living *Monochamus* (pupae or juvenile stages) in order to avoid any risk of dispersal via sample transport (Anses 2019). Each sample, representing around 250 g of wood or 800 mL of chips, is then divided into two duplicates and placed in hermetically sealed plastic bags to which water is added to prevent desiccation, then sent to a laboratory for analysis.

2.2.2 Monitoring of pine forest stands

Conifer stands are regularly observed to detect the presence of the PWN, especially when the species are known to be susceptible to the nematode, such as *Pinus sylvestris* or *P. pinaster*. Conifers located next to high-risk sites for the transit of processed wood are also placed under surveillance. The trees are usually monitored through visual observations from the ground, which can lead to the tree being sampled when wilt symptoms are detected. Dying trees that are still standing are sampled at ground level using a large wood drill bit. If felled, the samples are taken from the crown (chips or slices of branches cut with a saw). Like wood-based commodities, these samples are divided into two duplicates that are then sent to laboratories for analysis. There they are incubated for 14 days at 25 °C before analysis to encourage the multiplication of the PWN and thus enhance the probability of detection.

2.2.3 Monitoring of the PWN insect vector Monochamus spp.

Since 2013, traps (Crosstrap®) diffusing pheromones (Galloprotect®) have been placed in the largest and most sensitive stands of *Pinus* and next to high-risk sites in order to catch the PWN insect vectors, namely beetles belonging to the genus *Monochamus*. As these traps diffuse pheromones, they therefore capture only mature adult beetles. As these traps also contain an insecticide, they kill the insects caught to avoid any escape. Each trap is set up for 40 days from April to October (corresponding to the insect's flight period) and the insects are collected from the traps every 10 to 15 days. After this 40-day period, the traps are moved to another area needing to be monitored. The insects caught in the traps are sorted by the people in charge of trap monitoring, and only *Monochamus* spp. are sent to the laboratory to determine if they contain *B. xylophilus* (Fig. 1).

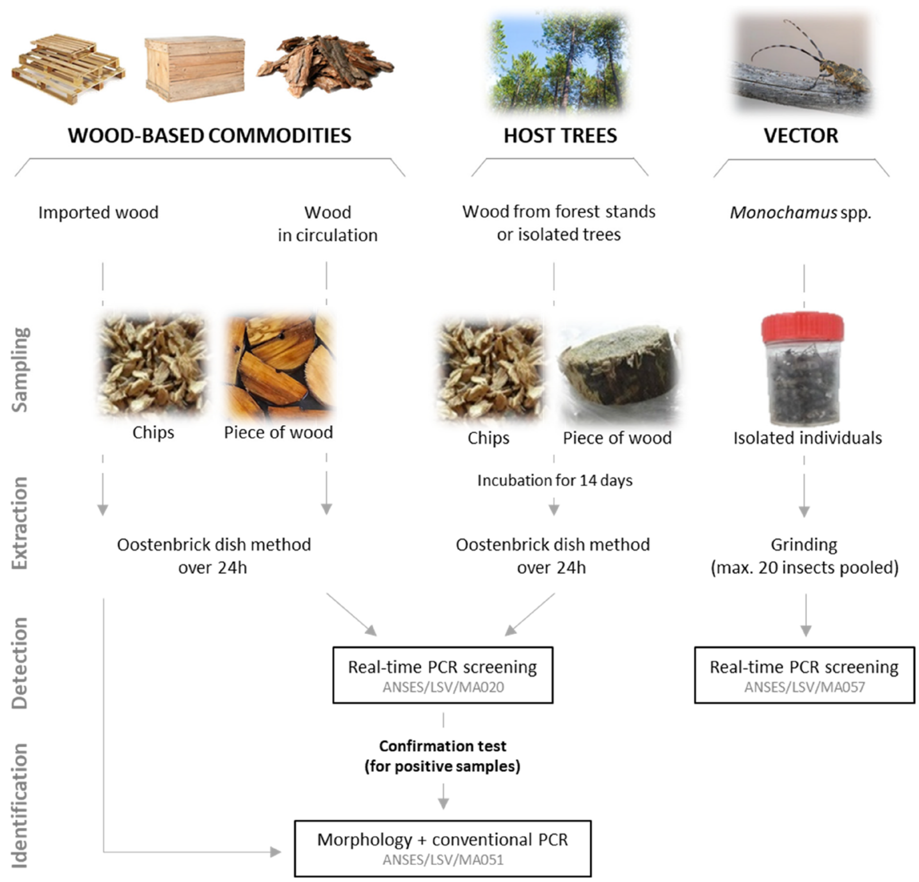

Fig. 1 Routine analytical process for *B. xylophilus* detection within the metropolitan France monitoring framework (in grey: the reference for the analysis method)

2.3 Detection and identification of PWN

Nematodes are extracted from the wood samples using the Oostenbrick dish extraction method (EPPO 2013). From 2000 to 2011, extraction was followed by the detection and identification of *B. xylophilus* in the samples using morphological characteristics as described in Braasch (2001); Ryss et al. (2005); Sarniguet et al. (2013). If *B. xylophilus* was detected or when results were doubtful, a molecular analysis method was applied to the duplicate sample, namely the conventional PCR analysis developed by Castagnone et al. (2005) or Matsunaga and Togashi (2004). Moreover, the morphological identification carried out was also useful in detecting other nematodes belonging to the *Bursaphelenchus* genus (at the species or group level). For some species, such as *B. leoni* or *B. poligraphi*, this identification was further confirmed or supplemented by a PCR–RFLP as described by Burgermeister et al. (2005, 2009). In this procedure, all the samples were analysed at the French NRL due to the taxonomical expertise required for the morphological identification. As the number of samples to be analysed has regularly increased over time (Fig. 2), this has led to the development of standardisable and validated analysis methods not requiring expertise in morphology, easily transferrable to the network of official laboratories.

Therefore, since 2011 for monitoring pine forest stands and since 2018 for monitoring wood-based commodities, samples are sent to one of the official laboratories which use the following analysis method: after the Oostenbrick dish extraction method, PWN DNA is detected by real-time PCR. This rapid screening test, developed by François et al. (2007), is published as an official method under the reference ANSES/LSV/MA020 and is described in EPPO's PM7/4 (3) (EPPO 2013). If the PCR test is positive for the PWN's presence, the duplicate sample is sent to the NRL for confirmation. This second test includes extraction according to the Oostenbrick dish method followed by a morphobiometric analysis of individuals in the genus *Bursaphelenchus* detected in the extract. Moreover, a conventional PCR analysis developed by Castagnone et al. (2005) or Matsunaga and Togashi (2004) is performed on isolated nematodes and can confirm the

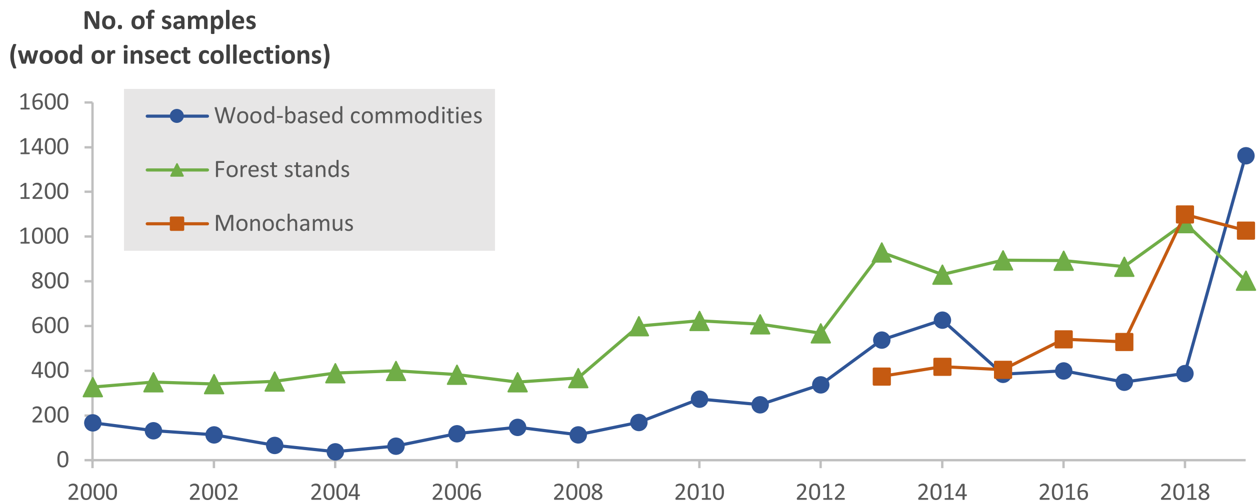

Fig. 2 Annual number of wood samples (wood-based commodities and host trees) analysed and number of collections from *Monochamus* sp. traps during French monitoring of the PWN from 2000 to 2019

presence of *B. xylophilus* in the sample. This process is identified under the reference ANSES/LSV/MA051. An exception is made for samples of wood imported from outside the EU, which are still sent directly to the NRL to be analysed using the morphobiometric and conventional PCR methods described above. This particular treatment depending on the kind of sample involved is due to the need to obtain quick results.

