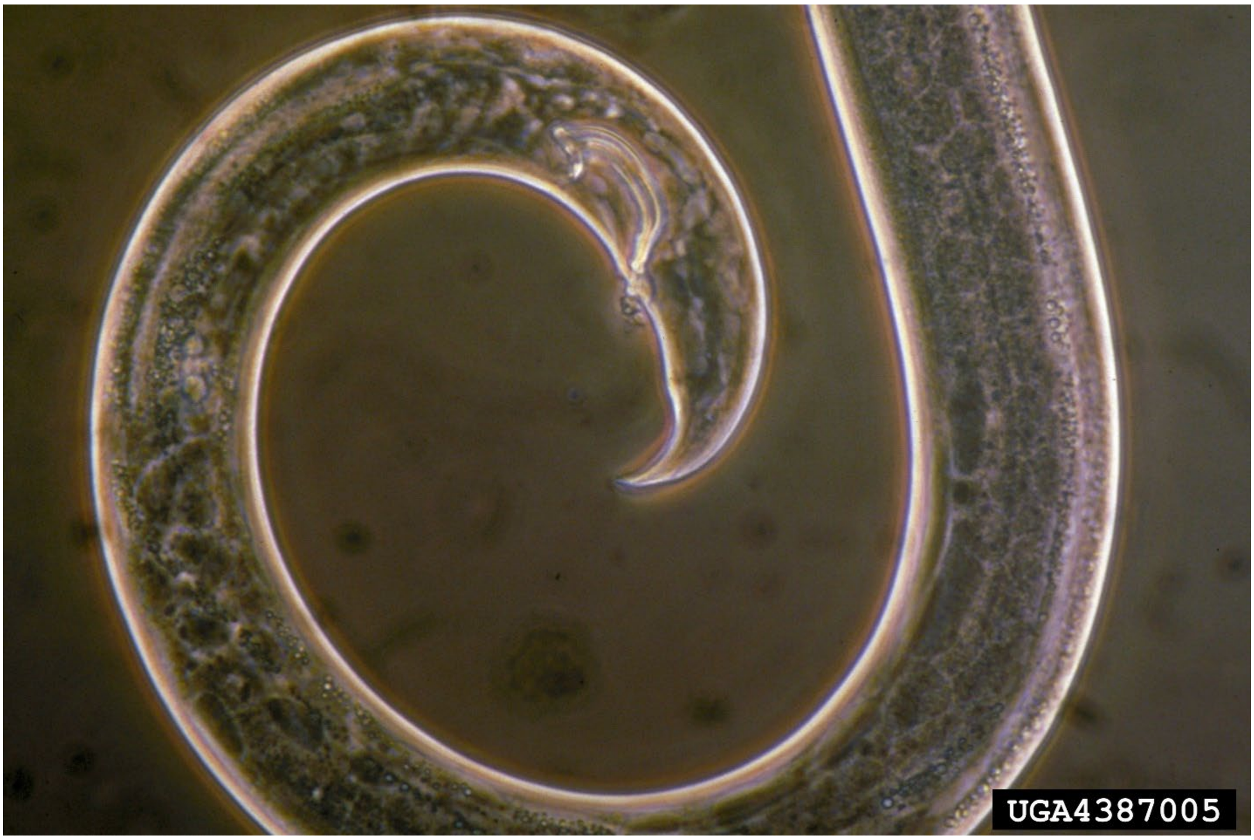
Pine wilt disease: Biosecurity readiness for New Zealand's plantation forestry

Stephanie Sopow, Belinda Gresham, Georgia Dickson and Nicolas Meurisse



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Microscopic image of pine wood nematode, the causal agent of pine wilt disease. The nematode is approximately 1 mm long.

Report information sheet

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Authors	*STEPHANIE SOPOW, BELINDA GRESHAM, GEORGIA DICKSON, NICOLAS MEURISSE* *SCION*
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Executive summary

Overview

The global expansion of pine wilt disease (PWD), caused by the pine wood nematode (PWN) *Bursaphelenchus xylophilus*, poses a serious threat to coniferous forests worldwide. PWD can be especially devastating to susceptible pine species, leading to tree mortality within weeks to months. This disease presents a significant biosecurity risk to New Zealand's plantation forestry sector and could swiftly impact the ability to export logs.

PWN, native to North America, first gained attention after accidentally being introduced into Japan in the early 1900s, causing a widespread epidemic of PWD, still requiring control efforts today. PWN spread from Japan to neighbouring countries China (1982), Taiwan (1985), and South Korea (1988). In China, the spread of PWD has accelerated in recent years, expanding into different host tree species and directly threatening nearly 60 million ha of pine forest resources.

Beginning in the 1980s, quarantine measures were implemented worldwide to prevent the distribution of PWN with the trade of wood products. In 1984, PWN was intercepted in Finland in wood chips imported from North America, raising concerns in Europe where susceptible pine hosts and suitable insect vectors are present. In 1993, the European Union went a step further and placed an embargo on untreated and unseasoned wood from all regions where PWN was known to occur. Despite the measures in place, PWN established in Portugal where it was first found in 1999, and costly eradication attempts over a ten-year period were unsuccessful. Subsequently, PWN has been detected and eradicated several times in the north of Spain since 2008.

PWN requires an insect vector to spread to new trees. The only known vectors are longhorn beetles in the genus *Monochamus*, and, as discovered in invaded regions, *Monochamus* species not previously associated with PWN have proven to become successful novel vectors.

To the best of our knowledge, PWN is not established in the Southern Hemisphere (CABI, 2022). It is prioritised on New Zealand’s Government Industry Agreement pest list for plantation forestry.

Key results and implications for New Zealand

Worldwide, PWD remains a major concern and is receiving much attention, with over 120 scientific papers published in 2023 alone. This review assesses the threat that PWN poses to New Zealand and identifies opportunities for improving preparedness.

There are two primary reasons for some ambiguity regarding the threat of PWD to New Zealand: 1) a historical perception that *Pinus radiata*, thought to have evolved along with the nematode in its native range, exhibits a high degree of tolerance to the nematode, and 2) the fact that no *Monochamus* species are known to have established in New Zealand.

While the primary damage in North America is indeed caused to exotic pines planted there, PWN is much more prevalent in eastern North America and evidence suggests the possibility that it may have spread from the east to the west and therefore may not have co-evolved with *P. radiata*. In addition, recent European studies have categorised *P. radiata* as medium to highly susceptible to PWN in these novel environments. Although pines are the primary hosts of PWN, other conifers are also susceptible and it is noteworthy that isolated deaths of Douglas fir, *Pseudotsuga menziesii*, caused by PWN have been reported in the United States.

While no *Monochamus* species are known to have established in New Zealand, members of this genus have been regularly intercepted, some potentially carrying PWN. Secondary vectors, known to carry PWN but not yet known to vector it, also represent a potential concern. PWN has been isolated from several secondary vectors, including *Arhopalus rusticus*, a close relative of *A. ferus*, which is present, widespread, and abundant in New Zealand.

Extensive modelling may be unable to predict spread, as seen in the recent unexpected spread of PWN northwards in China. The spread appears to be a result of both climate change and PWN being vectored by an additional species of *Monochamus* different from that in the original outbreak. Additionally, while *B. xylophilus* is currently thought to be the only species among the large *Bursaphelenchus* genus that is pathogenic to conifers, studies indicate varying degrees of pathogenicity of other species, and of hybrids, suggesting potential for other forms to become emerging pathogenic risks.

Significant learnings from elsewhere include the evidence from Europe that the phytosanitary precautions in place are not fully effective. Despite this, New Zealand lacks a dedicated surveillance programme for wood and bark boring beetles, representing a conspicuous gap in the biosecurity system.

Further work

This review supports the view that a readiness plan for New Zealand is warranted. Readiness may include better understanding the specific threat to New Zealand by screening our *Pinus radiata* germplasm for susceptibility, and by testing commonly encountered longhorn beetles for their ability to act as primary vectors. Various surveillance tools have been or are currently being developed, and these capabilities may be sourced in New Zealand. Up-to-date diagnostic protocols can be adopted, and laboratory facilities and supplies made ready. Details on recommendations are provided in the final section of the review.

New Zealand biosecurity readiness: Pine wood nematode and its vectors

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1. Introduction

The forestry and wood products industry is a vital component of the New Zealand economy, generating $6.58 billion in export earnings (year to June 2022), placing it as New Zealand's fourth-largest export earner (just behind horticulture) (MPI, 2024). The introduced Californian radiata pine, *Pinus radiata*, is the dominant plantation species, comprising approximately 90% of the commercial estate.

Despite the constant pressure of new biosecurity threats, New Zealand's geographic isolation and robust biosecurity system have undoubtedly contributed to its success in avoiding many damaging forest insect pests that have been demonstrated to be successful invaders elsewhere. Of the exotic pests of *P. radiata* established in New Zealand, the simultaneous introduction of the sirex wood wasp, *Sirex noctilio* F. (Hymenoptera: Siricidae) and its symbiotic fungus, *Amylostereum areolatum* (Chaillet ex Fr.) Boidin, before 1900, has been the most damaging (Bain et al., 2012).

Among the various nematode species present in New Zealand's *P. radiata* nurseries and plantations, at least three species utilise insects as transportation vectors: *Bursaphelenchus hildegardae*, *B. fungivorus* and the recently discovered *B. zealandicus*. These nematodes belong to the same genus as the highly pathogenic *Bursaphelenchus xylophilus* and have been found in association with dead or dying trees in New Zealand, however they were not determined as the causal agents. *Bursaphelenchus hildegardae* and *B. fungivorus* likely originate from Europe, exemplifying the threat of invasive *Bursaphelenchus* species in New Zealand. Their entry pathways and time of introduction remain unknown. A plausible scenario is through large-scale timber imports in the 19th or early 20th century, as it seems to have been an introduction pathway for various tree-associated microorganisms and natural enemies vectored by bark and wood-boring insects (Eshetu et al., 2023; Romo et al., 2017).

Pine-associated nematodes also include root-feeding species in the genera *Rotylenchus*, *Pratylenchus*, *Paratylenchus*, and *Trichodorus*, which have the potential to reduce seedling growth (Yeates, 1990). Other problematic introduced nematode species in NZ affect the pastoral, agricultural and horticultural sectors (Mercer et al., 2008).

The pine wood nematode, *B. xylophilus*, is not present in New Zealand. It is the causal agent of the pine wilt disease and is a serious threat to coniferous forests worldwide. Pine wilt disease can be especially destructive to susceptible pine species, causing tree mortality within weeks to months and causing significant economic losses.

The pine wood nematode is native to North America and has been spreading globally, despite best efforts to prevent its movement to new regions. In Japan, where it was first recognised as a highly damaging invasive agent and mitigation efforts have persisted for decades, annual wood volume losses have exceeded 2 million m^3 since 1970 (Mamiya & Shoji, 2009), peaking at 2.5 million m^3 in the early 1980s (Gruffudd & Jenkins, 2024). In 1993, the European Union (EU) placed an embargo on untreated wood from regions where PWN is known, and annual surveys for PWN (and delimiting surveys if detected) are now mandatory in the EU (Schroeder, 2019). This embargo caused a dramatic decline in softwood exports from the US and Canada, resulting in cumulated losses to North American exporters exceeding US$1B (actualised values) (Carnegie et al., 2018). The cost to eradicate isolated pockets of PWN-infested forests in Spain since 2008 is estimated at US$118 million, with the management of a single infested site costing nearly US$4.5 million between 2008 and 2010 (actualised values) (Gruffudd & Jenkins, 2024). The large-scale economic losses experienced in invaded regions because of direct damage to trees, as well as export barriers, provide a high incentive for vigilance in New Zealand.

2. An overview of nematodes and the genus Bursaphelenchus

2.1. Plant-parasitic nematodes

Nematodes, members of the phylum Nematoda, are unsegmented worms that constitute one of the most abundant groups of animals on Earth. Approximately 20,000 species are named, however, this figure likely represents only a small portion of their actual diversity. Nematodes exhibit diverse lifestyles; some are free-living and feed on microorganisms in soil, freshwater or marine environments, while others adopt parasitic relationships with animals or plants. They are typically microscopic, but some can be several metres long (Encyclopaedia Britannica, 2023). The life cycle includes an egg, usually four juvenile stages that appear superficially similar to the adult, and the adult (Wharton, 1986).

Nematodes of economic importance include cultivated plant parasites damaging crops and orchards, and animal parasites that reduce the health of animals. Some species are also beneficial, such as those used as biological control agents against insect pests.

Most plant-parasitic nematodes are associated with plant roots, however, some aggressive species in the genus *Bursaphelenchus*, such as the red ring nematode *B. cocophilus* Cobb affecting palm trees, and the pine wood nematode *B. xylophilus* (Steiner and Buhrer), are found in the aboveground parts of woody plants (Donald et al., 2016).

2.2. Bursaphelenchus spp.

The genus *Bursaphelenchus* contains over 100 described species, inhabiting soil or woody plants, in which the nematodes feed either on fungi, directly on plant tissue, or both.

