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REVIEW ARTICLE

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Pine wilt disease: A global threat to forestry

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Abstract

Pines are the most economically important trees in the world and, together with eucalyptus, they dominate commercial forests. But the success of a relatively small number of widely planted species, such as *Pinus pinaster*, the maritime pine, comes at a price. Pines are attractive to damaging pathogens and insect pests, including the pinewood nematode (PWN), *Bursaphelenchus xylophilus*, the causal agent of pine wilt disease (PWD). Originally described in Japan, PWD has caused widespread destruction to forests in countries such as China, Taiwan, Portugal, Spain and the United States. PWN causes irreparable damage to the vascular system of its pine hosts, leading to mortality within 3 months. Pine sawyer beetles (*Monochamus* spp.) are key vectors of PWD, introducing the PWN to healthy trees during feeding. Other organisms contribute to PWD spread and development, including bacteria, fungi and bark beetles. Control measures include tree felling to prevent vector transmission of PWN, insecticide treatments, trapping of *Monochamus* spp. and tree breeding for plant resistance. The PWN is a quarantine pathogen and subject to regular legislation and phytosanitary measures aimed at restricting movement and preventing introduction to new areas. Current research is investigating the use of biopesticides against PWN and *Monochamus* spp. This review examines the biology, epidemiology, impact and management of PWD through published research, grey literature and interviews with people directly involved in the management of the disease in Portugal.

KEYWORDS
Bursaphelenchus xylophilus, *Monochamus*, pinewood nematode, *Pinus pinaster*, sawyer beetle

1 | THE NEMATODE AND ITS VECTORS

The nematode genus *Bursaphelenchus* spp. belongs to the order Rhabditida and family Aphelenchoididae, which consists of nematodes that feed on plants and fungi. *Bursaphelenchus xylophilus* was originally described in Japan as *B. lignicolus* (Mamiya & Kiyohara, 1972) and *Aphelenchoides xylophilus* in the United States (Steiner & Buhrer, 1934) before Nickle et al. (1981) confirmed that they were in fact the same species. There is a close and dependent relationship between the pinewood nematode (PWN) and its pine sawyer beetle (*Monochamus* spp.) vectors.

The lifecycle of the PWN is complex (Figure 1) and comprises phytophagous and mycophagous stages (Fonseca et al., 2015). In the mycophagous stage, nematodes feed on blue-stain fungi such as species of *Ophiostoma sensu lato*, commonly introduced by bark beetles while colonizing trees. Such fungi occur in abundance around the larval chambers of *Monochamus* spp., which leads to an accumulation of second stage juvenile (J2s) PWNs in this locality. During

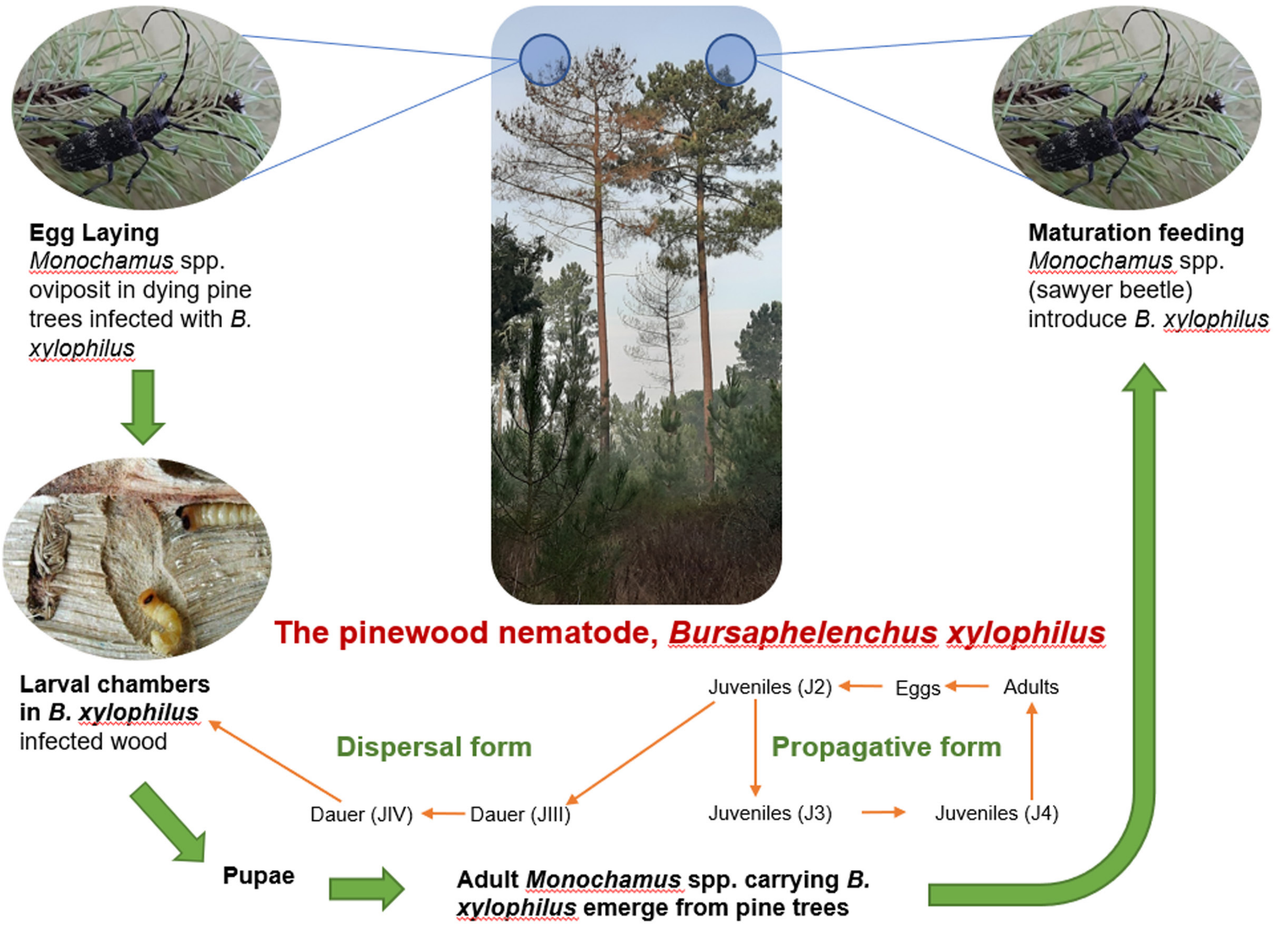

FIGURE 1 A summary of the life cycle of *Bursaphelenchus xylophilus* in relation to the activity of the pine sawyer, *Monochamus* spp. Photographs courtesy of INIAV, Portugal.

adverse conditions, including low food availability, the J2s moult into dispersion juvenile stages (JIII and JIV). The juvenile stage 4 (JIV) is a dauer stage and is characterized by having a thicker cuticle and dome-shaped head with a poorly developed stylet and medium bulb. Dauer juveniles do not feed and are coated in a protective substance that helps them adhere to their insect vectors. Nematodes accumulate in the wood surrounding the pupal chambers and are in direct contact with the pupae.

Gonçalves et al. (2021) showed that compounds from the cuticle of *Monochamus* spp. enable PWN to distinguish their vector from other non-vector cerambycids. Once in contact with their vector, the PWNs migrate under the elytra and into the respiratory system (tracheae) of the beetles which then head off to feed on healthy trees. The period when the young adult beetles feed on the bark of the 1-year-old young shoots of healthy *Pinus* trees is known as maturation feeding and typically lasts for 10–15 days (Togashi, 1990). The nematodes are introduced into the trees through the lesions caused by the feeding of the insects. And so, the disease cycle begins again.

Once inside the young shoots, the nematodes moult into adults within 48 h, before migrating into the vascular system and resin canals, where they feed on the epithelial and parenchyma cells. A detailed description of the pathogenesis of PWNs is given by Myers (1988), Kuroda (1989) and Kuroda (2012). PWNs appear to have two migration events once they enter the tree. First, a limited number of nematodes (those that escape the sticky oleoresin deposited in resin canals) migrate to the phloem, xylem and cambium. Second, reproduction begins as the feeding lesions enlarge and oleoresin production ceases, and many more nematodes migrate to the vascular tissues.