Under the monitoring programme for the PWN insect vector *Monochamus*, insects are sent to an official laboratory to be tested for *B. xylophilus* using the real-time PCR method based on the PCR analysis developed by François et al. (2007). Only the rear part of the insect is analysed as nematodes are mostly found in the respiratory system (Lai 2008; Naves et al. 2006). All the trapped insect vectors are analysed by groups of 20 individuals (at once) in order to ensure high detection sensitivity. The method used is available on the ANSES website under the reference ANSES/LSV/MA057 and is also described by EPPO (PM7/4 (4), forthcoming).

2.4 Risk maps of PWD expression

PWD expression following an infection of a susceptible pine by *B. xylophilus* can be very dependent on weather conditions, especially temperature (Rutherford and Webster 1987). Gruffudd et al. (2016) described an evapotranspiration model, based on an initial work of Evans et al. (2003), which predicts the European regions that are likely to succumb to PWD. Gruffudd et al. (2016) also showed that the use of only one meteorological parameter, the mean summer temperatures (MST) (i.e. the average temperature over June, July and August), was enough to very accurately predict the risk of PWD expression or not at a particular location. More precisely, areas where MST ≥ 19.14 °C are very likely to be affected by PWD, and conversely, no PWD symptoms are expected for areas where MST < 19.14 °C. In addition to this 'lite' model to predict PWD risk, the authors also developed a 'latency model' to predict whether there is a high probability of latency in PWD symptoms in a specific location by using two meteorological parameters, the MST and the mean annual temperature (MAT) (i.e. the average annual). Indeed, they showed that if MST < 23 °C and MAT < 14 °C, delayed wilt expression (of at least one year) is expected.

For this paper, we thus applied these thresholds to find out the distribution in metropolitan France of the three kinds of areas regarding PWD expression (i.e. asymptomatic areas, latency areas, symptomatic areas) and to know how these areas evolved from 2000 to 2019. More specifically, areas were classified as asymptomatic areas for MST < 19.14 °C, as latency areas (i.e. symptomatic areas with latency) for 19.14 °C ≤ MST < 23 °C and MAT < 14 °C, and symptomatic areas (without latency) corresponded to areas where MST ≥ 19.14 °C and MAT ≥ 14 °C and MST ≥ 23 °C & MAT < 14 °C. For this purpose, we used the daily data of 'Météo France' (SAFRAN dataset, which is at an 8 km × 8 km resolution).

2.5 Data analysis

The data used in this research were collected from the Regional Directorate for Food, Agriculture and Forestry (French Ministry of Agriculture) and the laboratories that analysed samples in the framework of PWN monitoring (NRL and official laboratories). They consisted of information on the location of sampling and the country of origin of the commodity sampled (in the case of imported wood materials), identity of the host species (in the case of tree sampling) and the sample's status i.e. detection or not of *B. xylophilus*. Moreover, we had information on the other *Bursaphelenchus* species identified in the samples during the first part of monitoring pine forest stands (2000–2011). Finally, the maps of PWD expression were created with the software R (version 4.2.0) (R Core Team 2020).

3 Results

3.1 Sampling effort for PWN monitoring in metropolitan France from 2000 to 2019

3.1.1 Monitoring of wood-based commodities

From 2000 to 2019, 6037 samples of wood-based commodities entering or circulating within metropolitan France were collected and analysed in the framework of PWN monitoring. The number of samples remained under 200 per year from 2000 to 2009 and then progressively increased until 2014, when more than 600 samples were collected (Fig. 2). Between 300 and 400 samples were collected in the following years, peaking in 2019 to over 1300 samples (Fig. 2). The location of the sampling was known in almost all cases as only 3.2% could not be assigned (mainly due to a change in the laboratory information management system). We can note that the sampling effort was particularly high in the regions with the main national airports and seaports: Normandie, Occitanie, Île-de-France and Provence-Alpes-Côte d'Azur, with more than 700 samples collected over the period considered in each of these regions, representing 61.3% in total (Fig. 3a). The surveillance of wood packaging also included visual inspections, which were even more numerous than samples collected as a visual inspection does not necessarily lead to sampling. The total number of visual inspections is nevertheless difficult to estimate as they were not always recorded.

3.1.2 Monitoring of pine forest stands

During the 2000 to 2019 PWN monitoring of standing conifers in France, 11,940 samples were collected and analysed. The monitoring devices were stepped up during the period considered, in which we can observe three separate plateaux. During the first part of the monitoring programme, from 2000 to 2008, the number of samples collected remained stable, at around 300 to 400 per year (Fig. 2). In 2009, the sampling effort was increased, leading to around 600 wood samples being collected; it then remained at this level for 4 years. Finally, the third plateau is visible from 2013, with more than 800 samples each year even though a slight decrease was observed in 2019 (Fig. 2). Like for wood-based commodities, the location of the sampling was known for the vast majority of the forest stand samples, with less than 5% that could not be assigned. As shown in Fig. 3b, there is clearly a much higher sampling effort among stands in the south of the country (around 70% of samples) than in the north (30% of samples). We can also add that among the northern regions, the region Grand Est in eastern France can be distinguished from others, with its 849 samples (Fig. 3b).

3.1.3 Monitoring of Monochamus spp.

As part of the surveillance programme for *Monochamus* in metropolitan France, insects were collected through a trapping network. In total, this network was responsible for 4396 insect collections (emptying of traps) from 2013 to 2019, leading to the collection of a total of 66,357 insects belonging to the *Monochamus* genus. Although insects were collected on 375 different occasions during

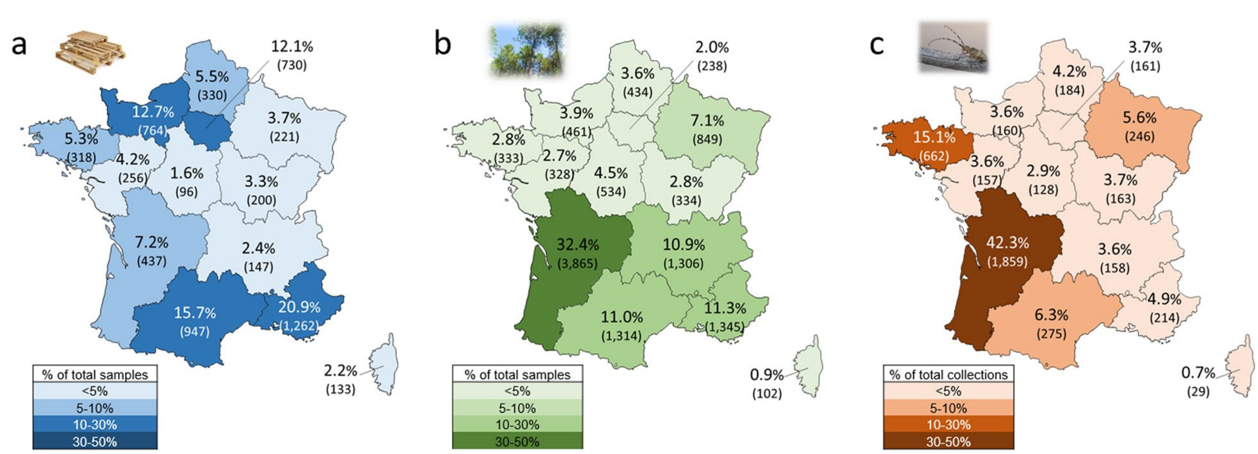

Fig. 3 Sampling effort in the framework of French monitoring of the PWN from 2000 to 2019 for **a** wood-based commodities, **b** samples of wood collected from standing trees and **c** collections of insects from *Monochamus* spp. traps. For each map, we have indicated the proportion of samples for each region (out of the total sampling), while the number of samples is indicated in brackets

the first year of the monitoring programme, the sampling effort regularly increased in the following years, with notably more than 500 collections in 2016 and 2017 (Fig. 2). Insect trapping was then considerably reinforced, with more than 1000 annual insect collections in 2018 and 2019. According to the geographical area, high disparities may be observed in the number of insect collections from 2013 to 2019. During this time, in two western regions—Bretagne and Nouvelle-Aquitaine—the sampling effort was particularly sustained, with more than 600 insect collections (Fig. 3c). With 1859 collections, Nouvelle-Aquitaine notably accounts for more than 40% of all sampling during the monitoring programme. This region also accounts for the highest number of *Monochamus* insects collected (i.e. 40,011). A lower sampling effort is observed for the rest of the country with fewer than 300 insect collections from traps in each of the other regions (Fig. 3c). We can nevertheless note that the sampling devices allowed more than 5000 *Monochamus* spp. to be collected in the two southern regions of Provence-Alpes-Côte d'Azur and Occitanie.