Within this large genus, few are considered significant tree pathogens. The pathogenicity of species of *Bursaphelenchus* is not clearly related to their phylogeny (Kanzaki & Giblin-Davis, 2018). Instead, pathogenicity is suggested to have evolved in accordance with the life history traits of the vector species (Kanzaki & Takemoto, 2012). Kanzaki and Giblin-Davis (2018) distinguish three main clades and discuss their hosts (principally Pinaceae), their vectors (usually beetles in the Curculionidae, including Scolytinae (bark beetles), and Cerambycidae) and their virulence (only qualified as moderate or high in four species). A summary of these relationships for key *Bursaphelenchus* pathogens of pine trees is provided in Table 1. The most virulent *Bursaphelenchus* seem to be associated with wood-boring insects that are more prone to attack healthy tissue, such as *B. xylophilus/mucronatus* associated with *Monochamus* beetles. However, it is worth noting that moderate to high virulent *Bursaphelenchus* are recorded across all clades, and these are vectored by both cerambycids and bark beetles.

The sister species *B. xylophilus* and *B. mucronatus*

Bursaphelenchus xylophilus exhibits high genetic diversity and variable pathogenicity, with both virulent and avirulent strains reported, and virulence is thought to be closely related to nematode reproductive potential (Filipiak et al., 2021; Menéndez-Gutiérrez, Villar, et al., 2021).

Bursaphelenchus mucronatus Mamiya & Enda is the closest taxonomic relative to *B. xylophilus* and, despite *B. mucronatus* being native to Eurasia and thus evolving in geographic isolation, the two can hybridise (Riga et al., 1992). The pathogenicity of *B. mucronatus* is reported as being highly dependent on susceptibility of the host species (Akbulut et al., 2007), with most considering it innocuous (e.g. Mamiya & Enda, 1979) to weakly pathogenic (e.g. Zhang et al., 2004). In contrast, Bakke et al. (1991) reported high mortality of inoculated *Pinus sylvestris*, however, these were seedlings subjected to high temperatures in a greenhouse. The hybrid between *B. mucronatus* and *B. xylophilus* occurs naturally where the two now overlap geographically, and the pathogenicity of the hybrid is reported as being similar to that of *B. xylophilus* (Y. L. Li et al., 2021).

Table 1: Pathogenicity to pine trees of *Bursaphelenchus* in different clades. In clade I, the vectors are mostly bark beetles, in clade III, they are mostly longhorn beetles, while variable beetle groups seem to be vectors in Clade II. Adapted from Kanzaki and Giblin-Davis (2018) and Zhao et al. (2024).

Species	Pathogenicity to pine trees	Natural hosts	Known vectors
Clade I			
B. sexdentati	Weak-moderate	Pinaceae	*Ips sexdentatus* (Coleoptera: Curculionidae: Scolytinae)
B. poligraphi	Suspected	Pinaceae	*Polygraphus poligraphus* (Coleoptera: Curculionidae: Scolytinae)
B. hildegardae	Suspected	Pinaceae	*Hylastes ater* (Coleoptera: Curculionidae: Scolytinae)
B. eggersi	Suspected	Pinaceae	?
B, tusciae	Suspected	Pinaceae	*Hylurgus ligniperda* (Coleoptera: Curculionidae: Scolytinae)
B. yongensis	Suspected	Pinaceae	*Cryphalus fulvus* (Coleoptera: Curculionidae: Scolytinae)
B. hellenicus	Weak	Pinaceae	*Tomicus piniperda, Hylurgus ligniperda, Ips sexdentatus* (Coleoptera: Curculionidae: Scolytinae)
B. niphades	Suspected	Pinaceae	*Niphades variegatus* (Coleoptera: Curculionidae)
B. hoffmanni	Suspected	Pinaceae	*Pityokteines curvidens* (Coleoptera: Curculionidae: Scolytinae)
B. anamarius	Weak	?	?
B. leoni	Weak		*Dryocoetes autographus* (Coleoptera: Curculionidae: Scolytinae)
B. zealandicus	Suspected	Pinaceae?	?
Clade II			
B. kiyoharai	Weak	Various broad-leaved and coniferous trees	*Xyleborus seriatus* (Coleoptera: Curculionidae: Scolytinae)
B. fungivorus	Suspected	?	?
B. cocophilus	Weak-moderate (native range) Moderate-high (invasive range)	Arecaceae	*Acalolepta luxuriosa* (Coleoptera: Cerambycidae)
Clade III			
B. mucronatus	Weak-moderate	Pinaceae	*Monochamus* spp. (Coleoptera: Cerambycidae)
B. firmae	Weak	Pinaceae	*Monochamus* spp. (Coleoptera: Cerambycidae)
B. xylophilus	Weak-moderate (native range) Moderate-high (invasive range)	Pinaceae	*Monochamus grandis* (Coleoptera: Cerambycidae)
B. luxuriosae	Weak	Araliaceae	*Acalolepta luxuriosa* (Coleoptera: Cerambycidae)
B. okinawaensis	Suspected	Various broad-leaved (and coniferous?) trees	*Monochamus maruokai* (Coleoptera: Cerambycidae)
Unassigned clades*			
B. vallesianius	Weak	?	?

***Bursaphelenchus* species in New Zealand**

Four species of *Bursaphelenchus* are reported in New Zealand (Zhao et al., 2024). Of these, two have had their taxonomic identity confirmed by molecular methods:

- *Bursaphelenchus hildegardae* Braasch *et al.*, 2006 was first recorded in 2019 from *P. radiata* trees in the Central North Island. These records were associated with unexplained tree mortality and the presence of the bark beetle *Hylastes ater* Paykull (Scolytinae) (Zhao et al., 2021). This species was first described in 2006 from German samples collected in *Pinus sylvestris* and from the bark beetle *Hylurgops palliatus* Gyllenhal (Scolytinae) (Braasch et al., 2009).
- *Bursaphelenchus zealandicus* Zhao, 2024 was extracted from a dead *P. radiata* tree in the Central North Island in 2021, and represented a novel species described for the first time in New Zealand (Zhao et al., 2024). To date, this species is unknown elsewhere.

It is worth noting that these recent detections resulted from active industry-funded forest health monitoring efforts, elevated awareness of field inspectors, and available diagnostic expertise. Before these, two other species have been reported from New Zealand based on morphological identifications:

- *B. fungivorus* Franklin & Hooper, 1962, was reported in New Zealand in 1971 as having “recently been recorded from beds of cultivated mushrooms” (Dale, 1971). This species was first described on rotting gardenia flower buds infected by *Botrytis cinerea* in Wales (Franklin & Hooper, 1962), but has since been reported in association with pines, including from *Pinus* spp. and associated with the bark beetle *Orthotomicus erosus* (Wollaston 1857) (not present in NZ) in Spain (Arias et al., 2005), and from *P. pinaster* bark in Portugal (Fonseca et al., 2014). Braasch et al. (1999) reported pathogenicity of *B. fungivorus* to *P. sylvestris* with 70% mortality and 20% partial wilting in inoculated 3-year-old seedlings.
- *Bursaphelenchus eggersi* Rühm, 1956 was reported in 1966 in association with North and South Island samples of adults and frass of the bark beetle *H. ater* (Dale, 1967). However, it must be noted that the closely related species *B. hildegardae* was not yet described at the time, and the two species are extremely difficult to distinguish morphologically (Braasch et al., 2006), leaving open the possibility that *B. eggersi* was a misidentification of *B. hildegardae.* This idea is supported by the fact that *B. eggersi* has not been recovered in New Zealand since the original study by Dale (1967), despite targeted diagnostics in biosecurity surveillance programmes and the investigation of a collection of 20 dried specimens of *H. ater* collected before 2002 from Scion’s National Forestry Insect Collection (Zhao et al., 2021). *Bursaphelenchus eggersi* is known from several European countries, in association with *Pinus* spp., *Larix* spp., *Picea* spp. and *Abies alba* (D'Errico et al., 2014). Dale (1967) noted that an examination of *H. ater* specimens in Germany revealed not *B. eggersi*, but *B. chitwoodi*, and suggested that *B. eggersi* may have been introduced to New Zealand on its normal bark beetle host *H. palliatus*, but the beetle failed to establish and thus a new association was formed with *H. ater*.

Zhao et al. (2024) showed that all *Bursaphelenchus* species currently known from New Zealand are phylogenetically distant from *Bursaphelenchus xylophilus* and *B. mucronatus.*

3. Bursaphelenchus xylophilus

3.1. Taxonomy and Biology

Scientific name: *Bursaphelenchus xylophilus* (Steiner and Buhrer 1934) Nickle 1970

Synonyms: *Aphelenchoides xylophilus* Steiner & Buhrer 1934, *Bursaphelenchus lignicolus* Mamiya and Kiyohara 1972

Common names: pine wood nematode, pinewood nematode, pine wilt nematode

Phylum: Nematoda

Class: Aphelenchida

Order: Rhabditida

Family: Aphelenchoididae

The pine wilt disease (abbreviated PWD) first gained attention after an outbreak in 1905 in Japan, followed by ongoing mortality of pine forests over decades (Mamiya, 1988). However, it was not until 1969 that the first association was made between the disease and the occurrence of a new nematode, isolated in affected stands of *Pinus densiflora* Siebold & Zucc. and *P. thunbergii* Parl (Kiyohara & Tokushige, 1971; Tokushige & Kiyohara, 1969). The nematode was described at the time as a new species, *Bursaphelenchus lignicolus* (Mamiya & Kiyohara, 1972). It was only later realised that this represented an accidental introduction to Japan, and was identical to a native North American species first isolated from *Pinus palustris* in 1929 in Louisiana, USA (Mamiya, 1988), originally described as *Aphelenchoides xylophilus* (Steiner & Buhrer, 1934) and later transferred to the genus *Bursaphelenchus* (Nickle, 1970). The ability of *B. xylophilus* to induce a severe wilting disease on pine trees was reaffirmed in 1979 in North America when the nematode was rediscovered from a dead Austrian pine, *Pinus nigra,* in Missouri (Dropkin & Foudin, 1979; Nickle et al., 1981).

Bursaphelenchus xylophilus consists of two forms, an 'R' or round-tail form and an 'M' or mucronate form, in which the females have a projection on the tail. The 'M' form is mainly found in North America, along with the 'R' form, and is nearly identical in morphology to *B. mucronatus* (IPPC, 2016) The 'R' form is the virulent form that causes PWD (Zhao et al., 2013).

Biology

Bursaphelenchus xylophilus, commonly referred to as the pine wood nematode, or the pine wilt nematode (abbreviated PWN), is the causal agent of pine wilt disease (PWD). The nematode kills pines and other conifers by feeding on the vascular tissues and disrupting the uptake of water (CABI, 2022).

The PWD cycle is complex, with several organisms contributing to the success of the development and spread. The nematode, *B. xylophilus*, requires a suitable host tree, commonly in the genus *Pinus*, an insect vector for dispersal to new host trees, and at times feeds not directly on the tree but on various fungi growing in the dying tree (Vicente et al., 2021). While *B. xylophilus* is the primary agent of PWD, associated microbiota are increasingly believed to play key roles in the expression of the disease (Proença et al., 2017; Xue et al., 2019; Zhao et al., 2011; Zhao et al., 2013). Tree species, pre-existing health of the tree and environmental factors also affect pathogenicity (CABI, 2022).

Bursaphelenchus xylophilus has two developmental cycles that arise in differing conditions: the propagative cycle when conditions are favourable, and the dispersal cycle when conditions are unfavourable (B. N. Kim et al., 2020; Vicente et al., 2021). Within these two cycles PWN has four juvenile stages. In the propagative cycle, food is available for the nematode, and it reproduces within the tissue of the wood (B. N. Kim et al., 2020). As time goes on, the proportion of juveniles in the dispersal stage increases (Togashi & Shigesada, 2006). When the conditions become unfavourable due to overpopulation, dauer juveniles (dispersal juveniles), which have a body structure adapted for transport, enter the tracheae of newly formed adult vectors for transmission to new trees (B. N. Kim et al., 2020; Togashi & Shigesada, 2006; Vicente et al., 2021).

As vectors, *Monochamus* spp. transmit PWN to new hosts in two ways. Primary transmission is through adult maturation feeding on shoots and young branches of the host, and secondary transmission is through adult female oviposition in dead or dying host trees (Vicente et al., 2021). Arakawa and Togashi (2002) showed that PWN may also be transmitted by males with high nematode loads in proximity to oviposition wounds, and that the nematodes could move up to 10 cm along the bark surface to reach the wounded tissue and gain entry. Further information on the biology of the vectors is provided below.

Once the new host is infected, PWN begins the cycle again, rapidly developing and reproducing. The population of PWN survives by feeding on fungi growing in the dead host, with further vector species being attracted to oviposit on the dead material (Zhao et al., 2008). Through this association, the vector species benefit through an increase in suitable host trees for reproduction, while PWN benefits through an increased vector population, thus increasing transmission rates (Vicente et al., 2021). Figure 1 illustrates the life cycle of PWN and methods of transmission by vectors.