There is a clear mutualistic relationship between the PWN and its vector. The pine sawyer beetle (*Monochamus* spp.) lays its eggs in dying or dead trees affected by biotic factors such as pine wilt disease (PWD) in combination with bark beetles or abiotic factors such as forest fires, while the nematode requires *Monochamus* spp. for transmission (Futai, 2013). Trees with PWD are more prone to colonization by bark beetles, and in turn attract *Monochamus* spp. through their pheromones. There are further details to this lifecycle that highlight the intricate relationship between these organisms such as the importance of the larval chambers of the beetles for nematode development during the mycophagous stage, expanded upon below.

2 | NEMATODE IDENTIFICATION

Approximately 125 *Bursaphelenchus* species have been identified to date, with 70% of all species occurring in pine trees (Kanzaki & Giblin-Davis, 2018). The PWNs can be extracted from both wood samples and insects and identified using morphological/morphometric and/or molecular (PCR) methods. Immunodiagnostic methods such as ELISA have drawbacks due to cross-reactions with other *Bursaphelenchus* species (Fonseca et al., 2015). Morphological identification of PWN is best undertaken by individuals who have significant experience in nematode taxonomy (Braasch, 2001; Braasch & Schönfeld, 2015). There are also limitations to keys and identification schemes that depend on observation.

Useful morphological features to observe include the tail shape of the females (rounded and lacking a prominent point known as a mucron at the tail end), and reproductive features of the males (shape of spicules) and lateral lines along the body (Figure 2; Braasch & Schönfeld, 2015). Taking account of the time taken to undertake morphological identifications, as well as the skills required and the shortfalls of the existing taxonomic keys, molecular methods provide a reliable and rapid alternative to screening multiple samples. A variety of primer sets have been developed for species-specific PCR and real-time PCR and are detailed in the European and Mediterranean Plant Protection Organization standard for diagnostics for *B. xylophilus*–EPPO PM7/4(4) (EPPO, 2023).

3 | DISTRIBUTION OF *B. xylophilus*

The PWN is widely distributed in the northern hemisphere; it has been confirmed in coniferous forests in eastern Asia (China, Japan, Taiwan and Korea), Europe (Portugal including Madeira, and Spain) and North America (United States, Canada and Mexico). *B. xylophilus* is thought to have originated in the eastern states of the United States, where it has been recorded from 36 states. The nematode is also widespread in Canada, where it occurs in all territories and provinces, with the exception of Prince Edward Island.

The general consensus is that the PWN originated in the United States, later spreading to Japan, China, Korea and Taiwan and East Asia. From Asia, *B. xylophilus* was introduced to Portugal (Mota et al., 1999), the first country in Europe where PWD has become established, probably through the movement of timber and wood products. Portuguese isolates of *B. xylophilus* show low genetic variation and have been linked to one or two introductions from Asia (Rodrigues et al., 2017; Vieira et al., 2007). Genetic variation in *B. xylophilus* from North America is much higher, particularly in Canada, suggesting these are the countries where the nematode first occurred. Parallels can be found with European genotypes of potato cyst nematodes (*Globodera* spp.), which can be traced back to Andean countries in South America and their centre of origin (Plantard et al., 2008).

4 | THE VECTORS, *MONOCHAMUS* SPP.

There are approximately 150 species of *Monochamus*, a longhorn beetle, commonly referred to as pine sawyer beetles or sawyers (Naves et al., 2005). They typically feed on the wood of trees in the pine family (*Pinaceae*), as well as in decaying organic matter. The adult beetles of *Monochamus* (Cerambycidae: Coleoptera) are the sole vectors of PWN and important species are shown in Table 1, including those of quarantine importance to the European Union (EU). The lifecycle of *Monochamus* spp. is closely entwined with that of the PWN, highlighting the importance of the beetles

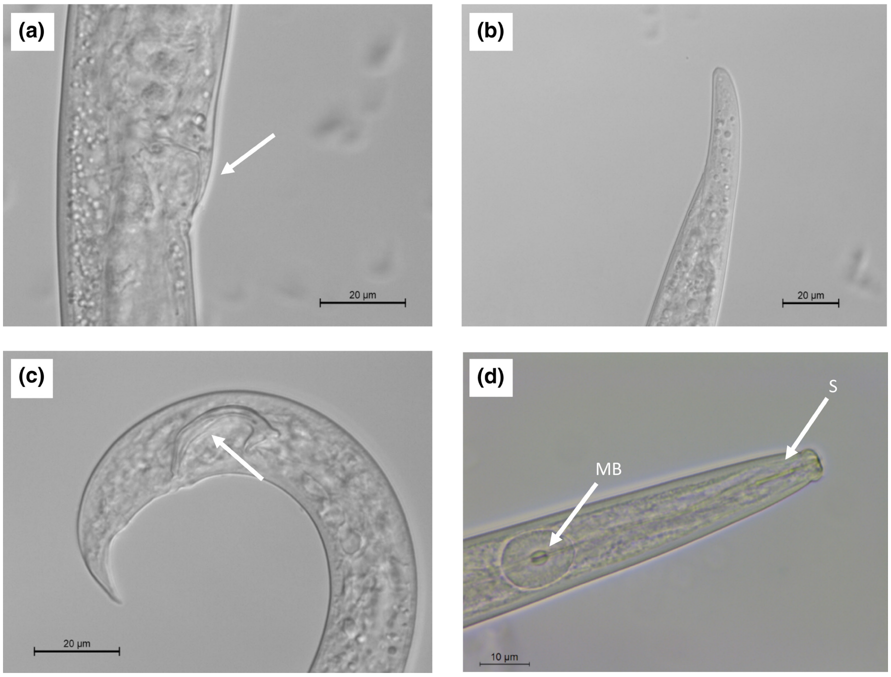

FIGURE 2 Useful features for identifying *Bursaphelenchus xylophilus*. (a) Vulval flap seen in females; (b) rounded tail of females, sans mucron; (c) arcuate tail of male with distinct spicules used in sperm transfer; (d) head region of the male showing weak stylet (S; spear) and prominent medium bulb (MB) with pump chamber (centre). Photographs courtesy of NemaINIAV, Portugal.

in the management of PWD. Pine sawyer beetles alone are unlikely to cause significant damage to conifers because they only oviposit in dead or dying trees. Some *Monochamus* spp. occur on deciduous tree species, but have not been reported to form associations with *Bursaphelenchus* spp.

Following emergence and the onset of maturation feeding, pine sawyer beetles typically fly several hundred metres, although during their life span they can travel as much as 10 km (Mas et al., 2013; Togashi & Shigesada, 2006). This represents a major challenge in trying to contain infested areas and is discussed later. Beetles carrying *B. xylophilus* can also be transported in sawn and round wood, another potential and substantial risk to regions where PWD has yet to occur.

TABLE 1 The occurrence of *Monochamus* species which transmit the pine wood nematode *Bursaphelenchus xylophilus* in the European Union and elsewhere.

Species	Present in the EU	Occurrence
M. alternatus	No	China, Japan, Korea, Taiwan
M. carolinensis	No	Canada, USA
M. clamator	No	Canada, USA
M. galloprovincialis	Yes	Asia, Europe, northern Africa
M. marmorator	No	Canada, USA
M. mutator	No	Canada, USA
M. nitens	No	Japan
M. notatus	No	Canada, USA
M. obtusus	No	Canada, USA
M. rosenmelleri (urussovi)	Yes	China, Japan, northern Europe, Russia
M. saltuarius	Yes	China, Japan, Korea, Taiwan
M. scutellatus	No	Canada, USA
M. titillator	No	Canada, USA

Note: Key species are in bold.