3.2 Detection of the PWN

Analyses performed on the wood from pine forest stands sampled from 2000 to 2019 and on *Monochamus* sampled from 2013 to 2019 did not reveal any *B. xylophilus* specimens. Nevertheless, while no PWN outbreak was detected in France during this period, the checks carried out during these two decades on wood-based commodities entering or circulating within the country intercepted 41 samples contaminated by living *B. xylophilus* (Table 1). This figure represents 0.66% of all the samples (6037) analysed during the period considered. These contaminated batches were from four different countries, though mostly from Portugal ($n=27$). The other contaminated products came from China ($n=4$), Morocco ($n=4$) and Canada ($n=2$), but the origin of the four pallets was unknown due to the absence of the stamp required by ISPM15. Moreover, more than 80% ($n=35$) of the samples concerned pallets used to transport auto parts, stones, food products or wood material, though some were free of goods at the time of the inspection (N/A in Table 1). The rest of the interceptions concerned dunnage

Table 1 Summary of samples of wood-based commodities entering or circulating within metropolitan France in which the PWN was detected

Year	No. of samples	Type of product	Country of origin	Goods transported[b]	Administrative region of interception in France
2000	1	Dunnage	China	N/A	Normandie
2001	1	Dunnage	Canada	N/A	Normandie
2008	2	Pallet	China	Stone	Provence-Alpes-Côte d'Azur
2010	1	Pallet	Portugal	Stone	Grand-Est
	4	Pallet	Portugal	N/A	Grand-Est
	2	Pallet	Portugal	N/A	Provence-Alpes-Côte d'Azur
2012	1	Pallet	Canada	Wood materials	Normandie
	3	Pallet	Morocco[a]	Food products	Occitanie
2013	1	Pallet	Morocco[a]	N/A	Occitanie
	1	Pallet	Portugal	N/A	Provence-Alpes-Côte d'Azur
2015	1	Pallet	Portugal	N/A	Bourgogne-Franche-Comté
2018	2	Tree bark	Portugal	-	Ile-de-France
	2	Wooden crate	Portugal	Stone	Grand Est
	5	Pallet	No ISPM15	N/A	Nouvelle-Aquitaine
	1	Pallet	Portugal	Stone	Grand Est
	1	Pallet	Portugal	Auto parts	Bourgogne-Franche-Comté
2019	1	Pallet	Portugal	Wood materials	Grand Est
	1	Pallet	Portugal	Auto parts	Auvergne-Rhône-Alpes
	1	Pallet	Portugal	Auto parts	Bretagne
	5	Pallet	Portugal	Auto parts	Hauts-de-France
	1	Dunnage	China	Garden furniture	Nouvelle-Aquitaine
	1	Pallet	Portugal	N/A	Pays de la Loire
	2	Pallet	Portugal	Auto parts	Bourgogne-Franche-Comté

[a] Moroccan pallets manufactured with wood from a contaminated country

[b] Wood-based commodities free of goods at the time of inspection are marked N/A

(transporting garden furniture or free of goods), wooden crates (transporting stones) and tree bark. A total of 19 interceptions of wood packaging materials were on materials not carrying any goods at the time of inspection. The number of wood products found positive for the PWN each year has tended to increase over the two decades of monitoring. Indeed, over half of the PWN-contaminated products concerned just two years, 2018 and 2019, with 11 and 12 positive cases, respectively. These figures may be compared with the total of 11 contaminated wood products found during the first ten years of the monitoring programme (Table 1).

3.3 Risk maps of PWD expression

Figure 4 shows the evolution of the three areas regarding the expression of PWD symptoms (symptomatic, latency and asymptomatic areas) in metropolitan France from 2000 to 2019. For convenience, the results were split into four periods of 5 years, but the area distributions are given for each year in the Figure 9 in Appendix.

For the period 2000–2004, around 58% of the country (i.e. 321,524 km^2) did not have weather conditions suitable for the expression of wilt symptoms (Fig. 4a). These asymptomatic areas were mostly located in the northern half of the territory and in mountains (Massif Central, Pyrenees and Alps). The rest of the country's weather conditions were conducive to the expression of PWD symptoms, either in the year of the infection (symptomatic areas) or with a delay of at least one year (latency areas). The symptomatic areas were very limited (i.e. 4.4% of the territory) and mainly located along the Mediterranean coastline, in the southeast of the country (Provence-Alpes-Côte d'Azur and Corse) whereas the latency areas, accounting for 37.0% of the territory, were located in the south-west, centre and east of the country (Fig. 4a).

For the periods 2005–2009 and 2010–2014, the symptomatic areas remained unchanged. However, some locations classified as latency areas in 2000–2004 turned into asymptomatic areas, which thus covered around 70% of

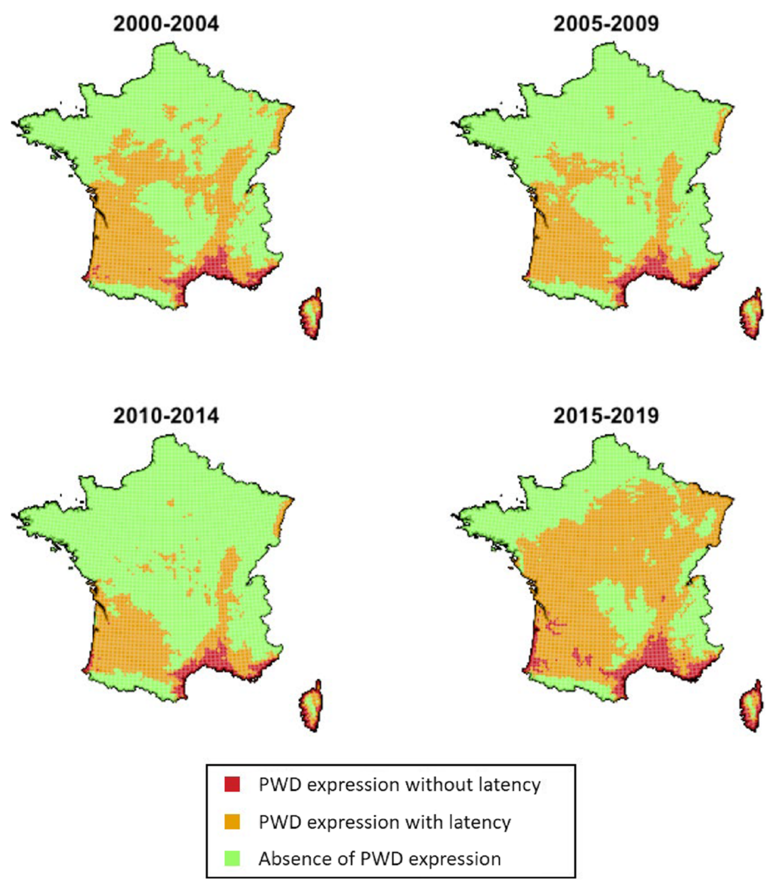

Fig. 4 Risk maps of PWD expression for metropolitan France from 2000 to 2019 according to the mean annual temperature (MAT) and mean summer temperature (MST). The construction of these maps was based on the works of Gruffudd et al. (2016) (see the Section 2 for further information)

the territory. The latency areas accounted for approximately 25% for these same periods (Fig. 4b, c).

In contrast, for the period 2015–2019, the asymptomatic areas were about half of what they were in the previous two periods. These areas only accounted for 35.4% of the territory, being restricted to regions along the English Channel and the mountainous regions of the Massif Central, Pyrenees and Alps (Fig. 4d). For this period, most of the country (i.e. 58%) was classified as latency areas whereas some parts of the southwest were classified as symptomatic areas for the first time; this category thereby covered 7.0% of the territory, almost double the figure for the other periods (Fig. 4d).

3.4 Detection of other *Bursaphelenchus* species during PWN monitoring

Although no *B. xylophilus* was detected in wood sampled from 2000 to 2019 under the monitoring programme covering metropolitan France, other *Bursaphelenchus* species were found. Indeed, the morphological analyses performed on 4596 samples collected during the first part of the monitoring programme (2000–2011) revealed that 191 of them (i.e. 4.15%) contained one or more individual(s) of this genus. In total, this represented 213 occurrences of endemic *Bursaphelenchus* spp. Over 70% of these nematodes were reported in southern France, especially in two southwestern regions—Nouvelle-Aquitaine and Occitanie—with, respectively, 83 and 39 reports (Fig. 5).

Among the 213 endemic *Bursaphelenchus* spp. discovered, identification stopped in 63 cases at the genus level (marked as '*Bursaphelenchus* sp.') whereas 150 were identified at the species level or, at least, until the group level defined by Braasch et al. (2009). Nevertheless, as *B. teratospicularis* is not attributed to a group by Braasch et al. (2009), we included this species in a *teratospicularis* group. In total, 12 species and eight groups of *Bursaphelenchus* were identified during the monitoring programme (Fig. 5). As there were very few individuals for some species, our analysis was carried out at the group level (by pooling the species belonging to the same group). Nematodes belonging to the *Bursaphelenchus* genus were found in all the regions except for Pays de la Loire. The highest diversities of *Bursaphelenchus* groups were found in the southern regions of the country, namely Nouvelle-Aquitaine ($n=6$ groups detected), ahead of Occitanie ($n=5$), Auvergne-Rhône-Alpes ($n=4$) and Provence-Alpes-Côte d'Azur ($n=4$) (Fig. 5).