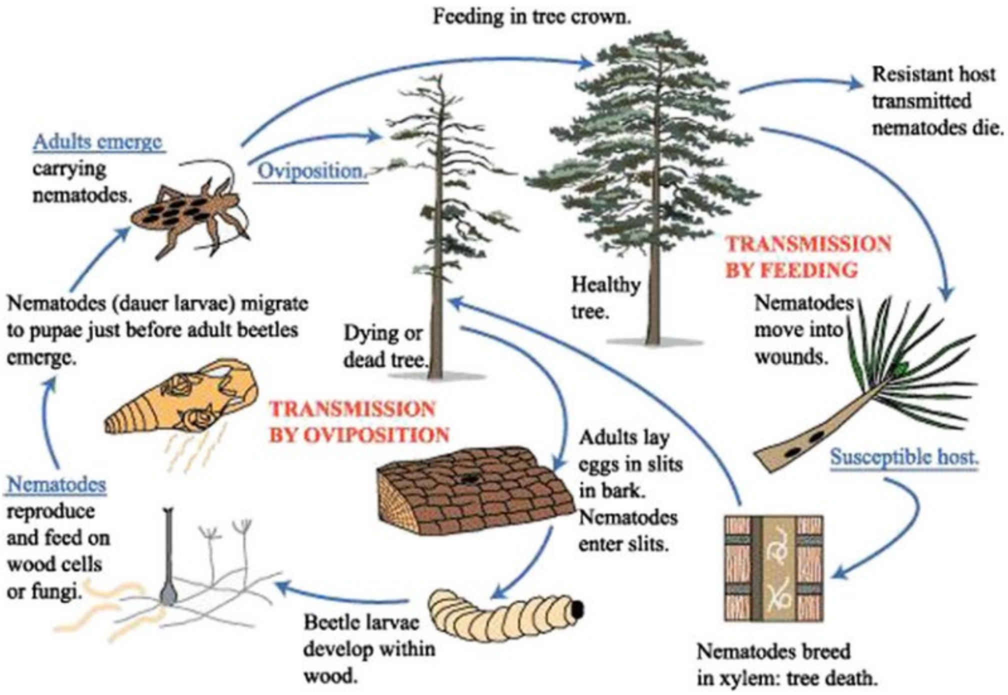

Figure 1: Life cycle of the pine wood nematode *Bursaphelenchus xylophilus*, and pine wilt disease (diagram courtesy of H. Evans).

PWD is caused by the phytophagous phase of PWN after being introduced to a susceptible host through feeding wounds made by adult *Monochamus* beetles on small branches. PWN moves through and multiplies in the cortical tissue and resin ducts of the host tree, causing a decrease in the secretion of resin and slowing needle transpiration (Carnegie et al., 2018). Eventually needle transpiration ceases altogether, with the needles turning yellow, and then red, persisting on the tree (Li et al., 2022) (Figure 2).

Figure 2 Evolution of symptoms of pine (*Pinus pinaster*) infested by *Bursaphelenchus xylophilus*, from healthy tree to dead (figure borrowed from IPPC diagnostic protocol (IPPC, 2016)). Photos courtesy T. Schröder, Julius Kühn-Institut, Germany.

The process is rapid, and tree death can occur within two to three months of infection (Carnegie et al., 2018; Li et al., 2022). Once the symptoms become visible, recovery of the host tree is no longer possible (Vicente et al., 2012). A combination of susceptible tree species, high temperature and low soil moisture conditions provides optimal conditions for the development of PWD (Gruffudd & Jenkins, 2024).

3.2. Insect vectors and associates

Vectors: longhorn beetles in the genus *Monochamus*

Scientific name: *Monochamus* spp.

Synonyms: *Monohammus*, *Monochammus*, *Monohamus*, *Meges*

Common names: sawyer beetles, sawyers, timber sawyers

Phylum: Arthropoda

Class: Insecta

Order: Coleoptera

Family: Cerambycidae

The spread of *B. xylophilus* requires an insect vector, and although the nematode has been isolated from several different insects associated with wood (e.g. Linit, 1988), only species belonging to the longhorn beetle genus *Monochamus* (Coleoptera: Cerambycidae) have been confirmed as true vectors successfully transferring the nematode to new hosts (Aikawa, 2008; CABI, 2022). *Monochamus carolinensis* (Olivier) is the primary vector in North America (Linit, 1988), *M. alternatus* Hope in Japan, China and Korea (Lee et al., 2018; Mamiya & Kiyohara, 1972), and *M. galloprovincialis* (Olivier) (Figure 3) in Portugal (Sousa et al., 2001).

Figure 3 An adult male *Monochamus galloprovincialis.* Specimen body length is 18.5 mm. [Image credit: Udo Schmidt]

Species of *Monochamus* are often associated with *Bursaphelenchus* spp., and it is presumed that most or all *Monochamus* species are capable of vectoring *B. xylophilus* (Pimentel et al., 2023), although the efficiency of different species as vectors can be expected to be variable, with differences in documented nematode loads, dispersal abilities and generation times (Akbulut & Stamps, 2012). *Monochamus carolinensis* and *M. alternatus* carry the highest nematode loads (with reported estimates of 19,000 and 15,000, respectively), with fewer reported loads for *M. galloprovincialis* (2000-2300), and the average load being less than 300 in other investigated *Monochamus* species (Akbulut & Stamps, 2012). Hashimoto and Sanui (1974) showed that 300 fourth-stage dispersal juveniles (JIVs) inoculation is sufficient to cause lethal infections in 8-year-old pine trees. According to calculations by

Togashi (2013), a single beetle carrying 10,000 nematodes initially, would be enough to infect a healthy tree. However, at lower loads (e.g., in the 1000's of JIVs), it would require the aggregation of several infective beetles to threaten healthy trees (Togashi, 2013). More recently, these studies were compared to the PWN-*M. carolinensis* system in the USA, which was found to be a less efficient vector than the PWN-*M. alternatus* system in Japan (Togashi et al., 2022).Table 2 shows *Monochamus* species reported as vectoring PWN.

Table 2: Species of *Monochamus* reported as vectors of *Bursaphelenchus xylophilus*. Table created from those in Pimentel et al. (2023) and Akbulut and Stamps (2012).

Species	Country	Host tree
Asia		
M. alternatus Hope*	China, Japan, Laos, North Korea, South Korea, Vietnam	*Pinus* spp., *Picea* spp., *Abies* spp., *Larix* spp., *Cedrus* spp.
M. grandis Waterhouse	Japan	*Pinus* spp., *Picea* spp., *Abies* spp., *Larix* spp.
M. nitens Bates	Japan	*Pinus* spp.
Asia / Europe		
M. saltuarius (Gebler)	China, Japan, South Korea, Austria, Germany, Italy, Lithuania, Poland, Russia, Switzerland, Ukraine	*Pinus* spp. (Asia), *Picea* spp.
M. sutor (Linnaeus)	China, Austria, Bulgaria, Denmark, Finland, France, Georgia, Germany, Italy, Norway, Poland, Serbia, Spain, Sweden, Switzerland, Ukraine	*Pinus* spp., *Picea* spp., *Abies* spp. (Asia), *Larix* spp. (Europe)
M. urussovi (Fischer-Waldheim)	China, Japan, North Korea, South Korea, Finland, Norway, Poland, Russia, Sweden	*Picea* spp., *Abies* spp., *Larix* spp., *Pinus* spp., *Betula* spp.
	Europe / North Africa	
M. galloprovincialis (Olivier)*	Austria, Finland, France, Germany, Greece, Italy, Poland, Portugal, Russia, Spain, Sweden, Switzerland, Turkey, Algeria, Morocco, Tunisia	*Pinus* spp., *Picea* spp.
North America		
M. carolinensis (Olivier)	Canada, Mexico, United States	*Pinus* spp.
M. clamator LeConte	Canada, United States	*Pinus* spp.
M. marmorator Kirby	United States	*Abies* spp., *Picea* spp.
M. mutator LeConte	United States	*Pinus* spp.
M. notatus (Drury)	United States	*Pinus* spp.
M. obtusus Casey*	Canada, United States	*Pinus* spp., *Pseudotsuga* spp., *Abies* spp.
M. scutellatus (Say)	Canada, United States	*Pinus* spp., *Abies* spp., *Picea* spp., *Larix* spp.
M. titillator (Fabricius)	Canada, United States	*Pinus* spp., *Picea* spp., *Abies* spp.

* Species of *Monochamus* recorded as feeding on *Pinus radiata* (Brockerhoff et al., 2023). This is not believed to be an exhaustive list but rather represents circumstances where these species have had the opportunity to encounter *P. radiata* and feeding has been documented.

The ability of *B. xylophilus* to easily adapt to novel vectors not native to North America is exemplified by the successes of the local species *M. alternatus* vectoring the nematode in Asia, and *M. galloprovincialis* in Europe (Akbulut & Stamps, 2012). The ability to adapt to a novel plant host has also been shown by the European congener, *B. leoni* Baujard, established in South Africa on a novel host, *Pinus radiata*, in the absence of its only known vector, the bark beetle *Dryocoetes autographus* (Ratzeburg) (Braasch et al., 1998). The transmission of PWN between *Monochamus alternatus* and *M. saltuarius* (Gebler) through interspecific mating behaviour has also been demonstrated (Togashi et al., 2019).

Transmission mechanisms associated with *Monochamus* species

Adult female *Monochamus* spp. chew oviposition holes in the bark of dead or weakened host trees to lay their eggs (Figure 4). The early instar larvae excavate and feed on the inner bark, cambium, and outer sapwood for several weeks. As the larvae grow older, they chew a characteristic oval-shaped entrance into the heartwood, which they bore deeply into before turning around and boring back towards the surface. Once mature, the larva creates a chamber near the bark where pupation occurs. New adults chew a circular emergence hole to exit the larval host material (Figure 5) and must feed on live material to reach sexual maturation before mating and oviposition (EPPO, 2024) (Figure 6).

Figure 4 Above: oviposition scar (arrowed) made by female *Monochamus alternatus* in *Pinus densiflora* bark. [Image credit: ©KATSUMI TOGASHI] Below: oviposition slits (arrowed) made by female *Monochamus carolinensis* in *Pinus* sp. bark. [Image credit: ©W. TERRELL STAMPS]

Figure 5 Newly emerged adult female *Monochamus saltuarius* near circular emergence hole in *Pinus tabuliformis* in China. [Image credit: Pan et al. 2020]

Figure 6 Maturation feeding damage made to a branch by newly emerged adult *Monochamus galloprovincialis.* Italy. [Image credit: ©FABIO STERGULC/UNIVERSITÀ DI UDINE, ITALY/BUGWOOD.ORG - CC BY 3.0 US]

Adult emergence occurs over an extended period; for example, in Japan the emergence period for *Monochamus alternatus* is around two months (Kobayashi et al., 1984). The duration of the life cycle varies, commonly taking one or two years to complete, but in some cases there may be two generations in a single year (e.g. Perishing & Linit, 1986), or the life cycle may take up to four years (e.g. Raske, 1972).

Associates: Other insect groups which are carriers of PWN

Other species known to carry PWN belong to the beetle families Cerambycidae, Buprestidae and Curculionidae (particularly the subfamily Scolytinae), and the termite family Termitidae (Wang et al., 2021; Zhao et al., 2008). Examples of carriers identified in China include the longhorn beetles *Arhopalus rusticus* Linnaeus, *Acanthocinus griseus* Fabricius, *Ac. gundaiensis* Kano, *and Aromia bungii* (Faldermann); in Japan, these species as well as the longhorn beetles *Acaloptera fraudatrix* (Bates), *Asemum striatum* Linnaeus, *Corymbia succedanea* Lewis, *Spondylis buprestoides* (Linnaeus) and *Uraecha bimaculata* Thomson have been identified as carriers (Linit, 1988; Mamiya & Enda, 1972; Wang et al., 2021). Insects present in New Zealand that may have the potential to become secondary vectors are discussed in the “Domestic pathways for spread” section below.

3.3. Host range and susceptibility

The primary host species of *B. xylophilus* are trees in the genus *Pinus* (Torres-Sánchez et al., 2023), but most coniferous species can be infected (Li et al., 2022). Known hosts are shown in Table 3 (Dwinell, 1993; EPPO, 2023a; Evans et al., 1996).