Pine sawyer beetles occur widely in forested regions in Europe, Asia and North America. In European countries, such as Portugal, the main vector of the nematode is *Monochamus galloprovincialis* (Figure 3), whereas in Asia transmission is mainly associated with *M. alternatus* (Japanese pine sawyer) and *M. saltuarius* (mainly in Korea). In North America, at least six species of *Monochamus* are associated with PWD, although *M. carolinensis* (Carolina pine sawyer) and *M. scutellatus* (white spotted sawyer) are considered to be the main vectors (Donald et al., 2016).

A modelling study by Estay et al. (2014) showed that the period of rainfall had the greatest influence on *Monochamus* spp. populations in North America and Eurasia. Cumulative day temperatures had, by comparison, little effect on most species included in this study. Either way, climate change increases the risk of new vector species of *Monochamus* establishing in areas where they are currently a low or negligible threat.

5 | VECTORS AND THEIR PINE HOSTS

In Europe, *M. galloprovincialis* is associated with six pine species, namely *Pinus halepensis*, *P. nigra*, *P. peuce*, *P. pinaster* (main host in Portugal), *P. sylvestris* and *P. uncinata* (Bonifácio et al., 2015; Naves et al., 2016). The host range of *M. alternatus*, the principal vector in

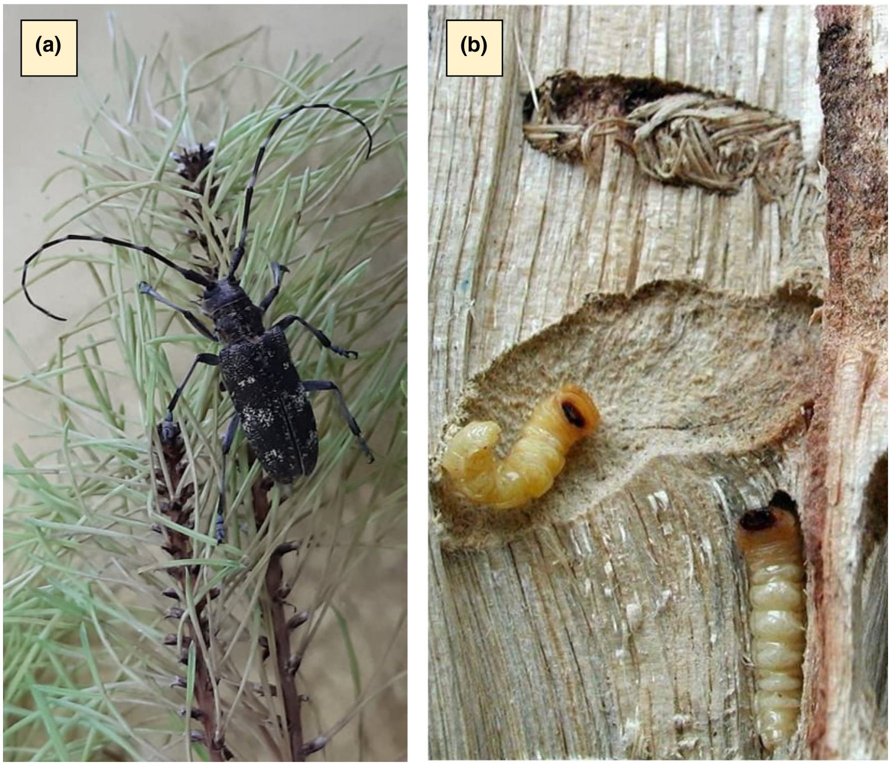

FIGURE 3 An adult beetle (a) and larvae (b) of the black pine sawyer (*Monochamus galloprovincialis*). Photograph courtesy of NemaINIAV, Portugal.

Asian countries, is much wider and it has been recorded on 22 species of pine as well as four species of fir, one cedar species and one larch species (Nakamura-Matori, 2008).

The beetles use volatile organic compounds (VOCs) emitted from foliage and bark to locate suitable host species for feeding. Studies have shown that *Monochamus* spp. exhibit clear preferences for different VOCs or profiles produced by different species of pine. The stone pine, *P. pinea*, produces a limited range of VOCs, dominated by limonene, a terpene. The maritime pine, *P. pinaster*, has a richer blend of VOCs, including variable amounts of α-pinene, β-pinene and δ-3-carene (Rodrigues et al., 2017). A study undertaken by Naves et al. (2006) in Portugal showed that *M. galloprovincialis* had the greatest preference for *P. sylvestris* (Scots pine) and lowest preference for *P. radiata* (Monterey pine). The VOC profile of *P. sylvestris* is 20%–60% α-pinene and 10%–50% δ-3-carene, whereas that for *P. radiata* is dominated by β-pinene (Bonifácio et al., 2015).

P. pinaster has three different chemotypes related to the ratio and occurrence of different VOCs. Gonçalves et al. (2020), also working in Portugal, found that VOC emissions increased when *M. galloprovincialis* was feeding on *P. pinaster* and that the population density of the vector may be a critical factor on how PWD is spread and develops. Research on VOCs has opened the possibility of using trap crops to lure the vectors away from pines and is discussed later in the review.

Pine sawyer beetles' choice of trees for oviposition is also related to release of VOCs; for example, *M. alternatus* responds to α-pinene (Bonifácio et al., 2015). *M. galloprovincialis* oviposits on thinner bark occurring on young trees or in the upper parts of mature trees in *P. pinaster*, whereas *M. sutor* prefers the lower, thicker bark of mature trees (Schenk et al., 2020). Visual signals triggered by the characteristic silhouettes of the host trees are also important, especially once sexual maturation is complete (Morewood et al., 2002).

Host availability also has to be considered when assessing the risk of disease spread by the introduction of non-endemic vector species (Schenk et al., 2020).

6 | MICROBIAL INTERACTIONS AND *B. xylophilus*

Other organisms play important roles in disease development and how the nematode affects its host. The two main groups to consider are bacteria and fungi. *B. xylophilus* carries its own bacteria but also interacts with endophytic bacteria found in pine trees. Blue-stain fungi that colonize the pupal chambers of *Monochamus* spp. may also play an important role (Vicente et al., 2022).

6.1 | Nematode-related bacteria

Entomopathogenic nematodes (EPNs) are commonly used in the management of insect pests and slugs attacking horticultural and other high-value plants. Their success is partly due to the bacteria they harbour, such as *Photorhabdus* spp. and *Xenorhabdus* spp., gram-negative bacteria associated with *Heterorhabditis* and *Steinernema*, two popular types of EPNs. Once inside their insect hosts, EPNs release (regurgitate) their associated bacteria, which secrete toxins that suppress the insect's immune system, breakdown insect tissues and induce reproduction of the EPNs (Dziedziech et al., 2020).

Parallels can be drawn between PWD and EPNs. *B. xylophilus* harbours bacteria belonging mainly to the *Enterobacteriaceae* and *Pseudomonadaceae* (Nascimento et al., 2015; Vicente et al., 2015). In Portugal, the dominant bacterial genera associated with the PWN are *Burkholderia* and *Pseudomonas* (Proença et al., 2010) whereas in Japan, *Bacillus* and *Pseudomonas* predominate (Kawazu, 1998). In China and Korea, the majority of bacteria associated with *B. xylophilus* belong to the *Enterobacteriaceae* (Morais et al., 2015).

The first evidence that the PWNs are themselves vectors came from Oku et al. (1980). These authors showed that a toxin associated with wilting was produced by a species of *Pseudomonas* found on the cuticle of *B. xylophilus* (described as *B. lignicolus* in the paper). The secretion of toxic metabolites by the bacteria contributes not only to pathogenesis but to altered virulence of the nematode (Morais et al., 2015). Proença et al. (2017) described some of the phytotoxic metabolites produced by bacterial species associated with *B. xylophilus* and development of PWD. For example, *Bacillus* spp. produce phenylacetic acid, while *Burkholderia* spp. secrete a siderophore known as pyochelin.