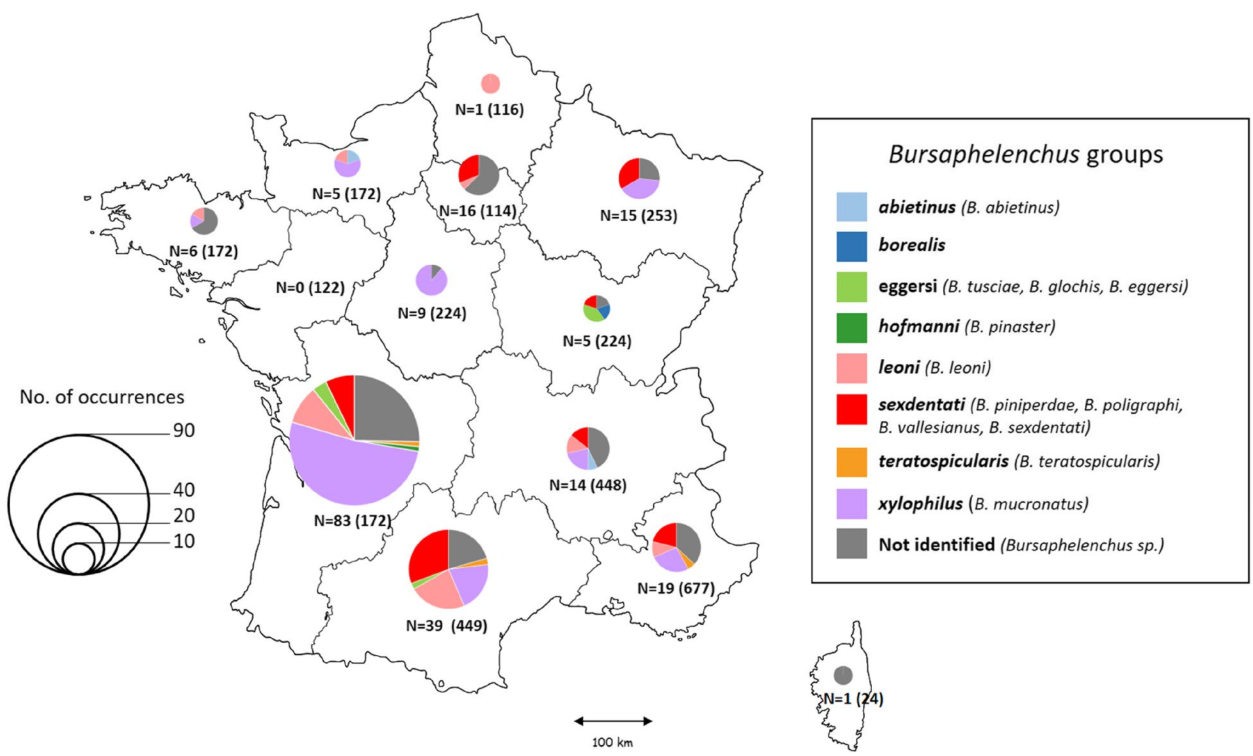

Fig. 5 Geographical distribution of *Bursaphelenchus* groups in metropolitan France, with the assignment of species/group when available, detected from 2000 to 2011 during PWN monitoring of host trees. The size of each pie chart is based on the number of nematodes collected in each region. Under each chart, we indicate the number of occurrences (N) and the total number analysed in this period (in brackets)

The *xylophilus* group (represented by the species *B. mucronatus*) was the most abundant one detected during the monitoring period with 77 reports, far ahead of the *sexdentati* (*B. piniperdae*, *B. poligraphi*, *B. vallesianus*, *B. sexdentati*) and *leoni* (*B. leoni*) groups, which accounted for 35 and 25 reports, respectively. Together, these three groups represented more than 90% of the reports (Fig. 5). Species from the *xylophilus* and *leoni* groups were distributed throughout the country, but were found more often in southern regions such as Nouvelle-Aquitaine (Fig. 5). Present in southern France, the *sexdentati* group was also reported in central and eastern regions (Ile-de-France and Grand Est, respectively). Moreover, species belonging to the *eggersi* group (*B. tusciae*, *B. glochis*, *B. eggersi*) were recorded six times in three different regions, not only in the southern part of metropolitan France but also in the east (i.e. Bourgogne-Franche-Comté). The other five groups were rare, with only one or two detections during the monitoring period: the *abietinus* group (*B. abietinus*), for example, was only detected twice, once in Normandie and once in Auvergne-Rhône-Alpes (Fig. 5).

The *Bursaphelenchus* nematodes identified through the monitoring programme were found on five host trees: four *Pinus* species (*Pinus sylvestris*, *P. nigra*, *P. halepensis* and *P. pinaster*) and *Abies grandi* (Table 2). This concerned 134 occurrences, as the species identity of the host tree was not known for 79 cases and thus marked as *Pinus* sp. without further detail. Most of the *Bursaphelenchus* spp. were found on *Pinus sylvestris*, *P. pinaster* and *P. nigra*, with at least 35 reports for each (Table 2). Moreover, at least five groups of *Bursaphelenchus* were detected on each of these three *Pinus* species even if no tree was found to host all eight groups detected during this monitoring programme. Members of the *sexdentati* group were reported on the four *Pinus* species and on *Abies grandi*. Nematodes belonging to the *xylophilus* and *leoni* groups were detected on the four *Pinus* species, whereas the *eggersi* group was found on three (*P. sylvestris*, *P. nigra* and *P. pinaster*). Moreover, two host pines were reported for each of the *abietinus* (*P. sylvestris* and *P. pinaster*) and *teratospicularis* groups (*P. nigra* and *P. halenpensis*). Finally, only one host was reported for the *borealis* group (*P. nigra*) while the host was not known (*Pinus* sp.) for the *hofmanni* group (Table 2).

4 Discussion

The French PWN monitoring described in this paper has been considerably reinforced over time, in line with changes in European or French regulations, such as the obligation to look for the PWN in its insect vector imposed in 2012 by the European Union (Council directive 2012/535/EU 2012). Despite this major sampling effort, the monitoring programme carried out in

Table 2 Host distribution of the *Bursaphelenchus* spp. detected from 2000 to 2011 in the framework of PWN monitoring in metropolitan France

***Bursaphelenchus* groups**	***Bursphelechus* species**	***Pinus sylvestris***	***Pinus nigra***	***Pinus halepensis***	***Pinus pinaster***	***Abies grandi***	***Pinus sp.***	**Total**
Abietinus	*B. abietinus*[a]	1	0	0	1	0	0	2
Borealis	*Bursaphelenchus sp.*	0	1	0	0	0	0	1
Eggersi	*B. tusciae*[a]	1	1	0	1	0	0	3
	B. glochis[a]	0	0	0	0	0	1	1
	B. eggersi[a]	1	0	0	0	0	0	1
	Bursaphelenchus sp.	0	0	0	1	0	0	1
Hofmanni	*B. pinasteri*	0	0	0	0	0	1	1
Leoni	*B. leoni*	5	5	2	7	0	4	23
	Bursaphelenchus sp.	0	1	0	1	0	0	2
Sexdentati	*B. piniperdae*[a]	2	0	0	0	0	0	2
	B. poligraphi[a]	1	3	1	3	0	3	11
	B. vallesianus[a]	1	1	0	0	0	0	2
	B. sexdentati[a]	0	1	0	1	0	0	2
	Bursaphelenchus sp.	3	1	1	4	1	8	18
Teratospicularis	*B. teratospicularis*[a]	0	1	1	0	0	1	3
Xylophilus	*B. mucronatus*	11	12	1	13	0	40	77
Not identified	*Bursaphelenchus sp.*	14	8	7	13	0	21	63
	Total	40	35	13	45	1	79	213

[a] Species detected for the first time in France

metropolitan France has not yet revealed any PWNs in host pine stands or in the PWN's insect vector, *Monochamus* spp. This finding shows that France is still free of *B. xylophilus*, as is fortunately the case for most European Union countries (EPPO (2022). In order to detect the PWN as early as possible, each EU country is required to organise its own national survey, highly comparable to that applied in France. These surveys notably involve sampling forest stands, especially close to high-risk sites for PWN introduction (Calin et al. 2013, 2015; Karmezi et al. 2022; Torrini et al. 2020). If the results of an annual survey reveal the presence of the PWN in a susceptible tree, member states shall take appropriate measures to eradicate the parasite or restrict its spread such as the clear-cut zones applied in Portugal and Spain following the PWN outbreaks (de la Fuente et al. 2018).