Table 3: Host list for *B. xylophilus.*

***Abies** amabilis*, *A. balsamea*, *A. firma*, *A. grandis*, *A. sachalinensis*
***Cedrus** atlantica*, *C. deodara*
***Chamaecyparis** nootkatensis*
***Larix** decidua*, *L. gmelinii* var. *olgensis*, *L. gmelinii* var. *principis-ruprechtii*, *L. kaempferi*, *L. laricina*, *L. occidentalis*, *L. olgensis*
***Picea** abies*, *P. engelmannii*, *P. glauca*, *P. jezoensis*, *P. mariana*, *P. pungens*, *P. rubens*, *P. sitchensis*
***Pinus** armandii*, *P. ayacahuite*, *P. banksiana*, *P. bungeana*, *P. caribaea*, *P. cembra*, *P. clausa*, *P. contorta* var. *murrayana*, *P. cooperi*, *P. densiflora*, *P. echinata*, *P. elliottii*, *P. engelmannii*, *P. estevesii*, *P. fenzeliana, P. halepensis* subsp. *brutia*, *P. halepensis* subsp. *halepensis*, *P. jeffreyi*, *P. kesiya*, *P. koraiensis*, *P. lambertiana*, *P. leiophylla*, *P. luchuensis*, *P. massoniana*, *P. montezumae* var. *hartwegii, P. monticola*, *P. morrisonicola*, *P. mugo*, *P. muricata*, *P. nigra*, *P. oocarpa*, *P. palustris*, *P. patula*, *P. pentaphylla*, *P. pinaster* subsp. *escarena*, *P. pinaster*, *P. pinea*, *P. ponderosa*, *P. pungens*, *P. radiata*, *P. resinosa*, *P. rigida*, *P. rudis*, *P. strobiformis*, *P. strobus*, *P. sylvestris*, *P. sylvestris* var. *mongolica*, *P. tabuliformis*, *P. taeda*, *P. taiwanensis*, *P. thunbergii*, *P. virginiana*, *P. wallichiana*, *P. yunnanensis*
***Pseudotsuga** menziesii*
***Tsuga** canadensis*

In Europe, *P. nigra*, *P. sylvestris* and *P. pinaster* are susceptible to PWN (Vicente et al., 2012). In Japan, *P. thunbergii, P. densiflora* and *P. luchuensis* are highly susceptible (Mamiya, 1983). In China primarily *P. massoniana* is affected, however, 17 species of *Pinus* and *Larix* can be naturally infected by PWN (for example, *P. tabuliformis*, Figure 7, (Pan et al., 2020)) and there is concern that other species of *Pinus* and *Abies* could prove susceptible as the nematode spreads (Xu et al., 2023). In North America, exotic tree species planted as ornamentals are most affected, despite the nematode being widespread in natural coniferous forests (CABI, 2022).

Figure 7 A stand of *Pinus tabuliformis* affected by PWN and *Monochamus saltuarius* in China. Images borrowed from Pan et al. (2020).

Susceptibility of *Pinus radiata*

Pines that co-evolved with the nematode in North America are generally thought to exhibit a degree of resistance under normal climatic conditions and in the absence of other stressors (Lawson & Sathyapala, 2008). However, there are several conflicting reports regarding the susceptibility of *P. radiata*. For instance, one seedling inoculation study reported *P. radiata* as moderately susceptible (Nunes da Silva et al., 2015), while another seedling inoculation study placed *P. radiata* in a highly susceptible group based on resin canal traits facilitating PWN migration through the stem and the formation of tracheid embolisms (Rodríguez-García et al., 2023).

Mature trees are considered a more accurate measure of susceptibility than seedlings or young trees (Lawson & Sathyapala, 2008), and Furuno et al. (1993) also deemed *P. radiata* as being highly susceptible after examining nematode-induced mortality of a range of exotic pines planted in and around Tokyo. In Spain *B. xylophilus* was found naturally infecting and causing PWD in *P. radiata*, with 69 of 94 trees exhibiting crown dieback testing positive (Zamora et al., 2015). These results suggest PWN may have evolved in eastern North America, without overlap with the native distribution of *P. radiata* (Refer to Section 6.1 “Global spread and distribution”). Climate also affects the susceptibility of different host species, with changes in temperature and precipitation already documented as influencing the spread of PWN in northeast China (Wang et al., 2023).

It is difficult to predict the susceptibility of *P. radiata* to PWN in New Zealand where the species has undergone many years of genetic selection, which can harm resistance mechanisms (Dungey et al., 1997). Until the currently utilised germplasm is screened for resistance to PWN, the level of uncertainty regarding the risk of PWD in New Zealand remains high (Lawson & Sathyapala, 2008).

4. Surveillance

4.1. Tree infestation symptoms

Trees infested with PWN do not initially show any symptoms, although physiological changes within the tree begin almost immediately. The first observable symptoms of PWD are needle wilting and discoloration. Needles lose their lustre and change colour from green to grey-green, yellow, red, reddish-brown and finally brown (Li et al., 2022). Dead needles are retained, and the disease typically progresses from the top of the tree downward (unlike needle diseases).

The damage caused by the nematode feeding on cells within the resin ducts prevents water movement and reduces resin production within the tree (DAFF, 2020). As a result, a decrease in resin flow can be observed from wounds. Resin flow may be qualitatively categorised using the Oleoresin Exudation Method, an auxiliary diagnostic tool for suspect-infested standing trees (Li et al., 2022).

Tree mortality usually occurs within three months of infection, depending on the site affected, and is a strong surveillance indicator of PWD. On occasion, however, only an individual branch will die (referred to as a ‘flag’), with no further disease progression until the following season (DAFF, 2020; Donald et al., 2016). Tree damage often occurs in clusters, due to the year-to-year spread of disease to neighbouring trees. This pattern also often reflects the spatial distribution of resistant and susceptible trees or local stress factors, such as drought or windthrow, which increase vulnerability to attack by insect vectors (Togashi, 1991). This has important implications for surveillance, as certain sampling methods may risk overlooking clumped infestations. For these reasons, a suitable sampling method for PWN can be based on systematic, regular sampling that provides the advantages of simplicity and robustness to detect the presence of clusters of attacked trees (Meurisse & Pawson, 2019).

4.2. Attack symptoms and monitoring of insect vectors

Signs of insect vector (*Monochamus* spp.) attack or growth of blue stain fungi within wood also suggest potential PWN infestation. *Monochamus* attack symptoms include: oviposition slits/craters in the bark of the tree (oviposition sites range from the trunk through to small branches around two centimetres in diameter); the flat-headed larvae and/or their feeding galleries (which cut through both cambium and sapwood) under the bark; frass dribbling from trees; oval-shaped holes made by larvae as they bore deeper into the sapwood, and circular adult exit holes in the bark (Kobayashi et al., 1984; Wilson, 1975).

There is scant evidence to suggest that *Monochamus* spp. are attracted to light, however, they respond well to synthetic host volatiles, pheromones and sympatric insect kairomones (bark beetles) (Ethington et al., 2018). Baited traps may be successfully used to detect adult *Monochamus* spp. in the field, with consideration for variation in each species' emergence period and dispersal behaviour (See section below, “Treatment and risk management options for *Bursaphelenchus xylophilus*: Vector trapping”).

4.3. Detection through remote sensing methods

Although more applicable for monitoring the spread of an established population of PWD, in recent years there has been increased focus on the development of remote sensing methods which allow more timely implementation of control measures, as unremoved infected dead pine trees allow further spread of PWD (Lim et al., 2022). Much of the published literature originates from research undertaken in either China or South Korea. Remote sensing technology is currently in use in the PWD epidemic areas of China, which are located mainly in the mountainous areas of the southeast (Li et al., 2022).

The use of high-resolution spectral data collected from remote sensing platforms in combination with powerful data science technology allows trees in the first pre-symptomatic stages of PWD to be detected up to three months before any symptoms are evident (Guillen-Climent et al., 2020; Yu et al., 2022). The science is complex, with researchers evaluating various combinations of spectral data, remote sensing platforms and computational models. Hyperspectral data, as well as visible red, blue and green spectral data, are the most useful. A range of remote sensing platforms, including airship, UAV (unmanned aerial vehicle, such as drones), and satellite have been utilised with varying success, for example, Mantas et al. (2022) used spectral data from Sentinel-2 satellite imagery to detect areas of tree decline in maritime pine (*Pinus pinaster*) forest in Portugal, but could not differentiate decline related to PWD from other sources. UAV-based remote sensing provides high-resolution data at a relatively low cost but is limited in terms of coverage area compared to high-altitude satellite-based platforms, which are less accurate for PWD detection due to the low spatial-temporal resolution and the impacts of adverse weather conditions (Li et al., 2022; Zhang et al., 2022). Using UAV-based hyperspectral and LiDAR systems, Yu et al. (2022) and Wu et al. (2023) determined the optimal PWD detection period in *Pinus koraiensis* and *P. thunbergii* plantation forests in northeast China. Y. Zhou et al. (2022) constructed a multi-band image-fusion infected pine tree detector based on deep learning, which could accurately detect infested trees, especially at the early stage.

Despite extensive field studies, an accurate method using only UAV-collected images for the detection of each infection stage is yet to be developed (Y. Zhou et al., 2022). Ideally, such a method would be suitable for biosecurity responses, particularly if the affected area is still quite localised. Moreover, there is a pressing need for remote sensing classification methods that can be easily adapted to new contexts, such as in developing incursion scenarios where no pre-existing images of infested trees in the new environment are available for calibration.

5. Diagnostics

5.1. Nematode sample collection

Pine wood nematode can be present in most woody parts of a host tree including the roots (but not needles, cones or seeds), or in an insect vector. It may be difficult to detect in the early stages of PWD as its distribution within an individual tree can be patchy, and the number of nematodes present may be very low (Donald et al., 2016; Nakabayashi et al., 2018). It may be necessary to take multiple samples from different parts of the tree to improve the likelihood of detection (Donald et al., 2016), and it is recommended that wood samples be incubated before attempted extraction to boost the number of nematodes present. Incubation involves keeping wood samples warm (around 25°C) and humid for at least 14 days (EPPO, 2023b).

Collection of wood samples, shavings or wood chips from sawmills or wood stockpiles may also be used for PWN detection, though it is difficult to determine the original site using such samples (IPPC, 2016).

5.2. Nematode extraction and identification

Typical work flows for nematode extraction and identification using traditional morphological or molecular methods from wood and insect vector samples are outlined in Appendix A, borrowed from the EPPO Standard of Diagnostics for *Bursaphelenchus xylophilus* (EPPO, 2023b)

Nematode extraction

The traditional procedure for nematode extraction is Baermann's funnel method, which relies on the migration of living nematodes out of a water-soaked sample over a period of 24-48 hours. This time-consuming method (or modified versions) has been in use for over 100 years. Recently, more rapid extraction methods which preserve nematode morphology have been developed, for example, the aseptic nematode extraction method from pine seedlings of Mannaa and Seo (2023).

Nematode morphological identification

Morphological identification of nematodes is complex, takes a relatively long time and requires specialist taxonomic knowledge (Li et al., 2022). Keys are based on adult features such as the shape of the spicule in the male, and tail shape and presence of a vulva cover in the female (Islam & Bakker, 2014; Li et al., 2022). If only juvenile nematodes are present in a sample, these must first be reared to adult on *Botrytis cinerea* fungal culture.

Both the European and Mediterranean Plant Protection Organization (EPPO) and the International Plant Protection Convention (IPPC) have produced PWN Diagnostics Standards, which are regularly reviewed and updated. Two short morphological keys to screen nematodes to the *Bursaphelenchus xylophilus* group and a short morphological key for identification of *B. xylophilus* within the 'xylophilus-group' are provided in the EPPO *Bursaphelenchus xylophilus* Standard on Diagnostics (EPPO, 2023b).

Morphological identification is complicated by the existence of two forms of *B. xylophilus:* the usual round tail ('R' form) and the mucronate ('M' form) (very similar to *Bursaphelenchus mucronatus kolymensis* – European type), found mainly in North America (IPPC, 2016), as well as several morphologically similar species in the *xylophilus* group (Gu et al., 2011; B. N. Kim et al., 2020). Morphological identification is further complicated as environmental stressors can alter the shape and size of certain morphological features. For example, in Portugal the shape of the female tail changed after several generations of laboratory rearing on one substrate, but not the other (EPPO, 2023b). Exposure to chemicals, such as monoethylene glycol (used as a wet preservative in vector beetle trap samples), can also alter nematode morphology (Berkvens et al., 2017).

In New Zealand, nematodes submitted for morphological identification to the Ministry for Primary Industries' Plant Health and Environment Laboratory are frequently redirected to the nematode taxonomic expert Zeng Zhao, based at Manaaki Whenua – Landcare Research. However, MPI's Plant Health and Environment Laboratory is developing capability in morphological identification of nematodes and is well-equipped to conduct DNA analysis.

Nematode molecular identification

In recent years, several molecular identification methods have been developed, to address the challenges of morphological identification. These include Restriction Fragment Length Polymorphisms

(RFLPs), Polymerase Chain Reaction (PCR), Nested PCR (n-PCR), Real-Time PCR (RT-PCR), Random Amplified Polymorphic DNA (RAPD), Sequence-Characterized Amplified Region (SCAR), Loop-Mediated Isothermal Amplification (LAMP), Enzyme-Mediated Amplification (EMA), and Recombinase Polymerase Amplification (RPA) (B. N. Kim et al., 2020; Li et al., 2022; Wang et al., 2022). Most PCR or isothermal amplification detection methods require time-consuming processes. More rapid methods have been developed, for example Cha et al. (2019) developed a diagnostic method using RPA and a novel gDNA (DAP) extraction buffer, and Q. Zhou et al. (2022) developed a diagnostic method using RPA combined with lateral flow dipstick (RPA-LFD) which can be completed within 30 minutes. Both of these rapid methods are able to be used in the field.

The molecular method of Matsunaga and Togashi (2004) can reliably differentiate the mucronate 'M' form of *B. xylophilus* from the separate taxon *B. mucronatus kolymensis*. Leal et al. (2005) designed a molecular method which detected *B. xylophilus* directly from concentrated Baermann funnel wood sample extracts. The molecular method of Burgermeister et al. (2009) has been effective in identification of the widest range of *Bursaphelenchus* species (IPPC, 2016).