Pine trees are naturally rich in resins that contain compounds toxic to nematodes, such as terpenoids (α-pinene), resin acid, benzoate, phenolic compounds and stilbenoids. Bacteria associated with *B. xylophilus* can protect the nematodes from these nematicidal defence metabolites (Vicente et al., 2015). When *B. xylophilus* invades parenchyma cells, production of terpenoids, benzoic acid and ethylene may increase. Some bacteria break down these nematicidal compounds via xenobiotic degradation (Cheng et al., 2013). The microbiome of *B. xylophilus* has the necessary enzymes to degrade α-pinene, for example, a feature lacking in the nematode.

There are different views on the origins of these bacteria. One is that they are introduced by *B. xylophilus* and are an integral part of the disease complex responsible for PWD. An alternative view is that the bacteria are endophytic—already inside the tree—and only attach to the nematodes when they colonize pine tissues.

6.2 | Endophytic bacteria and pine wilt disease

Endophytic bacteria occur naturally in many plants. Beneficial roles include the promotion of plant growth and resilience to abiotic and biotic stresses (Afzal et al., 2019). In PWD, it is thought that endophytic bacteria help activate plant defences, for example through the secretion of lipases (Proença et al., 2010). Lipases, such as cutinase, are used by plant pathogens to degrade the plant cell cuticle during infection. Lysis results in the production of lipid compounds/fatty acids (Lee & Park, 2019), which act as signalling molecules in a cascade of cellular pathways, that trigger plant defence molecules such as pathogenesis-related (PR) proteins, reactive oxygen species (ROS) and phytoalexins. The trigger for this type of defence activation is commonly referred to as damage-associated molecular patterns (DAMPs). Certain endophytic bacteria species may also have a negative effect on pine tree health by contributing to PWD. Alves et al. (2016) compared the microbiomes of *M. galloprovincialis* and *B. xylophilus* with that of *P. pinaster*, one of the pine species attacked by PWD. The insect vector, nematode and *P. pinaster* had bacterial communities commonly associated with xenobiotic degradation, whereas those of *P. pinaster* promoted plant growth (nitrogen fixation). Only the bacteria associated with the vector and nematode contributed to pathogenesis. The authors suggested that endophytic bacteria may contribute to plant defence initially but later switch roles, from harmless to harmful.

6.3 | The role of fungi in pine wilt disease

A range of fungal genera have been reported from countries with pine trees affected by PWD (Kobayashi et al., 1974). Ophiostomatoid fungi (also known as blue-stain fungi) such as *Ophiostoma ips*, *Leptographium* spp., *Graphilbum* spp. and *Sporothrix* spp. have mainly been found in pine trees with PWD (Vicente et al., 2022) and are associated with tree decline and blue-stained timber (Vasconcelos & Duarte, 2015). The fungi are vectored by bark beetles and introduced to trees when the insects bore into the phloem of pine trees to create egg-laying galleries. The fungi benefit from transmission by the beetles and the larvae of the beetles benefit from a supplementary food source provided by the fungi. *B. xylophilus* switches to a fungus-eating (mycophagous) stage when infected pine tree hosts are declining or have already been killed by PWD.

Fewer *B. xylophilus* are vectored by *Monochamus* spp. when *Ophiostoma* fungi are absent from pine trees with PWD, underlining their importance in the disease cycle (Maehara et al., 2005). In vitro studies on *M. alternatus* in Japan confirmed that the presence of the blue-stain fungus *Ophiostoma minus* in artificial pupal chambers increased the number of *B. xylophilus* transmitted (Maehara & Futai, 1996). The community of fungi colonizing pine trees can also affect the multiplication of *B. xylophilus*. Maehara and Futai (2000) showed that the total number of *B. xylophilus* rapidly increased for 4 weeks after nematode inoculation when *O. minus*, *Macrophoma* spp. and *Trichoderma* spp. were also present. Other studies have shown that the presence of *Verticillium* spp. or *Trichoderma* spp. reduced the numbers of PWN.

In China, reproduction of *B. xylophilus* and growth and survival rates of *M. alternatus* increased when the fungus *Sporothrix* was present (Zhao et al., 2013). Nematode reproduction appeared to be stimulated by the fragrant diacetone alcohol associated with xylem tissue infected with *Sporothrix*. There are clearly complex interactions between the nematode, its vectors and other fungi (Zhao et al., 2014). Ascarosides are low-weight molecules found in pheromones secreted by free-living and plant-parasitic nematodes to mediate behaviours such as dispersal, aggregation, dauer initiation and sexual attraction (Zhao et al., 2016). Unexpectedly, the ascarosides asc-C5 and asc-ΔC6 from *B. xylophilus*, and asc-C9 from the insect vector increased mycelial growth of *Leptographium pini-densiflorae* and two species of *Sporothrix* under in-vitro conditions (Zhao et al., 2018). Asc-C5 also increased hyphal density of *Sporothrix* and *L. pini-densiflorae*, as well as sporulation by *L. pini-densiflorae*.

7 | SYMPTOMS

Classic symptoms of PWD begin with yellowing and reddish browning of the pine needles (Forest Research, 2020) within the crown and brittle branches in the later stages. Symptoms appear around 3 weeks after infection by the nematode. Tree mortality can occur within 1–3 months. The severity of symptoms and speed of disease development are influenced by temperature, host plant and the time of year (Fonseca et al., 2015). PWD is associated with higher temperatures—places where mean summer temperatures exceed 20°C—and water stress (drought) (Hirata et al., 2017; Mamiya, 1983). Trees can also be infected by the nematode without showing visual decaying symptoms for some months.

8 | LOSSES AND ECONOMIC IMPACT

8.1 | Asia

PWD was first recorded in Japan over 115 years ago (Yano, 1913) and it is estimated that up to 2 million m^3 are lost each year due to PWN infestations. Approximately 28% of the forest is infected despite tens of millions of dollars being spent on control each year in Japan (Donald et al., 2016). PWD was first recorded in China in 1982 and has since spread to 10 provinces where the infected area is estimated to be around 80,000 ha, resulting in the death of 50 million trees. The death of over 1 million trees per annum in China between 1995 and 2006 has been suggested (Zhao, 2008). PWD was first reported in South Korea in 1989, in the harbour city of Pusan, on Japanese black pine (*P. thunbergii*) and Japanese red pine (*P. densiflora*) (Yi et al., 1989), and is estimated to cause losses in the region of US $8 million each year (Shin, 2008). The first report of PWD in Taiwan was in 1985 on luchu pine (*P. luchuensis*) in the prefecture of Taipei. Since then, PWD has spread to 14 provinces and cities, leading to the death of around half a billion pine trees and damage to 300,000 ha of forest (Pan, 2011).

Increased genetic diversity in populations of *B. xylophilus* is linked to significant variation in virulence and hence extent of damage caused by PWD. One study in Japan found that mortality of pine seedlings (*P. thunbergii*) varied from all dying to all surviving, depending upon the population of *B. xylophilus* (Kiyohara & Bolla, 1990).

8.2 | Europe

In Portugal, *B. xylophilus* was first confirmed from declining trees in the Iberian Peninsula during a survey of aphelenchid nematodes between 1996 and 1999 (Mota et al., 1999). In 2008, PWN had moved into central Portugal and by 2009 it had reached the island of Madeira (Fonseca et al., 2012). The discovery of the disease in the Setúbal peninsula led to a catastrophic destruction of pine forests. A colossal €80 million ($85.88 million) has been invested in a national PWD eradication programme (Forestry Commission, 2017).

Pine forests occupy a significant area within Portugal and are an important natural economic resource, providing timber (*P. pinaster*) and other wood products (Mota et al., 2009). Partially due to PWD, the area covered by *P. pinaster* in Portugal decreased by approximately a third, from 1.25×10^6 ha (late 1980s) to 715,000 ha (ICNF, 2015). In Spain, where pine is widely planted, limited outbreaks were recorded in 2008, 2010 and 2012 (de la Fuente & Saura, 2021; EPPO, 2013). Of most concern is the outbreak that occurred in the north-west, in Galicia, one of the most productive and economically important forestry regions in the country (Abelleira et al., 2011), together with Cantabria and the Basque Country. Since 2008, there have been nine reports of infestations in three Spanish provinces; three of these are active including Galicia (As Neves), Castilla y Leon (Lagunilla) and Extremadura (Sierra de la Malvana) (Ministerio de Agricultura, Pesca y Alimentación, 2023).