The natural arrival of the PWN in France via contaminated *Monochamus* insects from the closest infected countries (Portugal or Spain) seems unlikely, at least in the short term, due in particular to the barrier formed by the Pyrenean mountain range (de la Fuente et al. 2018). Nevertheless, such a way of introduction is not impossible, especially because western and eastern hillsides may represent corridors favouring the natural spread of the nematode from the Iberian Peninsula to France (Haran et al. 2015). However, the PWN is more likely to be introduced into France via imported wood contaminated by the nematode and its vector. Indeed, this is the most frequent way that the PWN enters a new area, one example being that the PWN, originally from North America, was successively imported into first Asia, then Europe (Mallez et al. 2014; Soliman et al. 2012). It should be remembered that the PWN was detected in about 40 batches of wood-based commodities entering into or circulating through metropolitan France from 2000 to 2019, representing a little less than 0.7% of the total samples analysed. These results were similar to those reported for wood-based commodities inspected from 2003 to 2005 in China by the Ningbo Entry-Exit Inspection and Quarantine Bureau, with 1% of infected batches (Gu et al. (2006). Almost all the contaminated wood materials sampled during the French PWN monitoring, mainly pallets, came from countries where the pest is known to occur, such as Portugal and Canada. The only exceptions were for pallets imported from Morocco but for which subsequent investigations revealed that they had been manufactured with wood imported from a country where the PWN was present. These results again highlight the risk of the circulation of PWN-contaminated wood material from infested to non-infested countries (Gu et al. 2006). It also emphasises the need to correctly apply phytosanitary treatment to such wood-based commodities, as required by international standards, in order to avoid the spread of products contaminated by the PWN, which is known to be highly resistant (Gu et al. 2006).

Any introduction of the PWN into France would be extremely risky due to the country's large areas of host pines, especially in the south. Moreover, PWN vectors are already present on the territory, as reflected by the collection of longhorn beetles *Monochamus* spp. in all regions during the monitoring. If the *Monochamus* species collected during this monitoring programme was unknown, we assumed that it was mostly *M. galloprovincialis* or *M. sutor*, as these are the most common species found in France (Fan et al. 2018). Apart from *Monochamus* spp., other insects could also foster the spread of the PWN: these include the *Arhopalus rusticus* beetle, recently described as a vector for this pest (Wang et al. 2020) and already present in France (MNHN/OFB 2022). In addition, our study has revealed that the weather conditions in France during the period covered were particularly suitable for the development of PWD, especially for years with hot summers, which was the case for the last period considered (2015–2019). For such years, the French areas suited to PWD expression (with or without latency) were not only restricted to southern regions as previously reported (Gruffudd et al. 2016), but instead covered most of the country, except along the north-western coastline and in mountainous regions classified as asymptomatic areas. Our risk maps of PWD expression also show that a latency in symptoms was expected in most of these suitable PWD areas, whereas the symptomatic areas (i.e. wilt symptoms expected within the year of infection) were mainly located in the south of the country.

According to the level of PWD expression, the consequences can greatly differ in terms of direct risk for local coniferous forests and establishment of the nematode. In symptomatic areas, the risk is high for both aspects. Indeed, in such areas, the direct risk for forests is linked to infected trees that will develop PWD and could die within a few weeks (Rutherford and Webster 1987). Moreover, the PWN is likely to spread quickly in these areas as its vector, *Monochamus*, can transmit the nematode in two ways, namely on dying trees via the female's oviposition and on healthy trees through maturation feeding (Naves et al. 2006, 2007). Because of the high risk of the nematode's quick dispersal, it is crucial to detect as soon as possible its presence on symptomatic trees with wilt symptoms and in its vector. In areas where symptoms are expected, visual inspections could be supplemented by methodologies based on remote sensing technologies (Ali et al. 2019; Zhang et al. 2020). In latency areas, the risk for local susceptible pines is also high as the trees will develop PWD but because the mean summer temperatures are lower than those encountered in symptomatic areas, the wilting of infected trees is expected to be slower (Rutherford and Webster 1987). Nevertheless, these locations are particularly problematic regarding pest management as it is difficult to detect infestations in time for successful eradication (EPPO 2018). Indeed, as the expression of wilt symptoms is expected to be delayed, the PWN's presence could thus be overlooked if only symptomatic trees are sampled, as trees that first appear healthy could also be contaminated (Gruffudd et al. 2016). In asymptomatic areas, the PWN can remain within infected trees without causing any damage to the host, even several years after infection (Halik and Bergdahl 1994). Moreover, as *Monochamus* spp. will not be attracted by these infected but asymptomatic trees, this greatly limits the risk of PWN dispersal through its vector in such areas. Although limited, the risk nonetheless exists because of potential transmission by oviposition on timber, felling residues or weakened trees (e.g. diseased trees, forest fires) (EPPO 2018). Like latency areas, these asymptomatic areas can thus constitute reservoirs of inoculum for the PWN where the pest is difficult to detect.

The sampling effort made in monitoring the PWN in metropolitan France is determined according to different criteria such as the risk of introduction (e.g. presence of timber mills) and the presence of susceptible pine stands. Since 2018, the evaluation of this sampling effort is performed by the Plant Health Epidemiological Surveillance Platform; its working group dedicated to the pine wood nematode aims to monitor and help to improve the efficiency and effectiveness of epidemiological surveillance of this pest. It would be wise to take into account suitability for PWD expression in the strategy of PWN monitoring and especially to anticipate the evolution of PWD expression areas due to climate change. Indeed, our study clearly states the importance of having up-to-date data on PWD expression in relation with changes due to global warming. Indeed, the limits of areas regarding PWD expression are susceptible to quickly evolve over time, as was the case within our 20-year follow-up (2000–2019). The last few years of this period were particularly suited to PWD expression due to their hot dry summers and are in line with the rise in temperatures observed in recent decades and especially since the 2000s (IPCC 2022). It is predicted that this ongoing global warming will continue to intensify in the coming years (IPCC 2022) and we can thus hypothesise that the surface area currently suited to the development of PWD will continue to expand, possibly affecting even the currently asymptomatic regions of metropolitan France. This trend has been confirmed through other works indicating that under future climate scenarios, the distribution of *B. xylophilus* will inevitably increase not just in Europe (Gruffudd et al. 2018; Ikegami and Jenkins 2018; Robinet et al. 2011) but also worldwide (Hirata et al. 2017). Similar concerns have also been raised about other plant pathogens such as the vector-borne plant bacterium *Xylella fastidiosa* (Godefroid et al. 2019) or species belonging to the tropical group of root-knot nematodes. Initially established in subtropical to tropical regions, species like *Meloidogyne graminicola* have now been reported in Europe (Fanelli et al. 2017) and may become emerging pests on this continent in the future. Moreover, as climate change is expected to modify the distribution areas of organisms such as insects (Thomas et al. 2001), it could thus influence the distribution of PWN vectors such as *Monochamus* spp. Nevertheless, it is difficult to anticipate changes because the spatial distribution of *Monochamus* spp. in France is not well documented except for *M. galloprovincialis* (Vincent et al. 2008b), and the effects of climate change on their distribution is not known.

The monitoring efforts applied in France to detect *B. xylophilus* have revealed *Bursaphelenchus* diversity, with the presence of 12 species across the country. All of them have already been reported in Europe where they are not associated with forest damage as they are only mycophagous under natural conditions (Calin et al. 2015; d'Errico et al. 2015; Karmezi et al. 2022; Torrini et al. 2020) (Table 3 in Appendix). Among them, three species had already been described in France: *B. pinasteri* (*hofmanni* group), *B. mucronatus* (*xylophilus* group) and *B. leoni* (*leoni* group) (Baujard 1980; Vincent et al. 2008b). The latter two species were found in many regions during the monitoring programme, confirming their cosmopolitan characters, as they have already been reported in many countries outside France, such as Austria, Greece, Italy and Spain (Baujard 1980; d'Errico et al. 2015; Karmezi et al. 2022; Torrini et al. 2020). The remaining nine *Bursaphelenchus* species detected were observed for the first time in metropolitan France (Table 2; Table 3 in Appendix). This was the case for *B. teratospicularis* (*teratospicularis* group), specimens of which were found in southern regions, which is not surprising as this species is generally found in the pine forests of southern Europe (Torrini et al. 2020). In most cases, the Pinaceae species on which *Bursaphelenchus* spp. were found during the monitoring programme had already been reported as hosts (Braasch 2001; Calin et al. 2015; d'Errico et al. 2015; Karmezi et al. 2022; Torrini et al. 2020). However, specimens of *B. poligraphi* and *B. abietinus* were found on some *Pinus* species that had never been reported as hosts until now, at least for European populations (Braasch 2001; Torrini et al. 2020) (Table 3 in Appendix).