PWN protein-based molecular detection methods include Two-Dimensional Gel Electrophoresis (2-DGE), Isozyme Analysis and Matrix-Assisted Laser Desorption/Ionization–Time of Flight Mass Spectrometry (Li et al., 2022). More efficient molecular procedures which amplify nematode DNA directly from beetle vector tissue (without the need to extract nematodes (X. R. Wang et al., 2011), and that directly detect the nematode from host wood, bark and insect vector (Cardoso et al., 2012) have also been developed.

5.3. Insect vector identification

Scion has diagnostic capability and an excellent collection of adult and larval reference specimens to enable rapid morphological identification of any suspected *Monochamus* beetles or other potential incursion. The Scion Molecular Laboratory has capability in molecular identification of insects if this is required. It is worth noting that the widely used "universal" Folmer primers work well for this beetle genus, and this primer pair is used by Scion in the first instance for any insect molecular diagnostics.

6. PWN risk to New Zealand

6.1. Global spread and current distribution

PWN is considered native to North America (Canada and the USA), but the precise region is unknown. In the USA, western and eastern native pine forests are geographically isolated by the Great Plains, while in Canada the boreal forest, containing *P. banksiana* and *P. contorta*, extends across the continent at high latitudes. PWN is abundant in native pine forests on the eastern side of the continent but is rarer and patchier in the west (CABI, 2022), with some first reports being relatively recent: Nebraska in 2000 (Gleason et al., 2000) and Colorado in 2006 (Blunt et al., 2014). This suggests the possibility that the native range of PWN may not overlap with that of *P. radiata*, which originates from a narrow range along the coast of California and Mexico's Baja California peninsula. This speculation is further supported by the high degree of resistance exhibited by native eastern North American pines (CABI, 2022), whereas exotic species commonly planted as ornamentals in urban areas (*P. mugo*, *P. nigra* and *P. sylvestris*) across the continent are highly susceptible (Atkins et al., 2021). In the USA, PWD is most problematic in the Midwest (Gleason et al., 2000), with infested exotic pines potentially acting as a bridge assisting the spread of PWN westward. Although PWN was reported in California in 1980 (Holdeman, 1980), an extensive survey throughout the native range of *P. radiata*, with particular attention to stressed trees and over 100 samples taken, found no evidence of the nematode (Bain & Hosking, 1988).

The first record of PWN outside of its native range was in Japan in 1905 (B. N. Kim et al., 2020; Xu et al., 2023). In the 1980s PWN spread to the neighbouring countries China, Taiwan and Korea (Carnegie et al., 2018; B. N. Kim et al., 2020). PWN was detected in Mexico for the first time in 1992 (Dwinell, 1993). PWN was detected in Europe for the first time in the late 1990s, invading Portugal from East Asia (Mota et al., 1999). Spain has reported multiple outbreaks since 2008 (Abelleira et al., 2011; Torres-Sánchez et al., 2023), and PWN has rapidly spread in Northeast and Northwest China, in recent years (Xu et al., 2023). A Nigerian record is considered unreliable. The current known global distribution of PWN is shown in Figure 8 (EPPO, 2023a), and the distribution of native pines and pine plantations in Figure 9 (Lantschner et al., 2017).

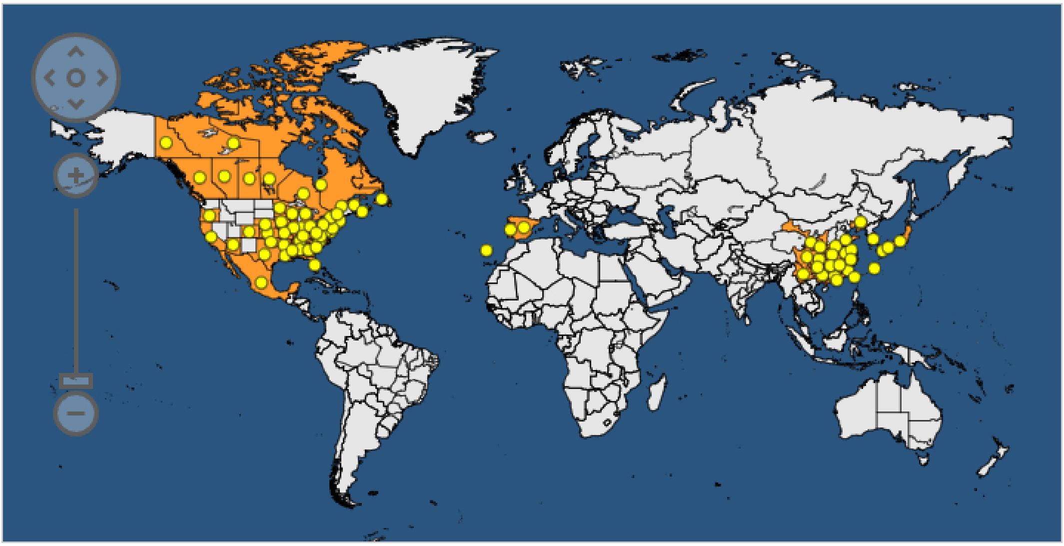

Figure 8 Global distribution of *Bursaphelenchus xylophilus*. Source: (EPPO, 2023a).

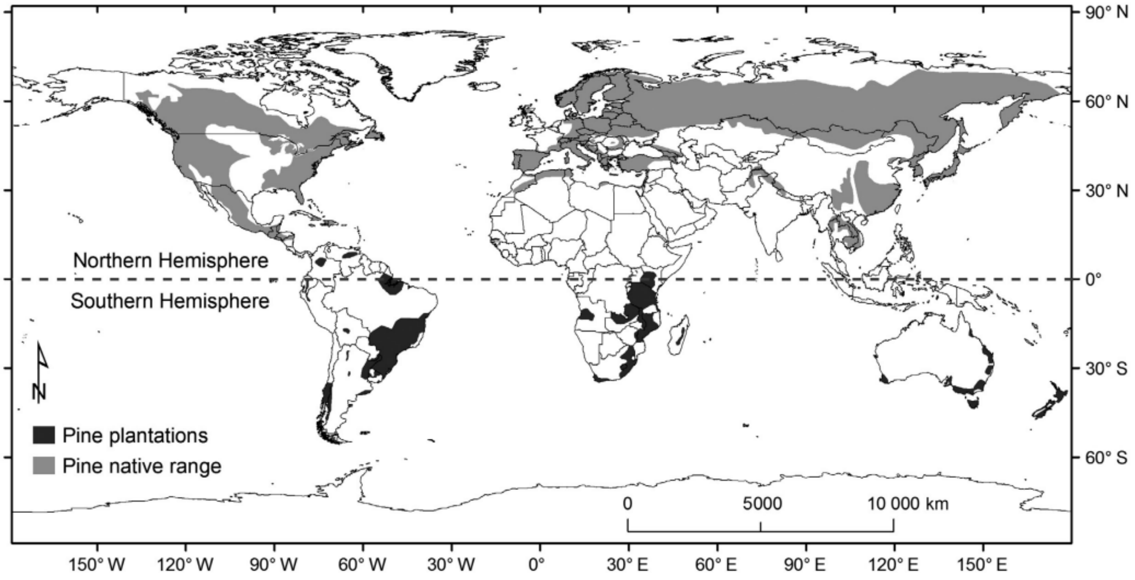

Figure 9 Native range of pines, and distribution of pine plantations in the Southern Hemisphere. Figure borrowed from Lantschner et al. (2017).

All areas where PWN is invasive are in the Northern Hemisphere and have widespread native populations of *Monochamus* spp., with *M. alternatus* in Japan and *M. galloprovincialis* in Spain demonstrating the ability for newly associated species to develop into effective vectors (Arakawa & Togashi, 2002; Schenk et al., 2020). There is no confirmed occurrence of PWN in the Southern Hemisphere, and no exotic species of *Monochamus* are recorded as invading and establishing. There are a small number of species of *Monochamus* native to Africa (Gorring & Farrell, 2023), and one Columbian species (Schenk et al., 2020). However, these regions have no native pines (Lantschner et al., 2017).

6.2. Border interceptions

No species of *Monochamus* have established in New Zealand or Australia. However, both countries have documented several interceptions of multiple species of *Monochamus* from different regions. New Zealand interceptions include *Monochamus alternatus*, *M. galloprovincialis*, *M. grandis, M. notatus*, *M. obtusus*, *M. sartor* (Fabricius), *M. scutellatus*, *M. sutor*, *M. titillator*, and some individuals only identified to the generic level (Turner et al., 2020) (Table 4). Additional species intercepted in Australia include *Monochamus impluviatus* Motschulsky (Lawson & Sathyapala, 2008), *M clamator* and *M. urussovi* (Turner et al., 2021).

Table 4: New Zealand border interception records for *Monochamus*

Species	Number	Commodity	Geographic origin
Scion's BUGS database 1950-2000			
M. alternatus	14	Casewood, dunnage	China, Japan, Unk
M. galloprovincialis	3	Casewood	Europe, Italy
M. grandis	1	Pallet	Unk
M. notatus	1	Casewood	Canada
M. obtusus	2	Casewood, dunnage	USA, Unk
M. sartor	4	Casewood, container, pallet	Italy, Russia, Sweden, Unk
M. scutellatus	16	Cable drum, casewood, dunnage	Canada, North America, Unk, USA
M. sutor	12	Bearer, casewood, dunnage, pallet	Europe, Italy, Japan, Russia, Spain, UK
M. titillator	8	Casewood, dunnage, pallet	North America, USA
Monochamus sp.	50	Batten, cable drum, casewood, log, pallet	Africa, Canada, China, Europe, Italy, Japan, Russia, Singapore, UK, Unk, USA
MPI border interception files 2000-2017			
M. alternatus	3	-	-
M. galloprovincialis	3	-	-
Monochamus sp.	8	-	-
Total	**125**	**-**	**-**

In 1995 a live female *Monochamus alternatus* was found in Christchurch. Laboratory testing found that it was not carrying any nematodes, and no further specimens were found by searching the immediate vicinity. Fresh pine billets were placed near the discovery to attract any nearby *M. alternatus*, as a further check for presence. No signs of any cerambycid infestation were found and it was concluded that the single female found was a 'one off' occurrence (Bradbury, 1995).

6.3. Potential entry pathways into New Zealand

Nematode-infested wood

The increase in the global trade of wood products and materials has facilitated the intercontinental spread of PWD, with wood packaging material as the main entry pathway for PWN and its insect vectors (Sousa et al., 2011; Vicente et al., 2021; Zhao et al., 2023). Despite the continuous reinforcement of quarantine measures from the 1980s, PWN spread from Japan to China, Taiwan and Korea, and has invaded and established in Portugal and Spain (Abelleira et al., 2011; Mota et al., 1999).

While there are reports of interceptions directly from invaded countries, for example into France from Portugal in 2018 in bark and wood packaging (ANSES, 2020), PWN may also be intercepted from trade transit countries. Interception records from China between 2010 and 2014 have also identified France, Germany, India, Italy and Malaysia as countries of origin for infected wood packaging material (Figure 10) (Zhao et al., 2023). However, PWN is not present in these countries.

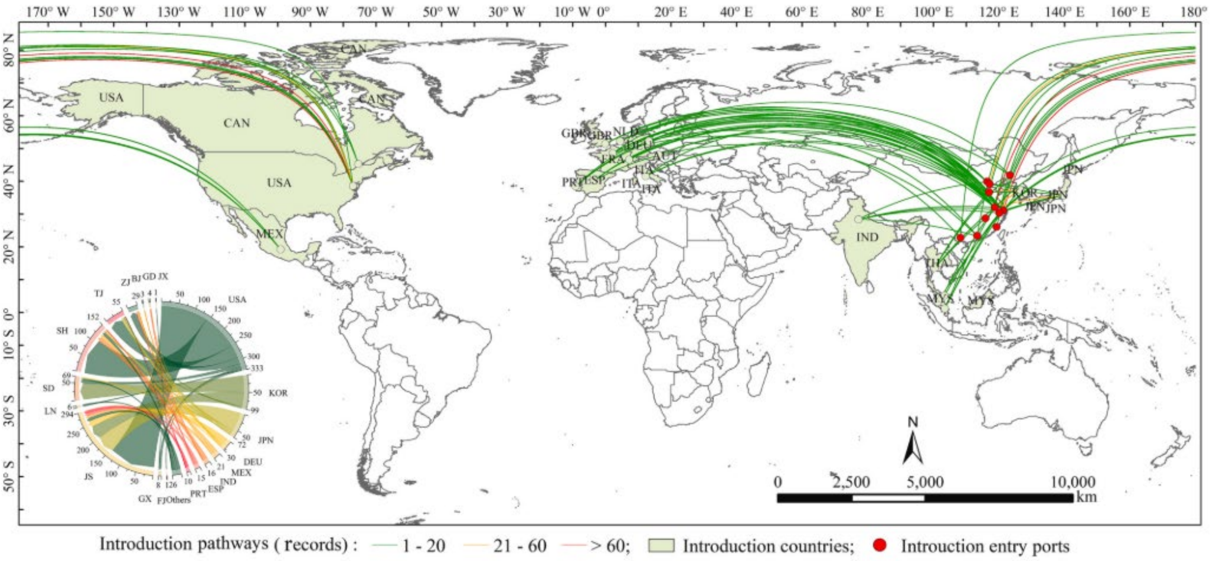

Figure 10 Introduction pathways (inspection records), source countries, and entry ports of pine wood nematode (*Bursaphelenchus xylophilus*) during 2010-2014. USA: United States. CAN: Canada. MEX: Mexico. PRT: Portugal. ESP: Spain. FRA: France. ITA: Italy. AUT: Austria. DEU: Germany. GBR: United Kingdom. NLD: Netherlands. IND: India. THA: Thailand. MYS: Malaysia. KOR: South Korea. JPN: Japan. FJ: Fujian. GX: Guangxi. JS: Jiangsu. LN: Liaoning. SD: Shandong. SH: Shanghai. TJ: Tianjin. ZJ: Zhejiang. BJ: Beijing. GD: Guangdong. JX: Jiangxi. Figure borrowed from Zhao et al. (2023).