Soliman et al. (2012), developed a model to estimate the potential impact of PWD in Europe. If the disease was unregulated, damage to forestry could amount to approximately €22 billion ($23.62 billion) between 2008 and 2030. Under EU regulations, *B. xylophilus* is a quarantine pest for more than 40 countries.

8.3 | North America

PWD is responsible for economic losses in North America, but for different reasons compared to Europe and Asia. *B. xylophilus* is indigenous to the United States and Canada, and although it does not affect native pine trees, the EU imposed a ban in 1993 on the import of untreated and unseasoned wood from all regions where PWN is known to occur, including the United States and Canada (Donald et al., 2016). Since the ban, exports from Canada and the United States have dropped by up to two thirds. Import restrictions are estimated to have caused losses of up to $150 million in the United States and up to $700 million in Canada (Carnegie et al., 2018).

As well as causing losses in timber and wood products, PWD also threatens employment in the forestry sector. The death and felling of trees for phytosanitary reasons will disrupt wildlife and forest ecosystems. Loss of forests also weakens resilience to climate change, due to the role of trees in the absorption of CO_2 emissions.

9 | KEEPING THE DISEASE AT BAY

PWD is caused by a complex series of interactions between the PWN, its vectors and associated bacteria and fungi. Individual control methods focus on different components and are described below, but these are only effective within an overall management strategy (Figure 4). The three main stages of this strategy are prevention, suppression and eradication.

Some control methods are not economically or environmentally viable, such as chemical control. Others require further development before they can be reliably put into practice, such as the use of biopesticides.

Sousa et al. (2015) recommend the use of models or decision support systems to formulate management strategies, in which the following would be evaluated: (a) existing extent and intensity of damage; (b) available control methods; (c) the cost–benefit ratio of using different control methods; and (d) when control methods should be used, to ensure optimal control. The issue of climate change and pest risk analysis is also currently an important factor to consider (Gruffudd et al., 2019).

9.1 | Phytosanitary measures, legislation and national responses

In Europe, *B. xylophilus* is categorized by the European Plant Protection Organization (EPPO) as an A2 quarantine pest—one that is present in the broad region but with a restricted distribution. A quarantine pest is one of potential economic importance to areas where it is absent, but also areas where the pest is present but not widely distributed, or officially controlled.

Measures to prevent the movement of infested plants, timber and other pine products have been vital in limiting the spread of PWD in Europe, especially Portugal. EPPO has published specific phytosanitary measures to prevent the introduction of PWN and its vectors and eliminate or limit their spread if it is detected. The main focus is on movement of wood and wood products, including round wood, sawn wood, processed wood, wood chips, harvesting residues and wood packaging. Pines are widely used for making palettes. Such material cannot be moved from or transported through areas known to be infected with *B. xylophilus*. Timber used for wood packaging must first be carefully debarked; bark remnants must not exceed 3 cm deep, regardless of the log length. The processed logs are then heat-treated using steam following procedures set down by the International Standard on Phytosanitary Measures (ISPM) on treatments of exported wood (ISPM 15; see FAO, 2018).

Wood products and timber can also be treated with methyl bromide, a powerful but dangerous fumigant. Methyl bromide is classed as a greenhouse gas and its use has been declining globally since it was added to the 'Montreal Protocol on Substances that Deplete the Ozone Layer' in 1992, ratified by 196 countries for phase out by 2015. Methyl bromide treatment is widely discouraged (and its use and sale banned by the EU in 2005). Despite bans, derogations

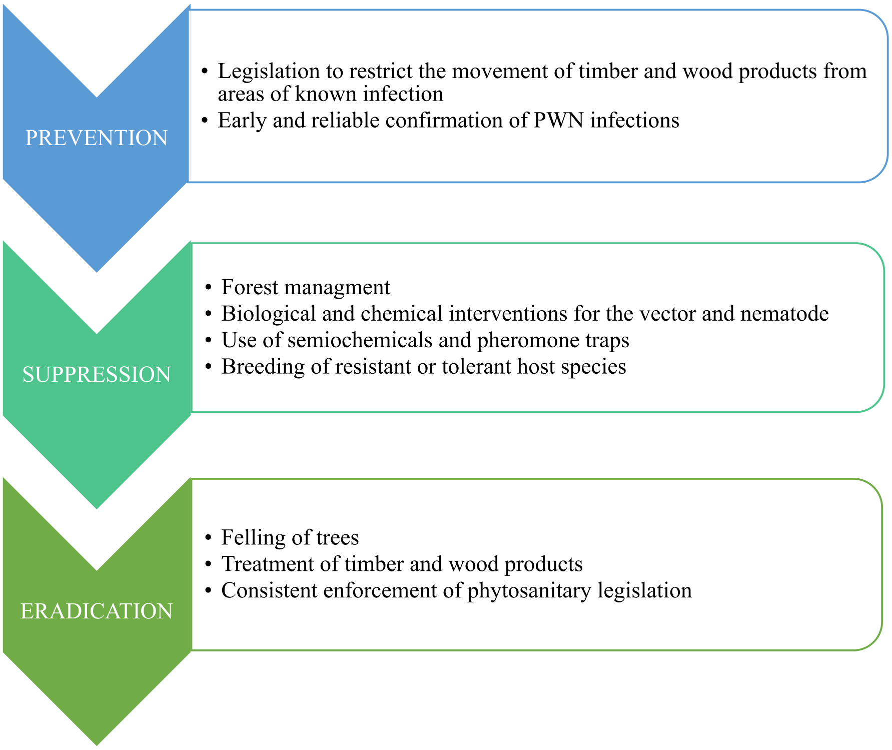

FIGURE 4 General management strategy for pine wilt disease and control options.

may be granted for quarantine purposes. An alternative is sulfuryl fluoride, included in ISPM 15 for treatment against the PWN and insect-vectors (Bonifácio et al., 2015).

All phytosanitary treatments add costs that have to be recovered by the forestry industry, part of the on-going economic impact of PWD. The need for such treatments will continue to increase because the threat from PWD is increasing. The greatest risk will be for countries where the climatic conditions are conducive for *B. xylophilus*, *Monochamus* spp. vectors are present and there are abundant host trees available (Gruffudd et al., 2019).

In Europe, PWN has been intercepted in contaminated timber or wood products, for example in Finland and Germany. In the 1990s, the EU increased inspections at ports for wood products coming from the United States. Unfortunately, less vigilance was applied to similar products from Asian countries, where PWD was established. So, it was perhaps inevitable that the nematode would eventually be found in Europe.

In 1999, trees infected with *B. xylophilus* were detected in the Setúbal Peninsula, 20 km south of Lisbon, Portugal, during a survey for an EU project (RISKBURS, 1996–1999). Despite creating a restriction zone with a 30 km radius, the disease continued to spread. Further action was taken to limit the spread of PWD. In 2006, the Portuguese plant health (phytosanitary) authorities introduced a 3 km wide strip or cordon sanitaire around *P. pinaster* plantations, where foresters were obliged to remove trees. In 2008, a national survey identified PWD in Coimbra, around 200 km north of Lisbon in central Portugal, and in parishes neighbouring this new discovery.

Felling of diseased or storm-damaged trees is a statutory requirement. Non-compliance carries fines of up to €3400 for members of the public and €44,000 for forestry businesses (ICNF, 2023). Felling has been carried out over prescriptive zones such as the 3 km clear cut belt (CCB) established in 2007 to create a barrier between areas affected by PWD and those without infections.

All areas where pines occur in continental Portugal are demarcated as potentially having PWD and a 20 km buffer zone (BZ) has been created along the border with Spain. The authorities further demarcated Infested Zones within which separated Intervention Zones (where PWN was known to occur) were created. From 2012, Portugal adopted EU policies for PWD management. These focus on management measures in BZs, whose aim is to prevent spread of *B. xylophilus* beyond Portugal, yet these have had limited effect. In 2014, Portugal introduced new national legislation to apply control measures in Intervention Zones.