Interestingly, knowledge of *Bursaphelenchus* diversity in a given area can directly improve surveillance of the PWN, in particular to determine which species to target when developing detection tools based on either molecular biology or morphology. Indeed, it is imperative that such tests—especially PCR tests used for PWN screening and therefore applied to either wood or insect extracts—are reliable and do not cross-react with species likely to be sampled instead of *B. xylophilus*. Indeed, the real-time PCR developed by François et al. (2007) and applied to the detection of *B. xylophilus* in samples collected as part of the French monitoring programme is, for example, very specific and has never shown any cross-reactivity with other *Bursaphelenchus* species reported in this paper. Furthermore, for samples in which the screening test indicates the presence of the PWN, the analytical process requires a confirmation using both morphology and conventional PCR applied to nematodes extracted from the samples (rather than directly on wood or insect extracts), thus eliminate the risk of false positive results. Moreover, it is valuable to know the distribution and frequencies of native species sharing the same resources (host plants or vector) as a given plant pest in order to carry out a risk analysis of this pest. Such species can limit the establishment of a plant pest due to competitive interactions for the available resources (Garcia et al. 2018). For instance, Jikumaru and Togashi (2004) had reported an inhibitory effect of *B. mucronatus* on *B. xylophilus* boarding *Monochamus alternatus*. These findings therefore suggest that the spread of the PWN could be limited if it was introduced into an area where *B. mucronatus* is widely distributed, as is the case in France. This nevertheless remains hypothetical, as subsequent experiments have shown that *B. xylophilus* was more competitive than its closely related species for boarding *M. galloprovincialis* (Vincent et al 2008a). In the future, it may be interesting to go further by focusing on the potential competition between *B. xylophilus* and other *Bursaphelenchus* species.

5 Conclusion

This work proposes some ideas to improve PWN surveillance in France and other countries:

- Maintain a strict surveillance of wood-based commodities, especially when they come from infected countries. Such commodities represent the highest risk of introducing the PWN into a new area as they can be infected despite international standards requiring their phytosanitary treatments.
- Take into account the suitability for PWD expression in the PWN monitoring strategy.

    ∘ Although sampling focused on wilting trees is suitable for symptomatic areas, it is less relevant in latency areas and, *a fortiori*, in asymptomatic areas where it is more valuable to sample healthy trees and wood cuts.
    ∘ Anticipate the evolution of PWD expression areas due to climate change. This could be done by applying models to different hypothetical climatic scenarios as described in the literature (Tuomola et al. 2021).

- Increase our global scientific knowledge of PWN vectors—especially *Monochamus* spp., but not only (e.g. *A. rusticus*)—and their distribution, as such information is still scarce. This includes species already known to be present on the territory and those which could be established. This additional knowledge would be useful for refining trapping and traps, if needed, to collect the different vectors.
- Maintain a reliable and adequately sized network of laboratories for the analysis of samples in order to keep abreast of the reinforcement of monitoring (especially if a PWN outbreak were to be detected). This entails developing, optimising and validating detection methods using molecular biology, morphobiometry or both combined if necessary. Technological and scientific advances in analytical processes need to be regularly evaluated to benefit from a higher performance that can then be integrated into the PWN monitoring programmes.

Appendix

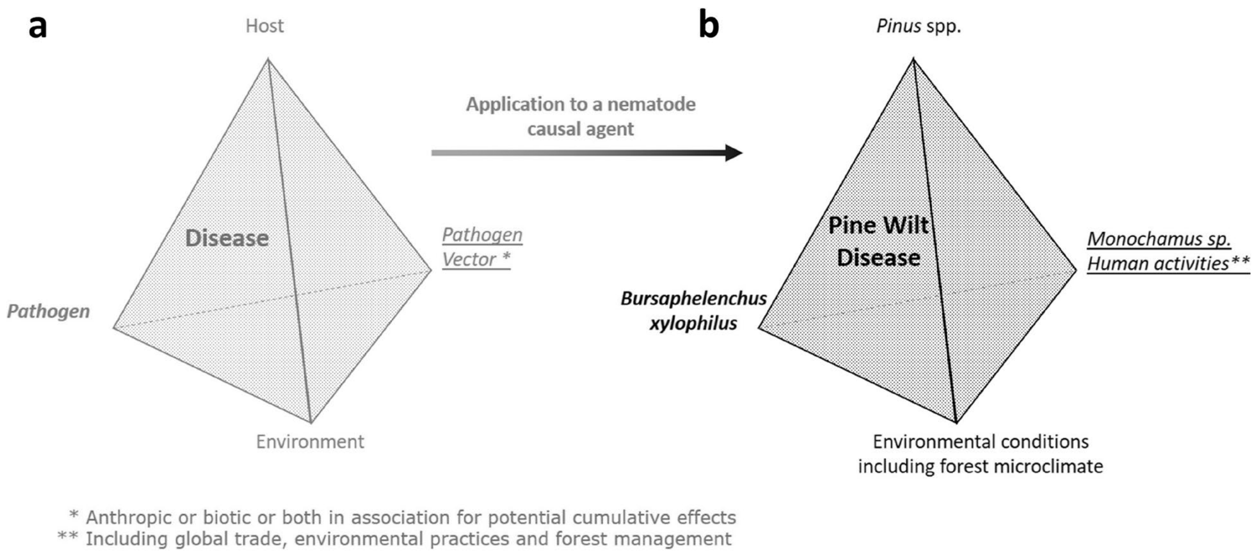

Fig. 6 Tetrahedron plant disease relationships applied to *B. xylophilus*, the nematode that causes pine wilt disease (adapted from Stevens 1960)

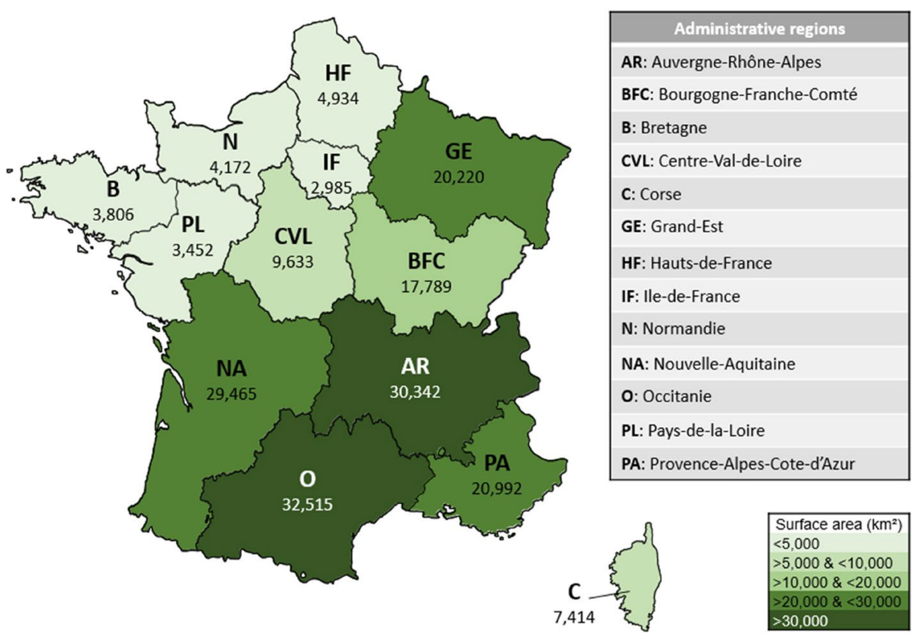

Fig. 7 Surface area (in km^2) of PWN-susceptible host trees in each administrative region of metropolitan France (Source IGN – BD Forêt, 2nd version, available on https://inventaire-forestier.ign.fr/spip.php?article646)