Insect vectors transported in commodities

Monochamus spp. vectors carrying PWN are another potential avenue for invasion and need not become an established population to present a risk. Theoretically, a single introduced gravid female vector could transmit PWN through oviposition wounds, as evidenced by Zhang and Linit (1998) who showed that even unmated females of both *M. carolinensis* and *M. alternatus* oviposit and infect new host trees in the process. As the nematodes develop and multiply, transmission by other species of vectors already present could lead to the establishment of the nematode. Likewise, in the absence of a vector, nematodes present in imported wood could be picked up by other potential vectors once in New Zealand.

Import Health Standards

Import Health Standards (IHSs) are issued under the Biosecurity Act 1993 and specify requirements to be met to effectively manage biosecurity risks associated with importing risk goods. An IHS specifies measures that must be applied in the exporting country and requirements that must be met by importers during importation and transit prior to biosecurity clearance, sometimes including post-clearance conditions.

The Import Health Standard: Wood Packaging Material from All Countries states that all consignments of wood packaging material must be a) free of live regulated pests, b) free of contaminants such as leaves and soil, c) made from debarked wood, and d) packed and/or shipped in a manner that prevents infestation and/or contamination by live regulated pests (MPI, 2023). This IHS references three International Standards for Phytosanitary Measures (ISPMs): ISPM 5 “Glossary of phytosanitary terms”, ISPM 12 “Phytosanitary certificates” and ISPM 15 “Regulation of wood packaging material in international trade”, as well as the “Official New Zealand Pest Register”.

ISPM 15 “Regulation of wood packaging material in international trade” (IPPC, 2019) describes the internationally accepted phytosanitary measures covering all forms of wood packaging material that may serve as a pathway for pests (including crates, boxes, packing cases, dunnage, pallets, cable drums and spools/reels) and consists of the use of debarked wood and the application of approved treatments described in the standard.

Wood packaging material (WPM) is considered the primary import pathway for the PWN and its insect vectors. Between July 2022 and June 2023, NZ imported approximately 686,000 containers (~930,000 twenty-foot equivalent units (TEUs)), with most arriving at the ports of Auckland, Tauranga and Lyttleton (MPI, Diagnostic and Surveillance and Service, 2023;). Based on these imports, an estimated 190,000 Tonnes, or 380,000 m^3, of WPM enters NZ annually (Spatial Pest Entry Risk Model (SPEAR), 2023). This is almost double the amount of WPM estimated by Sathypala (2004), and in line with increasing container port throughput over the last two decades.

MPI’s 24th Biosecurity Emerging Risks System Report (19 September 2022 to 21 August 2023) stated that PWN had been reported for the first time in three larch species in China, and that larch may be imported on the nursery stock or wood products pathway. It was concluded that this does not represent a biosecurity risk for New Zealand because i) infected nursery stock would be detected during post-entry quarantine, ii) no species of *Monochamus* occur in New Zealand and iii) there are specific measures for both the nematode and its vectors in the different wood product import health standards. However, an element of uncertainty remains regarding the effectiveness of ISPM 15. There has been at least one case in Australia where *Monochamus* carrying *Bursaphelenchus* sp. have emerged from pallet material stamped as ISPM 15 compliant (Lawson & Sathyapala, 2008).

Other risk pathways for PWN, and to a lesser extent its insect vectors, include nursery stock, seeds and cones, processed wood, and other types of wood items. These pathways were all rated “low” by Sathypala (2004). Wood chip imports require phytosanitary treatments and are considered a “negligible” risk pathway for PWN and its vectors (Sathypala, 2004). ISPM 39 “International movement of wood” (IPPC, 2017a) describes the recommended phytosanitary measures covering raw wood commodities and material resulting from the mechanical processing of wood: (1) round wood and sawn wood (with or without bark); and (2) materials resulting from the mechanical processing of wood such as wood chips, sawdust, wood wool and wood residue (all with or without bark). Similarly, ISPM 38 covers “International movement of seeds” (IPPC, 2021), and ISPM 40 “International movement of growing media in association with plants for planting” (IPPC, 2017b). ISPM 41 covers “International movement of used vehicles, machinery and equipment” (IPPC, 2017c), a relatively well controlled pathway, but representing high volumes for New Zealand.

6.4. Domestic pathways for spread

Biological dispersal

An insect vector is required for biological spread of the nematode and infestation of new material. Both Australia and New Zealand remain wary of the potential for insects already present, belonging to other genera or families of wood boring insects, to become primary vectors of PWN should it become established. Lawson and Sathyapala (2008) suggest the longhorn beetles *Arhopalus ferus* (Mulsant) and the native *Hexatricha pulverulenta* (Westwood) as the most likely candidates to vector PWN in New Zealand. Both are widespread and common in *P. radiata*, but primarily feed on dead material.

Arhopalus ferus has been recorded as attacking live trees but this is rare and probably limited to fire-damaged trees, whereas *H. pulverulenta* feeds on bark at the margins of damaged areas (Hosking, 1978b) and occasionally feeds on green twigs (Brockerhoff et al., 2016). The native longhorn beetle *Oemona hirta* is another potential vector and attacks live trees, however it is normally associated with hardwood trees and attacks on softwoods are rare (Hosking, 1978a). The Scion/FOA Forest Health Database contains 18 records of *O. hirta* from *P. radiata*, spanning from 1981 to 2023.

If a species of *Monochamus* became established in New Zealand and PWN was either imported with it or the two became acquainted within New Zealand, the potential for spread of PWN could be estimated based on known biology of the species of *Monochamus* or of congeners. *Monochamus* spp. adults have an initial dispersal flight and become less active once they reach a suitable host tree. Activity is strongly influenced by environmental conditions, for example in *Monochamus alternatus,* the Japanese timber sawyer, adult activity ceases below 18^{o}C, and it is reported that they do not fly on rainy days (Kobayashi et al., 1984). Data from mark-recapture tests of *M. alternatus* found that most adults moved less than 100 metres, with a few travelling up to 2.4 kilometres; data from repeated field observations from small island groups estimated that adults often fly approximately 800 metres, up to a maximum of 3.3 kilometres (Kobayashi et al., 1984). Togashi (1990) found that the proportion of beetles that fly away from the pine stand where they were released increased as stand density decreased. Adults are long-lived, with female *Monochamus alternatus* reared in outdoor cages in Japan having a mean longevity of 66 days (Togashi & Magira, 1981) and mated adult females reared in the laboratory having a mean longevity of 178 days (Zhang and Linit 1998).

Anthropogenic dispersal

The spread of PWD in China has been analysed using 40 years of data, revealing both continuous and leapfrog spread patterns (Hao et al., 2025). This suggests that human activities, such as timber transport, packaging materials and infrastructure projects, significantly contributed to long-distance introductions, with outbreaks accelerating in forestry-based and economically developing regions.

Similarly, the movement of infested wood could contribute to PWN moving throughout New Zealand. Infested trees can be asymptomatic carriers, and thus, the spread could happen unknowingly with the movement of wood. The pathways would include the transport of logs and log sections, thinned trees and firewood. Wood from a single stand, or even a single tree, may go to as many as four or five different destinations (for example, to a structural mill, pruned log mill, pulp mill, or for export), and wood originating from one forest often passes through other forests (P. Hall, pers. comm). Logs and other wood material may sit at a processing site for weeks to months before processing, allowing time for colonisation by wood boring beetles. Yet, despite the potential for movement of infested wood within New Zealand and the opportunities for insect colonisation, PWN could not be dispersed further unless a suitable vector was also present.

Environmental effects

The effects of climate change are expected to be favourable for PWD, with warmer temperatures leading to disease expression (Ikegami & Jenkins, 2018; Iki et al., 2020), while cool weather inhibits the transmission process of PWN (Jikumaru & Togashi, 2000). Mean summer temperatures of over 20°C is reported as a critical threshold for PWN reproduction and vector flight activity (Zhao et al., 2007).

Trees stressed by increased temperatures and drought are more attractive to the *Monochamus* vector species, thus boosting their population growth. Some species of *Monochamus*, for example *M. carolinensis*, exhibit facultative diapause and can complete several generations per year as long as ambient temperatures are high enough (Akbulut & Stamps, 2012). For those species that exhibit obligatory diapause and require one to three years for development regardless of ambient temperatures, extended periods of warmth could result in adults emerging earlier in the year and having longer periods of activity, which could in turn lead to an increase in nematode transmission.

With warming, development time for PWN may be reduced, more rapidly killing trees and increasing the number of nematodes available for transport to uninfected trees, thus resulting in a higher percentage of insects vectoring the nematodes (Akbulut & Stamps, 2012). Other factors have also been found to be positively associated with PWN, such as infestations of primary bark beetles (Kinn & Linit, 1992), or to increase the prevalence of *Monochamus* vectors, such as forest fires (Atkins et al., 2021).

6.5. Previously conducted risk assessments for Australia and New Zealand

Following the rapid spread of PWD in East Asia, the expansion of the disease in Europe in the 2000s, and the discovery of *Bursaphelenchus* spp. in several countries including Australia and New Zealand, concerns have been raised about the biosecurity risk posed by PWN. This led to an intensification of research focusing on PWN, other *Bursaphelenchus* species, and their vectors. As a result, detailed risk assessments have been published in several countries. The key risk components investigated in Australian and New Zealand risk assessments are outlined in Table 5.

Table 5: Compared ratings of the different factors of risk for *Bursaphelenchus xylophilus* and its *Monochamus* vectors in the most recent comprehensive risk assessments available for PWD in Australia and New Zealand.

Risk factor	Australia Carnegie et al. 2018	New Zealand Sathyapala 2004, Lawson & Sathyapala 2008
Entry likelihood	**PWN: High** **Vector: High** (extensive PWN range in the Northern Hemisphere, frequent vector interceptions, limited effectiveness of ISPM 15)	**PWN: High** (large amount of WPM sourced from regions where the PWN occurs) **Vector: High** (*Monochamus* spp. intercepted on numerous occasions)
Establishment likelihood	**PWN: Moderate** (three recent examples of *Bursaphelenchus* sp. establishment) **Vector: Low** (no evidence of prior *Monochamus* establishment)	**PWN: Low** (assumption of vector absence, and suspected unlikelihood of other insects ability to become novel vectors) **Vector: Low** (no evidence of prior establishment of *Monochamus* spp.)
Spread likelihood	**PWN (assuming vector): Moderate** (widespread distribution of *Pinus* spp., suitable climate in likely arrival locations and pine plantation regions) **Vector: High** (assuming initial establishment) (most favourable from central NSW northwards and in southwestern WA)	**PWN: Negligible** (assumption of vector absence) **Vector: Not assessed**
Severe impact likelihood	**PWD on pine plantations: Moderate** (*Pinus* spp. in Australia considered resistant or mildly susceptible, but uncertainty under severe drought/heat) **PWD on national value chain: High** (ban would be applied on log transport) **PWD on timber exports: Low**	**PWD on pine plantations: Uncertain** (susceptibility of selectively bred *P. radiata* in NZ remains unknown) **PWD on national value chain: High** (ban would be applied on log transport) **PWD on timber exports: High**

	(current export requirements, MeBr application, likely to control PWN and vectors)	(unprocessed logs, timber and wood would require heat treatment) **PWD on other hosts: High** (if endemic conifers proved susceptible, would have major social, cultural and environmental impacts; could impact exotic species in urban areas, increasing erosion and creating micro-climate modification, which could further impact indigenous ecosystems and biodiversity)

Notes: “PWN” = *Bursaphelenchus xylophilus*. “Vector” = *Monochamus* spp.

Entry likelihoods were assessed under a mitigated scenario (i.e. current phytosanitary measures applied); they would be different under an unmitigated scenario (e.g. absence or inefficient treatment of wood packaging material (WPM)). The estimates for establishment, spread and impact likelihood vary depending on whether they assume the widespread distribution of a suitable *Monochamus* vector (Carnegie et al., 2018) or assume the known vectors are absent (Sathypala, 2004) and that the potential for other insects present to become novel vectors remains unknown (Lawson & Sathyapala, 2008). These assessments did not provide an uncertainty rating, although it has been discussed.