The concern is that *B. xylophilus* will continue to spread, heightening economic impact and threatening important habitats and ecosystems in other parts of Europe. These include protected nature conservation areas containing forests that come under the EU's Natura 2000 scheme. Natura sites make up 23% of the total forested land in the EU (EEUSTAFOR, 2013), areas vital for protecting soil from erosion, regulating watersheds, storing carbon, maintaining local climates, protecting pollinators, as well as personal recreation (mental health), education and tourism.

There are no quarantine measures within North America, where *B. xylophilus* is indigenous, hence the absence of restrictions on movement of pine plants and products. However, both Canada and the United States follow recommended procedures set down by ISPM 15 (FAO, 2018).

In Japan, PWD is widespread, with the exception of the northern prefectures of Aomori and Hokkaido (Kosaka et al., 2001). Government efforts have focused on local outbreaks and vector control through aerial application of insecticides, a practice that began in the 1970s. A similar strategy is used in China, which also carries out rigorous inspections of imported timber and other pine products at ports (Zhao et al., 2020, 2021).

9.2 | Managing the disease: Current approaches

Various strategies have been used to suppress and limit the spread of PWN and its pine sawyer beetle vectors. They include host resistance, cultural approaches (how trees are managed), biological control and chemical control. In addition, much research has been carried out on novel methods, particularly biocontrol—see below.

Tree felling in spring is common in East Asia and Portugal, to prevent vector transmission of *B. xylophilus* by emerging pine sawyers. Symptomatic trees are felled during the autumn and winter then debarked, burnt and used for charcoal. In Portugal, some trees are chipped or buried (Sousa et al., 2015). Debarking is an effective treatment but it is costly. Burning of trees is a popular method, particularly in Japan, but needs to be carefully managed to avoid unwanted forest fires. In Japan, China and South Korea, methyl bromide fumigation of wood material is highly effective (close to 100% mortality of the beetles) but it is also expensive, dangerous to workers and harmful to the environment. Wood chipping is regarded as more sustainable and gaining in popularity as it allows contaminated wood to be used in producing pulp for paper production, for example.

The pine sawyer beetles can be treated using a variety of insecticides (neonicotinoid, carbamate, pyrethroid, organophosphate and benzoylurea). These are applied at ground level, aerially or via injections, providing systemic protection. Ground treatment is more challenging and involves the use of high-pressure spray guns to distribute insecticides up to 15–20 m (Kamata, 2008). Insecticide injections through the trunk can be problematic, particularly for species of pine that contain high amounts of resin, which limits the absorption of water.

Aerial treatments are typically applied by helicopter at 10 m above the forest canopy and have been used extensively in Japan to target *M. alternatus* from May to mid-June, when maturation feeding occurs. Two applications are typically needed. Good results are possible but come at a high cost and do not provide a lasting solution to disease control. There are also concerns regarding the environmental impact of some active ingredients in use. The neonicotinoid thiacloprid may affect non-target pollinator species. A recent article in the *Japan Times* (Anonymous, 2020) reported that the mayor of Matsumoto (Yoshinao Gaun), in the prefecture of Nagano, had decided to withdraw aerial spraying of acetamiprid, used to control the beetles. Pressure from five residents' associations led to ¥6.25 million (c. $42,425) being allocated to treat 29.2 ha of land but there was equal concern from the public about the safety of the insecticide, even though the atmospheric levels in the locality of the city were negligible. A questionnaire of city residents highlighted an even split of opinion with 49.3% supporting spraying and 45.3% against it.

9.3 | Managing the disease: New approaches

Public opinion and wider scientific concern about the use of insecticides have prompted efforts to find effective biocontrol agents, mainly for the vectors. Various strategies have been explored, including parasitoids, EPNs and entomopathogenic fungi. Ichneumon and braconid wasps looked to be possible parasitoids but suitable species have yet to be found. EPNs show greater potential for suppressing *Monochamus* spp. A study in Korea found that *Steinernema carpocapsae* (strain GSN1) was able to infect *M. alternatus* at a depth of 7.5 cm in pine logs (Yu et al., 2016). However, the search for EPNs needs to be widened and research undertaken to ensure that they are compatible with other control strategies (Sousa et al., 2015).

Beauveria bassiana has a long history as a fungal biocontrol agent of insect pests. It was first isolated from the cadavers of *M. alternatus* in Japan (Shimazu & Katagiri, 1981). Subsequent testing showed that strain F-263 was safe to use against pine sawyer beetles. An application system using fabric strips (fungal bands) was commercialized in 2007 in Japan under the registered trademark of BiolisaMadara (Shimazu, 2009). The fungal bands are applied to the ends of cut logs and kill 90% of emerging adult beetles within 15 days. Conidia of *B. bassiana* can also be sprayed on to the bark of the trees, used in bran pellets or as inoculated fabric strips.

Other novel control methods have examined the use of kairomones, volatile compounds associated with pine trees and pheromones, volatile compounds produced by *Monochamus* during mating. Known collectively as semiochemicals, these compounds are used to trap *Monochamus* spp. for monitoring and suppressing vector populations. Some traps and semiochemicals work better than others for different species of *Monochamus*. *M. galloprovincialis* responded best to a combination of kairomone, while bark beetles were attracted to the pheromones ipsenol (*Ips sexdentatus*) and methyl butenol (*Orthotomicus erosus*) (Ibeas et al., 2007). The combination of the host volatile, α-pinene and pheromones of bark beetles increased the attractiveness to both male and female *M. galloprovincialis*. Finally, the inclusion of the specific pheromone compound 2-undecyloxy-1-ethanol enhanced to the maximum the efficiency for trapping *Monochamus* beetles (Álvarez et al., 2015; Jactel et al., 2019; Pajares et al., 2010).

9.4 | Targeting the pinewood nematode

A tree resistance breeding programme began in Japan in 1978, led by the National Tree Breeding Center, Forestry Agency (Japanese MAFF). By 1984, they had identified PWD resistance in 92 lines of Japanese red pine (*P. densiflora*) and 16 lines of Japanese black pine (*P. thunbergii*). A similar programme was also undertaken in Anhui Province in China (Nose & Shiraishi, 2008). Some good results were obtained, but non-native pine species do not always adapt well to conditions in other countries (Sousa et al., 2015). Breeding and testing for resistant or tolerant tree lines is best done in the country of origin. More recent testing of 96 selected lines of maritime pine

(*P. pinaster*) found variation in resistance to that originally reported (Carrasquinho et al., 2018). The resistant lines were randomly selected from a phenotypic screening programme, highlighting potential problems with this approach. The use of molecular markers (quantitative trait loci) associated with resistance suggests a more reliable way of identifying suitable tree lines, although further research is needed.

Trunk injections of nematicidal chemicals into the trunk is a safer, more environmentally friendly and cheaper chemical control of PWN compared to aerial spraying of the vectors or use of fumigants. Takai et al. (2000) identified nAChR (nicotinic acetylcholine receptors) antagonists (abamectin, ivermectin, emamectin benzoate, milbemectin and milbemycin-5-oxime) as effective nematicides. Bi et al. (2015) observed that avermectin, emamectin benzoate and milbemectin suppressed nematode reproduction by reducing juvenile development. However, nematicides such as abamectins have low water solubility, which could reduce xylem transmission and spread beyond the injection site. Liu et al. (2020) found in vitro that fluopyram had better water solubility and killed more juveniles and adults of *B. xylophilus* than abamectin, and also reduced the hatching rate. More work is needed to see if these promising results are repeated when active ingredients are injected into trunks.