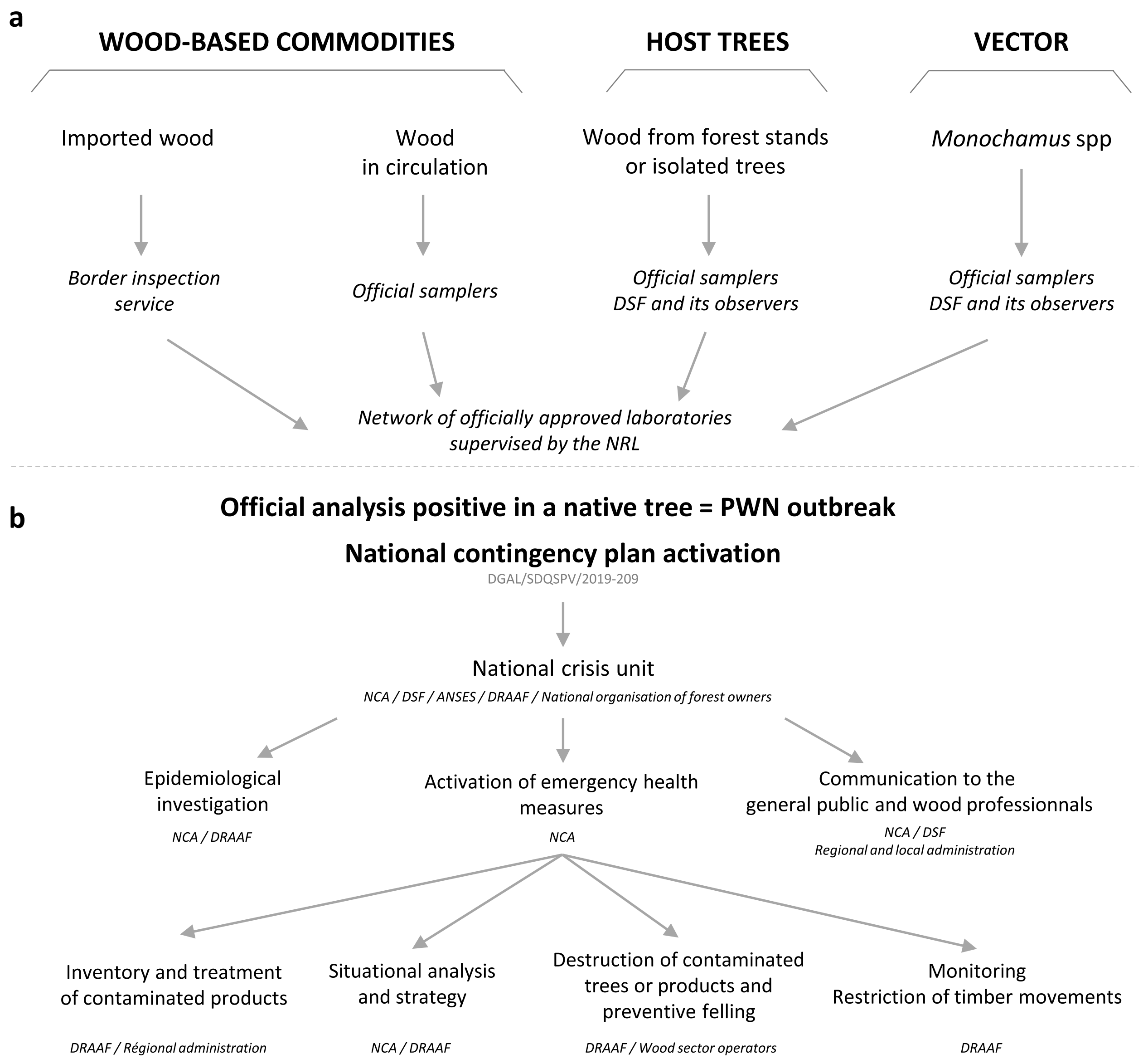

Fig. 8 Description of **a** French PWN epidemiosurveillance and risk assessment and **b** French risk management involving the PWN contingency plan; *ANSES: French Agency for Food, Environmental and Occupational Health & Safety, DGAL: French General Directorate for Food (Ministry of Agriculture), DRAAF: Regional Directorate for Food, Agriculture and Forestry (Ministry of Agriculture), DSF: Department of Forest Health (Ministry of Agriculture), NRL: National Reference Laboratory for nematology, ANSES, Official samplers: regional food and environment services or pest control organisation, NCA: National Competent Authority (Ministry of Agriculture—DGAL)*

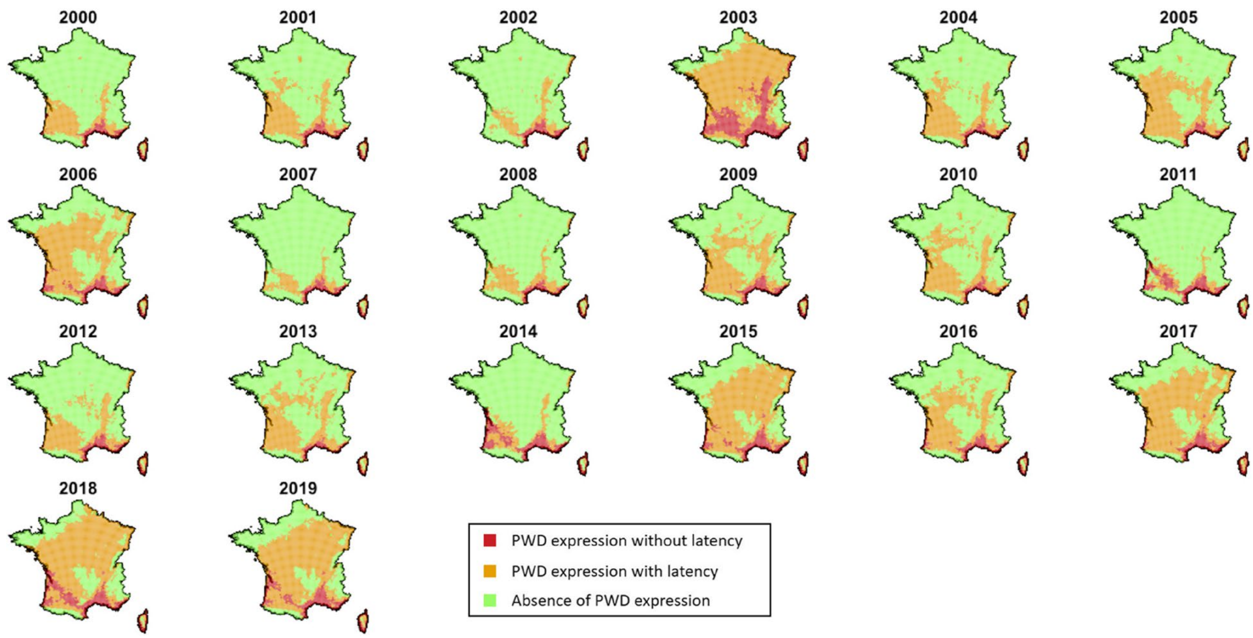

Fig. 9 Annual risk maps of PWD expression for metropolitan France from 2000 to 2019 according to the mean annual temperature (MAT) and mean summer temperature (MST). The construction of these maps was based on the work of Gruffudd et al. (2016) (see the Section 2 for further information)

Table 3 European distribution, reported hosts and insect vectors of the *Bursaphelenchus* species sampled during the monitoring of PWN in metropolitan France from 2000 to 2019

***Bursaphelenchus* species**	**Original description**	***Bursaphelenchus* group (sensu Braasch et al. 2009)**	**Country**	**Host**	**Insect vector**	**Reference**
B. abietinus	Braasch & Schmutzenhofer (2000)	*abietinus*	Austria	*Abies alba*	*Pityokteines spinidens, P. curvidens, P. voronzowi*	Schmutzenhofer (1981), Braasch & Schmutzenhofer (2000) *In* Braasch (2001)
			France	***Pinus sylvestris, P. pinaster***		This work
			Italy	*Abies alba*		Torrini et al. (2020)
			Romania	*Picea abies*		Calin et al. (2015)
B. eggersi	Rühm (1956)	*eggersi*	Austria		*Hylurgops palliatus*	Tomisczek (2000) *In* Torrini et al. (2000)
			France	*Pinus sylvestris*		This work
			Germany	*Pinus sylvestris, P. strobus, Picea exelsa, Larix leptolepis, P. abies*	*Hylurgops palliatus*	Rühm (1956), Braasch et al. (1999) *In* Braasch (2001)
			Greece	*Pinus pinaster*		Skarmoutsos & Skarmoutsos (1999) *In* Torrini et al. (2000)
			Spain	*Pinus pinaster, P. radiata, P. sylvestris*		Abelleira et al. (2003) *In* d'Errico et al. (2015)

***Bursaphelenchus* species**	**Original description**	***Bursaphelenchus* group (sensu Braasch et al. 2009)**	**Country**	**Host**	**Insect vector**	**Reference**
B. tusciae	Ambrogioni & Marinari Palmisano, (1998)	eggersi	**France**	*Pinus nigra, P. sylvestris, P. pinaster*		This work
			Germany	*Pinus sylvestris*		Schönfeld et al. (2001) *In* Braasch (2001)
			Italy	*Pinus nigra, P. pinaster, P. pinea*	*Hylurgus ligniperda*	Ambrogioni & Palmisano (1998), Carlett i(2008) *In* Torrini et al. (2020)
			Portugal	*Pinus pinaster*	*Hylurgus ligniperda*	Penas et al. (2002, 2004, 2006) *In* Torrini et al. (2020)
B. glochis	Brzeski & Baujard (1997)	eggersi	**France**	*Pinus sp.*		This work
			Poland	*Pinus sylvestris*		Brzeski & Baujard (1997)
B. pinasteri	Baujard (1980)	*hofmanni*	France	*Pinus pinaster*		Baujard (1980) *In* Braasch (2001); This work
			Germany	*P. sylvestris*		Schönfeld et al. (2000) *In* Braasch (2001)
			Spain	*Pinus pinaster, P. pinea*		Braasch (2001); Escuer et al. (2002, 2004a, 2004b),; In d'Errico et al. (2015)
B. leoni	Baujard (1980)	*leoni*	Austria	*Pinus nigra, P. sylvestris*		Braasch et al. (2000),Tomiczek (2000) *In* Braasch (2001)
			Cyprus	*Pinus brutia, P. nigra, P. pinea*		Philis (1996), Philis & Braasch (1996), Braasch & Philis (2002) *In* Torrini et al. (2020)
			France	*Pinus pinaster, P. sylvestris, P. halenpensis*		Baujard (1980) *In* Braasch (2001); This work
			Germany	*Pinus sylvestris*	*Dryocoetes autographus*	Braasch et al. (1999), Schönfeld et al. (2001) *In* Braasch (2001)
			Greece	*Pinus brutia, P. halepensis, P. nigra, P. pinaster, P. radiata, P. sylvestris*		Skarmoutsos & Skarmoutsos (1999), Michalopoulos-Skarmoutsos et al. (2004) *In* Torrini et al. (2020); Karmezi et al. (2022)
			Italy	*Pinus halepensis, P. nigra; P. pinaster, P. pinea, P. sylvestris*		Ambrogioni et al. (1994), Marinari Palmisano & Ambrogioni (1994),Ambrogioni et al. (1994), Ambrogioni & Caroppo (1998), Caroppo et al. (1998), Carletti (2008) *In* Torrini et al. (2020)
			Portugal	*Pinus pinaster*	*Pityogenes sp.*	Braasch (2001); Penas et al. (2004) *In* Torrini et al. (2020)
			Spain	*Pinus halepensis, P. pinea*		Escuer et al. (2002, 2004b) *In* d'Errico et al. (2015)