7. Control methods and risk management options for PWD

Due to the complexity of interactions between PWN, the host, the vectors and other insect and microbial associates (such as bark beetles and fungi, as well as bacteria associated with PWN and its vectors), management intervention for PWD must be tailored to match agreed biosecurity objectives and local contexts. For instance, eradication may be envisaged if the affected area is small and contained, and host trees can be readily identified and managed. Conversely, management options may shift rapidly to containment strategies when dealing with larger affected areas or when control measures are challenging to apply. In situations where PWN is not deemed eradicable, current control recommendations focus on reducing reservoirs of infection, isolating the vector and the nematode, and increasing host resistance (Kamata, 2008). These include: (i) the physical destruction or processing of infected material; (ii) vector suppression through pheromone trapping, insecticide applications, and biological control; (iii) nematode suppression through biological control; and (iv) tree breeding for tolerance and resistance to PWN. Effective management strategies typically combine local control methods with coordinated phytosanitary measures for timber and wood products. The available and investigated control methods are discussed below.

7.1. Physical control

The physical destruction of diseased material is a highly effective control method that can be applied at large scales. Killing the vector beetles before PWN transmission takes place is the most effective method for controlling PWN damage (Kamata, 2008). Destroying infected material prevents the transmission of PWN by preventing the emergence of *Monochamus* spp. adults. In China, the destruction of infected pines is the main control method for PWD, alongside inspection and quarantine measures (Yu et al., 2011). Infected trees are felled and then may be destroyed through chipping, burning, crushing and burying. Tree burning is considered one of the most efficient methods, and in East Asia and Portugal, burnt trees are occasionally used to make charcoal. However, great care is required to avoid forest fires, leaving this method restricted to low fire risk periods (Back et al., 2024; Kamata, 2008; B. N. Kim et al., 2020). In Portugal, diseased trees are also chipped or buried, the chipped wood used to make pulp to produce paper. Debarking prevents oviposition by *Monochamus* spp. (Back et al., 2024). Although physical control is a highly effective method, it is expensive, labour intensive and contributes to environmental pollution (Dou & Yan, 2022).

7.2. Chemical control

A variety of insecticides, such as acetamiprid, clothianidin, fenitrothion, thiacloprid and thiamethoxam, have been used to control *Monochamus* spp. (J. C. Kim, S. Baek, et al., 2020).

In Japan, felled trees are not always subjected to physical treatments but are also sprayed with chemical insecticide or fumigated (Kamata, 2008).Where felling is not an option, chemical application methods on standing trees include ground level spraying, aerial release, spot spraying and trunk injections (Back et al., 2024). Chemicals can be used as preventative sprays to protect host trees from the beetles or on already diseased trees to kill the larvae under the bark or emerging adults. In Korea, thiacloprid 10%SC is sprayed onto trees to control the adult beetles (J. C. Kim, S. J. Lee, et al., 2020). Spot spraying from a helicopter is also employed in areas where the infestation is small, or aerial spraying over the tree canopy is used in larger areas of infection. Trunk injections of nematicidal compounds such as emamectin benzoate, abamectin, fenitrothion and morantel tartrate have been widely used in East Asia to control PWN (Lee et al., 2023). Soil injections of fosthiazate were investigated by Fu et al. (2012) and were found to provide some protection against PWN. The main concerns with chemical control are the impact on the environment, human health, and ecological systems (C. Y. Wang et al., 2011). Good results can be achieved but do not provide a long-term solution to PWD (Back et al., 2024).

7.3. Vector trapping

Semiochemicals, a combination of volatile compounds associated with host trees and pheromones associated with *Monochamus* spp., have been investigated for trapping the vector beetles for early detection, population monitoring and control. An adult male-produced aggregation pheromone, monochamol, common to several *Monochamus* species, has been identified (Fierke et al., 2012), and its use in combination with monoterpenes and the bark beetle lures ipsenol and ipsdienol is highly effective in surveillance trapping (Chase et al., 2018). Response to trap and lure combinations are often species-specific, but a combination of monochamol, ipsenol, methyl butenol, and α-pinene paired with black cross-vane panel traps coated with Teflon, has been shown to attract *Monochamus* species in Europe, Asia and North America (Boone et al., 2019; Dodds et al., 2024).

Trap logs (i.e. the set-up of attractive billets from trees felled during the adult flight period) may also be placed in heavily infested locations for the mass trapping of adult *Monochamus* (IPPC, 2016). Where multiple tree species are available, the most attractive tree species should be chosen with consideration of the individual *Monochamus* targets. For example, in Japan adult female *Monochamus alternatus* have been shown to strongly prefer fresh *Pinus elliottii* logs for oviposition (Kobayashi et al., 1984), while *Pinus sylvestris* are preferred by *Monochamus carolinensis* in North America (Walsh & Linit, 1984).

Sites identified as epicentres should be the focal points of integrated pest management efforts. Vectors captured from epicentres early in the flight season have the highest probability for spread of PWN (Atkins et al., 2021).

7.4. Biological control

Biological control of *Monochamus* spp.

Entomopathogenic fungi have been under investigation as a control method for the PWN vector, in conjunction with or as a replacement to insecticides. Applications of *Beauveria bassiana*, *B. brongniartii*, *Metarhizum anisopliae*, *M. pemphogum* and *M. pingshaense*, resulted in fatality rates under laboratory conditions ranging from 37% to 100% (Dou & Yan, 2022). The entomopathogenic fungi can be applied through methods such as soil application, spraying, non-woven fabric strips, wheat bran granule and insect carriers (Hongjian et al., 2007; J. C. Kim, S. Baek, et al., 2020; Okitsu et al., 2000; Shimazu & Sato, 1995). In Japan, a commercial company sells fungal bands, applied to the end of cut logs, with a fatality rate of 90% of emerging adult beetles (Back et al., 2024). Natural insect enemies of *Monochamus* spp. have been investigated, on their own and in combination with entomopathogenic fungi (Dou & Yan, 2022; Kim et al., 2022). In China the field release of a parasitoid wasp, *Scleroderma guani* Xiao et Wu (Hymenoptera: Bethylidae), close to PWD infected trees led to a parasitism rate in *M. alternatus* of 20% (Kang et al., 2008). Other potential biological control agents include the parasitoid wasp *Spathius verustus* Chao (Hymenoptera: Braconidae), and the ectoparasitic beetle *Dastarcus helophoroides* (Coleoptera: Bothrideridae), both found parasitising *Monochamus alternatus* and *M. saltuarius* in Korea (Kim et al., 2022; Lim et al., 2012).

Biological control of PWN

Almost 100 nematocidal strains of fungi, bacteria and actinomycete microorganisms have been reported for PWN with 76 active substances identified (Dou & Yan, 2022). The endoparasitic fungus *Esteya vermicola* attracts the nematode towards its conidia by mimicking the host volatiles α- and β-pinene, and camphor (Pires et al., 2022; Vicente et al., 2021). The conidia then attach to the cuticle, penetrating and consuming the nematode (Pires et al., 2022; Ryss et al., 2011). *Esteya vermicola* has shown the ability to supress PWN in both greenhouse experiments and in the field (C. Y. Wang et al., 2011; Wang et al., 2018). Aggressive strains of *E. vermicola* can kill PWN in four to five days (Ryss et al., 2011). *Esteya vermicola* has been patented in both South Korea and the United States (Pires et al., 2022). A second species of *Esteya*, *E. floridanum*, was isolated in 2017 in the United States, with initial results showing potential to control PWN (Y. Li et al., 2021). Application methods of *Esteya* include spraying of a conidial suspension, inoculation of liquid or solid culture and inoculation of infected nematodes (Pires et al., 2022). Each method has varying levels of tree survival, depending on strength of the solution, timeframe applied and tree age. Yin et al. (2020) reported higher tree survival when *E. vermicola* was applied preventatively rather than after PWN inoculation.

Another nematode-trapping fungus *Drechslerella dactyloides* Drechsler, typically used against the tomato root-knot nematode, has been converted to a granular formulation which is known to control PWN when applied in pot and field trials. Z. Wang et al. (2011) identified two Korean isolates of the fungus with unusually high capturing ability against PWN. Efficacy is thought to be due to the unusually shaped conidia and more complex fungal 'traps' formed by these isolates.

Wang et al. (2012) investigated nematocidal *Bacillus thuringiensis* (Bt) strains from forests in Zhejiang, China. Bioassays showed that PWN was highly susceptible to a particular protein (Cry5Ba3).

Gene silencing

RNA interference (RNAi) (or, Post-Transcriptional Gene Silencing (PTGS)) is a natural mechanism for sequence-specific gene silencing. Xue and Wu (2019) investigated three cathepsin L-like cysteine proteinase (CPL) genes affecting PWN development. The silencing of these genes could reduce feeding, reproduction, and pathogenic functions in PWN.

7.5. Elicitors

Nunes da Silva et al. (2014) investigated the efficacy of applying chitosan, a natural biopolymer derived from chitin, as a soil amendment to PWN-infested *Pinus pinaster* and *P. pinea* trees. High molecular weight (MW) chitosan provided the best results for the highly PWN-susceptible *P. pinaster*, while low MW chitosan was more effective for the relatively PWN-resistant *P. pinea*. The exact reasons chitosan reduces disease severity are unclear, however, chitosan is known to support microbes that outcompete or inhibit plant-parasitic nematodes. Thus, treating PWN-inoculated *P. pinaster* plants with chitosan could hinder nematode migration and reproduction. It is also suggested that PWN relies on ectosymbiotic bacteria, especially Gram-negative ones such as *Serratia*, *Enterobacter*, *Ewingella*, and *Pseudomonas* (Roriz et al., 2011), which chitosan may interfere with.

7.6. Increased tree resistance

Genetic breeding for resistance against PWN has been conducted for multiple pine species. There are two methods used in breeding programs: selection breeding, where individuals are selected that are already showing resistance in PWN infected areas, or cross breeding resistant individuals to create new cultivars (Nose & Shiraishi, 2008). The first breeding program began in Japan in 1978 for resistance in *Pinus densiflora* and *P. thunbergii*, with these clones being used as resistant seedlings for planting (Kamata, 2008). Further breeding programs for *P. massoniana* and *P. luchuensis* have been carried out in China and Japan, as well as a breeding program for *P. pinaster* in Portugal (Nose & Shiraishi, 2008; Ribeiro et al., 2012). Menéndez-Gutiérrez et al. (2017) found no clear climatic pattern in a provenance study of PWN susceptibility in three- to four-year-old *P. pinaster*, but suggested susceptibility could be related to temperature peaks in summer. Recently, *P. radiata* was assessed for genetic variation in PWN susceptibility from a program in Spain and was found to show potential for breeding resistant strains (Menéndez-Gutiérrez, Alonso, et al., 2021).

Torres-Sánchez et al. (2023) suggested that populations originating from arid climates may be less susceptible to PWD. Other factors that may have a role in pine susceptibility include resin canal traits, oleoresin and other polyphenolic compound production, cell wall strength and lipid-soluble substances in needles (Iki et al., 2020; Menéndez-Gutiérrez et al., 2017; Rodríguez-García et al., 2023; Torres-Sánchez et al., 2023). For the long-term success of resistance breeding against PWD, it is important to maintain tree populations that are genetically diverse in combination with multiple resistance mechanisms.

7.7. Phytosanitary measures for limiting spread

Preventative measures against the spread for PWD focus on reducing the movement of infested wood. In regulated areas with buffer zones and clear-cut belts, fumigation of diseased material and heat treatment of infested logs is made mandatory (Back et al., 2024; Ryss et al., 2011). Stated in the EPPO standard PM 9/1 procedures for official control (EPPO, 2018), a regulated area of at least 20 km consisting of the infested area and a buffer zone is created upon discovery of PWD symptoms, where all susceptible material is prohibited to leave the area without prior treatment. Around each infested area, a clear-cut belt of at least 50 m radius is created, where trees are felled to survey the potential spread of PWN and act as a barrier between infested and un-infested areas (Back et al., 2024; EPPO, 2018). However, Gordillo and Kim (2012) suggest it may be possible to achieve success and maintain a level of forest productivity by selectively eradicating only infested trees (but immediately upon cessation of oleoresin production), taking advantage of the Allee effect in the absence of a widely distributed vector. In Europe, regulated areas are kept in place for 15 years after the last PWN detection. In South Korea, an area is not considered “clean” until 3 years has passed with no PWN detection in a monitored area (Ryss et al., 2011).

Fumigation of diseased wood is used to kill PWN and its vectors before transporting the material outside of infested areas. Methyl bromide fumigation is a very effective method however, due to it being a greenhouse gas and its danger to workers, its use is restricted.

An alternative to fumigation is to heat treat the wood by heating the logs until the core temperature is at or above 56°C for 30 minutes or more, as described in the EPPO standard PM 10/6 (EPPO, 2009). This however does bring an increased cost to the industry (Gonçalves et al., 2012). The installation of combined heat and power (CHP) units in sawmills, fuelled by wood shavings, has been investigated in Portugal as a cost-saving measure for required heat treatments for wood exports (Gonçalves et al., 2012).

8. *Risk management options*

8.1 Surveillance and detection

Methods for detection and identification of PWN and their advantages and limitations are outlined in Table 6. Selection of preferred methods can be made in advance of a possible incursion, with protocols, equipment and required consumables identified, along with capacity and capability.

Table 6: Summary of PWN detection and identification methods including advantages and limitations of each (borrowed from Li et al. (2022)). Notable omissions are detections notified by the general public, and eDNA as a detection tool.