Plant extracts containing essential oils, alkaloids, terpenoids, flavonoids and amino acids have also been tested in attempts to avoid the use of synthetic pesticides and some useful identified secondary plant metabolites with nematicidal activity have been found (Sousa et al., 2015). The alkaloid piperine in extracts of black pepper (*Piper longum*) has also shown promise as a nematicide with good effects on the mortality, fecundity and motility of *B. xylophilus* (Rajasekharan et al., 2020). Extracts had a marginal effect on plant seed germination while killing up to 90% of the nematode. Faria et al. (2013) extracted essential oils from 59 different species plants, representing 13 botanical families. Twenty of the essential oils were capable of causing ≥96% mortalities of *B. xylophilus* at 2 μL/mL. Essential oils from *Ruta graveolens* (common rue), *Satureja montana* (winter savory) and *Thymbra capitata* (Mediterranean thyme) were the most toxic with lethal concentrations (LC_{100}) at <0.4 μL/mL.

Endoparasitic fungi, such as *Esteya vermicola*, have been investigated for their ability to suppress plant-parasitic nematodes, including *B. xylophilus* (Pires et al., 2022). Some fungi use ascarosides to lure nematodes into their sticky hyphal loops, which then rapidly swell once a nematode is attached—hence the term ‘nematode-trapping fungi’. Other endoparasitic fungi might be able to parasitize eggs, juveniles and adults or secrete toxic metabolites. The majority of endoparasitic fungi have yet to be widely commercially released. If successful, spray application is the most likely route of application.

10 | PORTUGAL CASE STUDY: PERSONAL REFLECTIONS

The establishment of PWD and *B. xylophilus* in Portugal has had a major impact on research and the extent of general efforts to bring the outbreak under control. Several scientists were interviewed to learn more about their experiences and insights gained from a long involvement with the disease. The results of interviews with two key experts on PWD are presented below. A short list of questions was prepared in advance but interviewees were also encouraged to share information on other topics.

Interviewees were asked about their scientific background and involvement with PWD, and how the outbreak compared with other plant pandemics. Other topics covered included the main social, political and economic impacts and how the disease (and its management) had affected the environment. They were asked to assess the success of the national eradication programme, any future control measures that were needed and the significance of climate change with regard to PWD.

10.1 | Professor Manuel Galvão de Melo e Mota

NemaLab-MED & Department of Biology, Universidade de Évora

Professor Manuel Mota has been actively involved with PWD since its discovery on *P. pinaster* on the Setúbal Peninsula in 1999 (Mota et al., 1999). He has participated in two major EU projects, which he said were critical to the understanding and management of PWD. He is well known within industry and academic circles for his expertise on the disease. "In the beginning, it was very surprising how difficult it was to persuade the authorities that the nematode was present", explained Professor Mota. The outbreak was initially limited to a 10–20 km zone. Some of the scientists involved at the time pushed for immediate felling of the trees within the adjacent areas, but this was not received well and regulators frowned upon the idea. The PWD pandemic in Portugal may have turned out differently if stringent measures had been taken at this early stage. Professor Mota compared the success of incisive actions by Spain authorities when PWD eventually got this far.

He said that the nematode appears to have been introduced twice, once from Japan and once from China, probably on furniture or wood packaging brought to Portugal returning from Macau in the 1990s. Macau was originally a Portuguese colony, before reverting to special Chinese administration in 1999. "Between 1999 and 2010 was the most challenging period of the PWD pandemic", added Professor Mota. In 2006, the authorities introduced a 3 km strip around the infected area where pine trees were felled. In principle, this approach should have worked because of the limited flight movement of the pine sawyer beetles. However, trucks carrying infected wood were believed to have led to new infections outside the infected zone (IZ). "In 2008, the disease spread from the centre and the Portuguese National Republican Guard (GNR) were enlisted to perform a greater number of checks on wood trucks".

The largest economic and social impacts from PWD came in 2007–2008. Professor Mota likened the PWD pandemic with the current spread of *Xylella fastidiosa* across olive groves in southern

Europe. PWD continues to require economic investment in terms of monitoring (10,000 wood chip samples collected and tested every year), trapping of *M. galloprovincialis* (c. 2000 traps each year at a cost of €200,000 and the felling of approximately 1–1.5 million trees at a cost of €750,000–1,000,000). The total cost for PWN control is €1,165,000. He also said that the felling of trees has caused significant changes to the landscapes with likely impacts on plants and animals, although this has yet to be evaluated.

During the critical years of the pandemic there were several changes in government. "This meant that approaches to PWD management changed quite frequently. Such changes in political direction caused an instability with the national effort to manage the disease", reflected Professor Mota.

Since 2010, the forestry sector has learned to live with PWD. The ICNF (Institute for Nature Conservation and Forests) has a PWN Action Plan that integrates the Buffer Zone Plan, cooperation with Spain (Bilateral Operational Plan) and other efforts to manage PWD. Although there have been challenges, the PWN Action Plan has been successful in stabilizing spread of the disease.

On the future of PWD management, Professor Mota highlighted the importance of monitoring and intensification of routine transport checks by the National Guard. Infected trees should continue to be cut and burned or chipped promptly. Lure traps are also an important part of management. There is also the prospect of identifying new resistance or tolerance to PWN in pine provenances identified in a national breeding programme coordinated by INIAV (National Institute for Agricultural and Veterinary Research). Biocontrol of the insect vector (e.g., EPNs) was more likely to work than biocontrol of *B. xylophilus* although recent research on the endoparasitic fungus *Esteya vermicola* seems to be promising. Nematicides, such as emamectin benzoate, injected into trunks are effective but only for treating a limited number of trees.

Professor Mota drew attention to the findings of the EU project REPHRAME. Modelling of future scenarios for PWD clearly show that rising temperatures will increase the risk of PWD in northern Europe.

10.2 | Dr Luís Bonifácio

Instituto Nacional de Investigação Agraria e Veterinaria (INIAV)

Dr Bonifácio has worked as a Forest Entomologist since 1988, joining INIAV in 1997, under the leadership of Dr Edmundo Sousa, with Dr Pedro Naves joining just 1 year later. He has been involved in PWD since its discovery in 1999 and carries out applied research on the biology and management of the disease. His work takes him all over the country. He runs training courses for forest inspectors and works closely with José Manuel Rodrigues, coordinator of PWD policy at ICNF.

He has been active in all the main projects on PWD, including two major international projects, PHRAME and REPHRAME, both coordinated by Dr Hugh Evans of the Forest Commission. More recently, he has worked on a case study in Troia peninsula, a tourist resort based on the south bank of the Sado River, near the city of Setúbal (Setúbal District). He said this was a great example of how integrated management approaches can reduce PWD incidence below 10% where the attack by bark beetles was the main threat to the pine forest.

Dr Bonifácio summarized the timeline of the PWD pandemic in Portugal. From 1999 to 2006 the disease was circumscribed to a small restricted zone (RZ) within the Setúbal Peninsula of about 309,000 ha. The RZ comprises an IZ and a BZ that is at least 20 km wide. In 2006, the 3 km CCB was implemented by the EU, meaning that host conifer trees on the perimeter of the RZ had to be removed. He said this was "an awkward decision that many scientists and industry representatives did not feel happy with". There was no clear evidence that the 3 km CCB would prevent the movement of the vector. He added that the experience of CCBs in northern European countries, such as Finland, was that they did not work. "The CCB was a mistake both economically and environmentally", reflected Dr Bonifácio. Once PWD moved out of its initial area, it became more widespread, although there are many areas that are still not infected.

When PWD spread to the centre of Portugal this was largely attributed to the illegal movement of wood by trucks from affected areas. Diseased wood was believed to have been mixed with uninfected 'clean' wood. Presently, the main challenge is to prevent PWN occurring in the 20 km BZ along the Spanish border.

One of the main difficulties in managing PWD in Portugal is that 98% of the forests are privately owned, the remainder belonging to government. Many areas planted with pines, particularly in the north and centre of the country, are made up of a patchwork of small plots of around 0.5 ha. A good proportion of the land-owners only live on their properties for short periods throughout the year. It is difficult for the police to service notices to land-owners for not felling pine trees with clear symptoms of PWD. The records of forested land are out of date. Even forest associations have difficulties communicating with all the land-owners.