Bursaphelenchus species	Original description	*Bursaphelenchus* group (sensu Braasch et al. 2009)	Country	Host	Insect vector	Reference
B. piniperdae	Fuchs (1937)	*sexdentati*	Austria	*Pinus sylvestris*	*Tomicus piniperda*	Fuchs (1937) *In* Braasch (2001)
			France	*Pinus sylvestris*		This work
			Germany	*Pinus sylvestris, P. montana, P. exelsa*		Rühm (1956) *In* Braasch (2001)
			Netherlands	*Pinus sylvestris*	*Tomicus piniperda*	Fuchs (1937) *In* Braasch (2001)
			Slovakia	*Pinus sylvestris*	*Tomicus piniperda*	Tenkacova & Mituch (1987), Vilagiova & Mituch (1991) *In* Braasch (2001)
B. poligraphi	Fushs (1937), Goodey (1951)	*sexdentati*	**France**	***Pinus sylvestris, P. nigra, P. halenpensis, P. pinaster***		This work
			Germany	*Picea abies, Pinus exelsa*	*Polygraphus poligraphus, Hylurgops. palliatus*	Fushs (1937),Rühm (1956), Braasch et al. (1999) *In* Braasch (2001)
			Roumania	*Pinus sp*		Calin et al. (2013) *In* Calin et al. (2015)
			Slovakia	*Picea abies*	*Polygraphus poligraphus*	Tenkacova & Miush (1987) *In* Braasch (2001)
B. vallesianus	Braasch et al. (2004)	*sexdentati*	France	*Pinus sylvestris, P. nigra*		This work
			Greece	*P. nigra*		Lange et al. (2007) *In* d'Errico et al. (2015)
			Romania	*Picea abies*		Calin et al. (2013) *In* Calin et al. (2015)
			Switzerland	*Pinus sylvestris*		Braasch et al. (2004)
			Turkey	*Pinus sylvestris*		Akbulut et al. (2008b) *In* d'Errico et al. (2015)

Bursaphelenchus species	Original description	*Bursaphelenchus* group (sensu Braasch et al. 2009)	Country	Host	Insect vector	Reference
B. sexdentati	Rühm (1960)	*sexdentati*	Austria	*P. sylvestris*		Tomiczek (2000) *In* Braasch (2001)
			Cyprus	*Pinus brutia*		Braasch & Philis (2002) *In* Torrini et al. (2020)
			France	*Pinus nigra, P. pinaster*		This work
			Germany	*P. sylvestris*	*Ips sexdentatus, Tomicus piniperdia*	Rühm (1960), Braasch et al. (1999) *In* Braasch (2001)
			Greece	*P. brutia, P. nigra, P. pinaster, P. halenpensis, P. radiata, P. maritima*		Skarmoutsos & Skarmoutsos (1999), Skarmoutsos et al. (1998b), Michalopoulos-Skarmoutsos et al. (2004) *In* Torrini et al. (2020); Karmezi et al. (2022)
			Italy	*Pinus pinaster, P. halenpensis, P. pinea, P. sylvestris, P. nigra*	*Ips sexdentatus, Orthotomicus erosus*	Ambrogioni & Caroppo (1998), Carletti (2008) *In* Torrini et al. (2020); Caroppo et al. (1998) *In* Braasch (2001); Torrini et al. (2020)
			Lithuania		*Ips sexdentatus*	Voslilite (1990) *In* Braasch (2001)
			Portugal	*Pinus pinaster*	*Hylurgus ligniperda, Orthotomicus erosus*	Penas et al. (2002, 2004, 2006), *In* Torrini et al. (2020)
			Spain	*Abies alba, Pinus pinaster, P. pinea, P. sylvestris*		Abelleira et al. (2003), Escuer et al. (2004a, b) *In* d'Errico et al. (2015)
			Turkey	*Pinus brutia, P. maritima*		Akbulut et al. (2008a) *In* d'Errico et al. (2015)
B. teratospicularis	Kakuliya & Devdariani (1965)	teratospicularis	Croatia	*Cupressus sempervirens*		Braasch (2001)
			Cyprus	*Pinus brutia*		Braasch (2001)
			France	*Pinus nigra, P. halenpensis*		This work
			Georgia	*Pinus nigra, Picea orientalis*	*Orthotomicus proximus, Blastophagus minor*	Kakuliya & Devdariani (1965) *In* Braasch (2001)
			Germany	*Pinus sylvestris*		Braasch (2001)
			Greece	*Pinus halepensis, P. brutia*		Skarmoutsos & Skarmoutsos (1999) *In* Torrini et al (2020)
			Italy	*Pinus pinaster, P. halepensis, P. pinea*		Ambrogioni & Palmisano (1998), Caroppo et al. (1998) *In* Braasch (2001)
			Portugal	*Pinus pinaster*	*Orthotomicus erosus*	Braasch (2001); Penas et al. (2006) *In* Torrini et al. (2020)

Bursaphelenchus species	Original description	*Bursaphelenchus* group (sensu Braasch et al. 2009)	Country	Host	Insect vector	Reference
B. mucronatus	Mamiya & Enda (1979)	*xylophilus*	Austria	*Abies alba, Larix decidua, Picea abies, Pinus nigra, P. sylvestris*		Braasch et al. (2000),Tomiczek (2000) *In* Braasch (2001)
			Czech Republic	*Pinus sylvestris*		Braasch (2001)
			Finland	*Picea abies, Pinus sylvestris*	*Monochamus galloprovincialis, M. sutor*	Tomminen et al. (1989), Tomminen (1990) *In* Braasch (2001)
			France	*Pinus nigra, P. pinaster, P. sylvestris, P. halenpensis*	*Monochamus galloprovincialis*	Baujard et al. (1979) *In* Braasch (2001); Baujard (1980) *In* Vincent et al. (2008b); This work
			Germany	*Larix decidua, Picea abies, Pinus sylvestris*	*Monochamus galloprovincialis*	Braasch (1991); Braasch et al. (1999),Schönfeld et al. (2001) *In* Braasch (2001)
			Greece	*Pinus brutia, P. halenpensis, Abies borisii-regis, P. maritima*		Skarmoutsos & Skarmoutsos (1999), Michalopoulos-Skarmoutsos et al. (2004) *In* Torrini et al. (2020); Karmezi et al. (2022)
			Italy	*Pinus nigra, P. pinaster, P. radiata, P. strobus, P. sylvestris*	*Monochamus galloprovincialis*	Marinari Palmisano et al. (1992), Marinari Palmisano & Ambrogioni (1994), Ambrogioni & Caroppo (1998), Carletti (2008) *In* Torrini et al. (2020); Caroppo et al. (1998) *In* Braasch (2001)
			Norway	*Pinus sylvestris*		McNamara & Stoen (1988) *In* Braasch (2001)
			Poland	*Pinus sylvestris*		Brzeski & Baujard (1997), Brzeski & Brzeski (1997) *In* Braasch (2001)
			Portugal	*Pinus pinaster*		Penas et al. (2002, 2004) *In* Torrini et al. (2020)
			Spain	*Pinus halepensis, P. nigra, P. sylvestris*		Escuer et al. (2002, 2004a, 2004b) *In* d'Errico et al. (2015)
			Sweden	*Picea abies, Pinus sylvestris*	*Monochamus galloprovincialis; M. sutor*	Magnusson & Schroeder (1989) *In* Braasch (2001)
			Turkey	*Pinus nigra*	*Ips sexdentatus*	Vieira et al. (2004), Akbulut et al. (2006) *In* d'Errico et al. (2015)

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Conceptualization: A.M. Chappé, L. Folcher, H. Hotte and N. Mariette; Methodology: A.M. Chappé, H. Hotte, M.T. Paris and C. Sarniguet; Collection of the data: A.M. Chappé, M. Grosdidier, H. Hotte, E. Kersaudy and M.T. Paris; formal analysis and investigation: M. Grosdidier, L. Folcher, H. Hotte and N. Mariette; Writing—original draft preparation: A.M. Chappé, L. Folcher, H. Hotte, and N. Mariette; Writing—review and editing: G. Anthoine, A.M. Chappé, O. Colnard, L. Folcher, M. Grosdidier, H. Hotte, E. Kersaudy, E. Koen and N. Mariette; Supervision: L. Folcher. The authors read and approved the final manuscript.

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Availability of data and materials

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Declarations

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