Dr Bonifácio highlighted the economic importance of maritime pine (*P. pinaster*) in Portugal and the big impact PWD has had. "Maritime pine is a popular commodity with many companies across the world and an important source of national income", he said. PWD has reduced international trade while the requirement to treat forestry products for export meant that profit margins were significantly reduced. The felling of trees has resulted in excess availability of products such as woodchip. As the market became flooded, product prices declined.

PWD has also hastened a move away from pines to other forestry species. Blue gum (*Eucalyptus globulus*) was first introduced into Portugal in the late 18th century by Sir Joseph Banks, and in the 1950s it began to be planted for high-quality paper pulp. Since 2008, eucalyptus has replaced pine wood plantations, a move hastened by the spread of PWD. Dr Bonifácio shared estimates on the changes in tree species used in forestry (Table 2), showing the big decline in pines up to 2010. Paper pulp production from eucalyptus

TABLE 2 Changes in the forest area occupied by *Pinus pinaster* (maritime pine), *Quercus suber* (cork oak tree) and *Eucalyptus globulus* (blue gum tree) in Portugal from 1995 to 2010.

Tree species	1995 area (ha)	2010 area (ha)
Pinus pinaster	1,250,000	715,000
Quercus suber	660,000	735,000
Eucalyptus globulus	500,000	810,000

has continued to increase over the last 10 years. The Navigator Company produces high quality office paper and is one of Portugal's most well-known brands. A new law, introduced in 2017, requires Navigator and other companies in the paper pulp industry to release 1.5 ha of land for every 1 ha of new land rented.

Although PWD has had a major impact on pines in Portugal, some species remain unaffected. The stone pine or umbrella pine (*Pinus pinea*) is dominant in many parts of the Setúbal Peninsula, where PWD was first detected more than 20 years ago, yet remains unaffected by the disease. Unlike *P. pinaster*, the stone pine is not colonized by *M. galloprovincialis* because it produces non-attractive volatiles. Stone pine is a source of pine nuts and therefore economically important.

PWD has changed landscapes and insect populations in Portugal. "There are far more open habitats than before, particularly in central Portugal", said Dr Bonifácio. As these spaces regenerate they are mostly occupied by wild shrubs and some young pine trees, less attractive to the pine sawyer beetles because of their size. But the increase in dying or dead trees associated with PWD has resulted in increased populations of other bark beetles, mainly the six-toothed pine bark beetle (*Ips sexdentatus*) and the pine shoot beetles *Tomicus piniperda* and *T. destruens*. These increases have potentially negative implications for woodland across the country.

Dr Bonifácio commented on successes in tackling the PWD pandemic. The project in Troia was an excellent example of how the disease can be managed (Naves et al., 2018; Sousa et al., 2011). He linked success to two main practices. The first was to remove all dead trees, including fallen branches and upper canopies, before March and thus limit the number of emerging pine sawyer beetles (*M. galloprovincialis*). The next step was to use lure traps for 5–6 months in order to capture the flying insect vectors. When traps are adequately spaced and lures replaced every 6 weeks, trapping can reduce *M. galloprovincialis* by around 30%–40%. He emphasized that it was important not to use traps where there are healthy trees as these might lure beetles that are carrying *B. xylophilus*.

Government grants are available to help forest managers purchase traps. On the future of PWD management, Dr Bonifácio highlighted the *P. pinaster* breeding programme underway in Portugal and Galicia (Spain), and how chemical ecology approaches could become more important. But he was also wary about the deployment of trees resistant to PWD. "We need to be careful in introducing a limited number of provenances with resistance as this could add to selection pressure for variants of *B. xylophilus* and other native pine pests and diseases".

11 | CONCLUSIONS

PWD continues to exert major impacts in Asia and in Portugal. As well as the economic effects for the forestry industry, the loss of trees and changes to the landscape are bad news for nature tourism and recreation in natural environments. PWD has also had consequences for loss of tree habitats and forest ecosystems on which arboreal animals, plants and fungi depend. Loss of forests means less removal of carbon dioxide, the main cause of increasing global temperatures. The ability of pine forests to regulate soil erosion, mediate the water cycle through transpiration and absorb radiation have been compromised by PWD.

In North America, the probable origin of the pine wood nematode, direct losses due to PWD in forests are much less because of resistant or tolerant native species of pine. But indirect financial losses are much higher because *B. xylophilus* is classified as a quarantine organism by countries importing pine from the United States, requiring expensive pretreatment of timber and wood products. There is also a concern that direct losses may increase. There is evidence that PWD is spreading westwards from the Pacific coast and mountain ranges in the United States towards areas where susceptible introduced pine species are grown, such as Scots pine (*P. sylvestris*) and Austrian pine (*P. nigra*), according to Dr Luís Bonifácio.

The complex nature of PWD highlights the multiple challenges for disease management. The main biotic components are the nematode itself and its insect vectors. Bacteria also contribute to disease development, both directly and by eliciting defence pathways in plants. Blue-stain fungi and bark beetles also have an important role in the disease.

Steady progress has been made in combatting the spread of the disease. Felling trees and burning or chipping infested wood continues to be a crucial strategy, as is trapping of the insect vectors. Important advances have been made in the use of lures and trap design, particularly through improved knowledge of semiochemicals. In Portugal, annual trapping of vectors from March to November is an important part of ongoing efforts to manage PWD. Chemical control of vectors has had mixed success. Insecticides are effective in reducing vector populations but depend on area-wide applications. Aerial spraying undertaken in Asian countries such as Japan is expensive and unpopular with local communities. Non-target effects of insecticides are also causing concern.

Tree injection of nematicides, such as abamectin and avermectin, provides systemic protection for up to 3 years against *B. xylophilus* (Sousa et al., 2013). It is also expensive and more suited to small groups of trees. A significant amount of work has been undertaken on the biological control of *Monochamus* species and *B. xylophilus*. Biopesticides are gaining more attention as the use and range of licensed pesticides continues to decline. EPNs active against the vectors and endoparasitic fungi for the *B. xylophilus* show potential but more commercial development is needed. The successful commercialization of *B. bassiana* against black pine sawyer suppression in Japan is a promising step forward.

Despite all these efforts, the treatment of timber and wood products remains one of the main measures for combatting PWD, supported by legislation and scrutiny by plant health authorities and importing countries. Methyl bromide, a powerful fumigant, is banned or carefully restricted in many countries. It is a dangerous chemical and damaging to the environment because of damage to the ozone layer.

Tree breeding for resistance or tolerance has had limited success so far. Breeding programmes in Japan and China have made progress, although it is unclear if resistant species and cultivars developed there will succeed or meet the forestry needs of other countries. This highlights the importance of breeding efforts in Portugal to identify suitable *P. pinaster* provenances.

The case study in Portugal revealed that management programmes depend not only on availability of suitable and effective control methods but how these are put into practice. Political will and effective execution of strategies are also crucial in tackling PWD. Tree felling and trapping of *M. galloprovincialis* have worked well. A slow initial response to the discovery of PWN hindered attempts to limit the outward spread. Decisive action is needed in the crucial early stages of an outbreak. The forestry sector in Portugal has adapted to the disease with a shift towards the use of eucalyptus by the paper pulp companies. Major efforts to monitor and combat the disease have been hampered by difficulties in contacting land-owners and poor official records of holdings. Interviews with Professor Manuel Mota and Dr Luis Bonifácio were invaluable in gaining fuller insight into the impacts of PWD in Portugal, with experiences and reflections that are largely missing from the published literature on the disease.

PWD is an ongoing problem for those countries and regions that already have the disease and a major threat to pine forests in countries that so far have remained free of PWD. Continued vigilance by plant health authorities will continue to be of paramount importance in preventing entry of *B. xylophilus* and its vector. There have been numerous interceptions in Europe. *M. alternatus* was found on wooden furniture imported into the UK. It is unlikely that the vectors or the nematode would become established in cold temperate countries (such as the UK, Sweden or Finland) under current weather regimes. Yet increasing temperatures due to climate change clearly indicate future dangers and on-going challenges in keeping PWD at bay.

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