	Method	**Sample**	**Advantages**	**Limitations**
Direct inspection of pine trees	Manual check	Wild pine trees	Fast	Requires technical personnel expertise, possibility of subjective judgement
Morphological analysis	Microscope	Nematode	Low cost	Requires technical personnel expertise, possibility of subjective judgement
DNA-based methods	RFLP	Target DNA	Tool for analyzing the genetic variation of nematodes within and between species	Time-consuming, complicated, requires high-purity DNA
	n-PCR	Target DNA	High specificity	Time-consuming, difficult to detect and distinguish multiple species
	RT-PCR	Target DNA	Sensitive, reliable, safe and high throughput	Time consuming, equipment relatively expensive
	RAPD	Target DNA	Generates a large amount of information	Lacks repeatability, requires strict experimental reaction
	SCAR	Target DNA	High sensitivity	Time-consuming
	LAMP	Target DNA	Low cost, simple operation, low equipment demand	False-positive results
	RPA	Target DNA	Easy to use, results are accurate	Requires special detection equipment
Protein-based methods	2-DGE	Nematode protein	Fast, high resolution, can separate and display thousands of proteins at the same time	Time-consuming, difficult to distinguish the same protein spots
	Isozyme analysis	Nematode protein	High sensitivity	Tedious and time-consuming
	MALDI–TOF MS	Nematode protein	High sensitivity	Time consuming, requires specialized skills
VOC-based method	GC–MS	VOCs	Fast, allows repetitive, noninvasive, dynamic monitoring	Poor reproducibility
Spectral techniques	Satellite remote sensing	Wild pine trees	Fast, large-area detection, dynamic monitoring	Requires technical personnel expertise, difficult to capture detailed changes
	UAVs	Wild pine trees	Fast, large-area detection, dynamic monitoring	Requires technical personnel expertise

Surveillance trapping for *Monochamus* spp. in New Zealand ports and surrounding forested areas is highly recommended. Additionally, plans and preparation should be in place for rapid deployment of a larger scale trapping programme, should an incursion be detected. Black flight intercept traps coated with a lubricant such as fluon, fitted with wet collection cups containing propylene glycol, and baited with a blend of pheromones and kairomones are recommended as optimal for *Monochamus* spp. (Dodds et al., 2024). If possible, traps should be set up at the most likely entry sites (e.g. ports), at nearby forest edges and in the forest interior as different *Monochamus* spp. have different habitat preferences (Allison et al., 2019). Figure 11 illustrates this optimal trapping scenario for *Monochamus* spp. (Dodds et al., 2024).

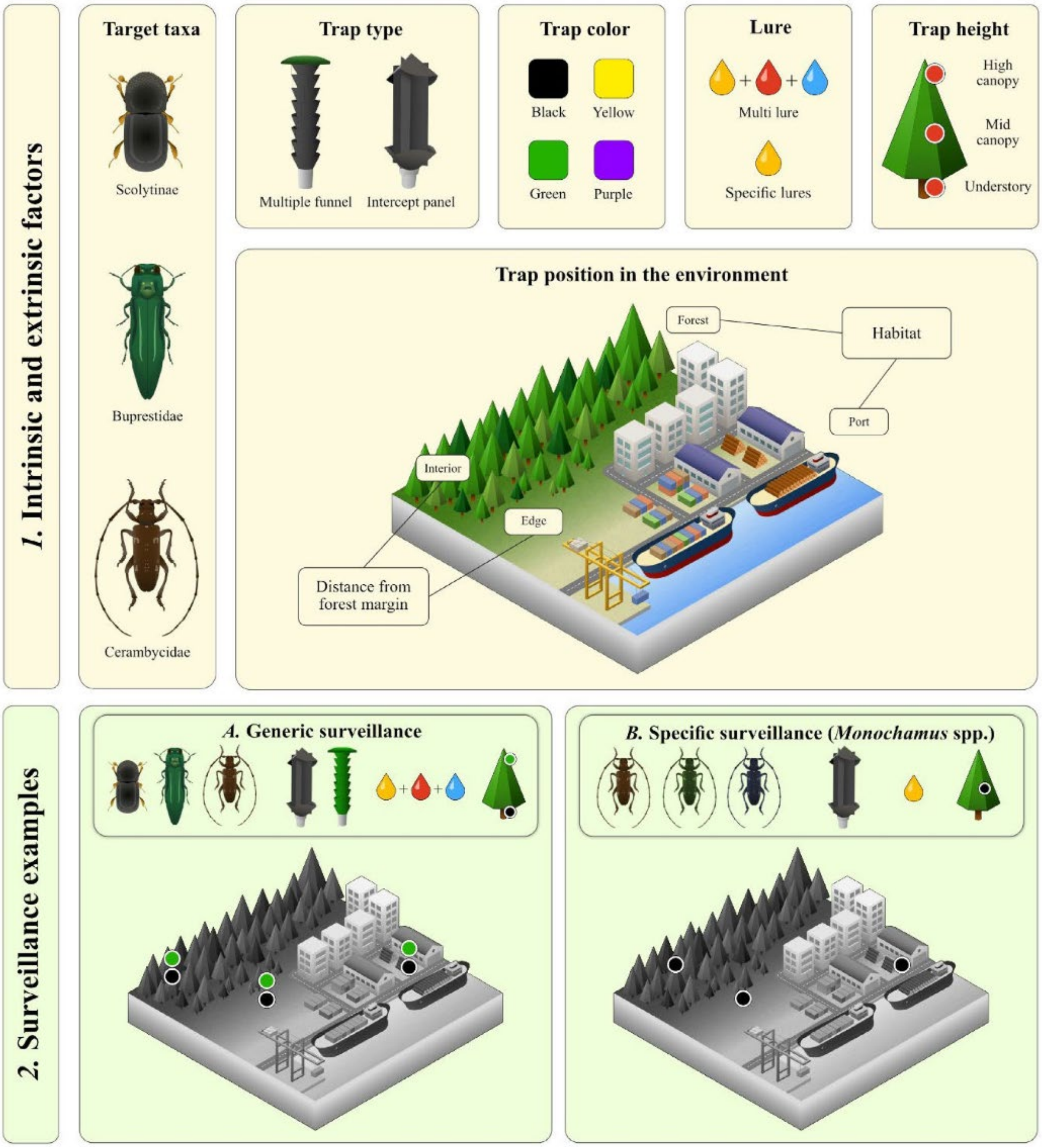

Figure 11 Example of how to exploit available knowledge on intrinsic and extrinsic factors affecting trap efficacy in surveillance programs. Here we use as case studies a generic surveillance targeting bark and ambrosia beetles, longhorn beetles and jewel beetles (**A**) and a specific surveillance targeting longhorn beetles of the genus *Monochamus* (**B**) carried out in a port area and its surrounding natural area (e.g., a forest). Information on the best trap type, trap colour, lure (multi-lure blend vs. specific lure), trap height, and horizontal position of the traps are provided. Black or green dots on the black and white landscapes represent the traps. Figure borrowed from Dodds et al. (2024).

The large size and striking appearance of *Monochamus* spp. bodes well for early detection in New Zealand, including by members of the public. Conversely, where these beetles are naturally present and not actively monitored because the nematode is not present, detection of an incursion of PWN may go unnoticed for a considerable length of time, as was the case with *M. galloprovincialis* and the accidental introduction of PWN into Portugal.

8.2 Incursion response

The incursion of *Bursaphelenchus hunanensis* in Melbourne, Australia is summarised as a case study in Appendix B and represents a highly relevant scenario for New Zealand. Responding to an incursion of PWN would necessitate delimiting surveys via trapping and sampling, implementing restrictions on the movement of infested or potentially infested wood, and destruction of infested wood. Social licence to operate could present a significant hurdle for an eradication campaign. Potential entry points for PWN and vectors are likely to be in urban environments, where host trees form an important part of the urban landscape, or may be on private property. Removal and destruction of these trees could be met with considerable resistance, particularly if the public is not well informed in advance. Since this would apply for several potential scenarios related to biosecurity threats, we recommend a general awareness campaign outlining potential actions that may be necessary in the event of an incursion. Already, New Zealanders are more biosecurity aware than most countries, so there is greater potential for acceptance.

8.3 Key research and management questions and relevance for New Zealand

Robinet et al. (2024) identified and prioritised 12 key research and management questions that need to be addressed to best evaluate and manage the invasion risk of PWN (Figure 12).

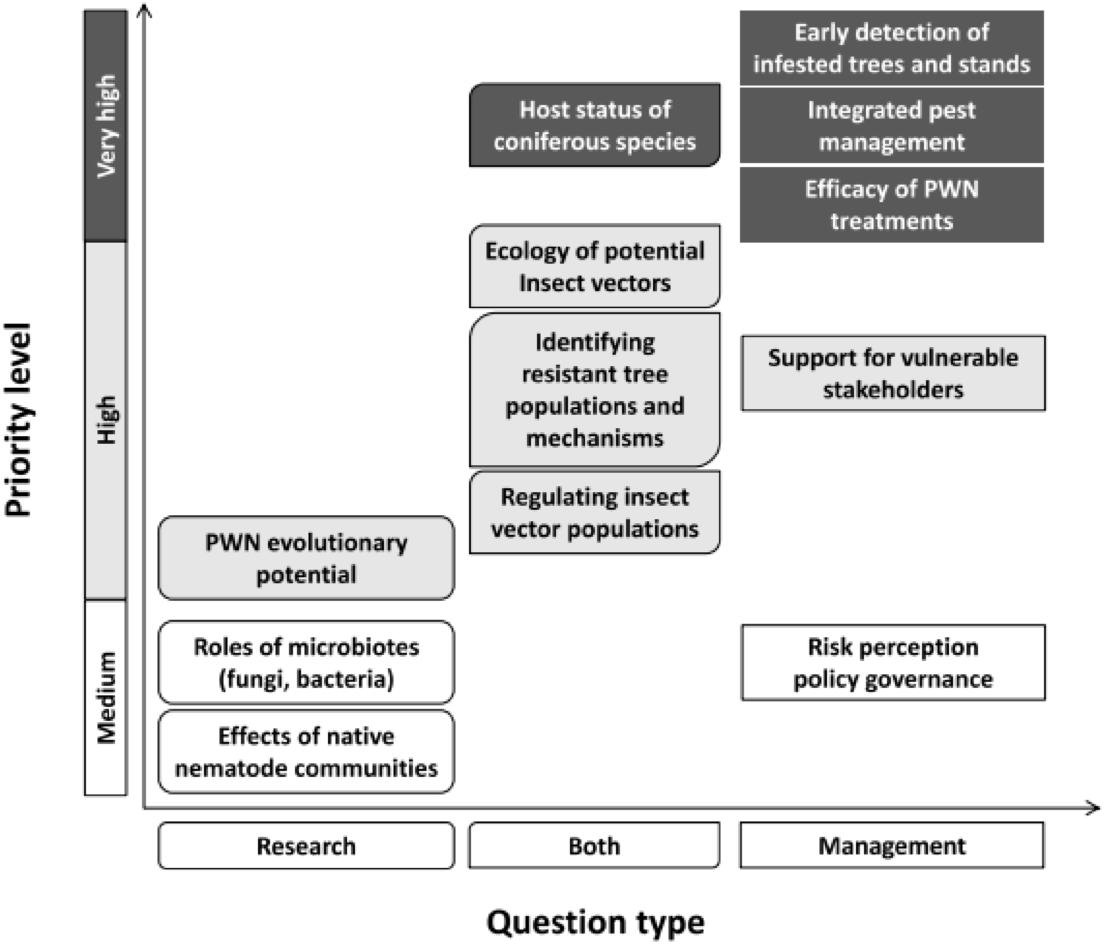

Figure 12 Ranking of priority questions as function of category (research, management, or both). These questions were identified in a European context by Robinet et al. (2024), but can be considered in other global and national contexts to address the threat of PWD.

Table 7 summarises the general and New Zealand-specific relevance of these questions regarding the following components of readiness: Risk assessment, Surveillance and detection, Incursion response, and Long-term management planning.

Table 7: Key research priorities identified by Robinet et al. (2024) in relation to the general areas of preparedness to PWN identified in this report. Research priorities are aligned to their main areas of relevance but can support other areas of preparedness. Research to improve Import Health Standards or Border clearance is not specifically addressed by these authors.

Preparedness areas		
Key priorities (Robinet et al.)	**General relevance**	**Relevance to NZ**
Risk assessment (Pest Entry, Establishment Spread and Impact)		
Ecology of potential insect vectors	The actual and potential distributions of vectors, their local abundance and other traits (such as assisted and natural spread capability, PWN load capacity, and host preferences) affect the risk of PWN globally.	The main vectors of PWN, *Monochamus* spp., are intercepted at NZ borders but not yet established. Pine insect pests and associated pathogens have been previously introduced in NZ. Limitations in pest mitigation efficacy on the wood packaging material pathways indicate the potential for the arrival of *Monochamus* and PWN. (Cf. sections 6.3, and 6.4)
Host status of coniferous species / Resistant tree populations	Pine and other conifers can be classified as resistant, susceptible, or intermediate hosts to PWN and its *Monochamus* vectors. This status is often established based on laboratory or nursery trials (e.g., inoculations of PWN in young tree seedlings), which are occasionally contradicted by field observations. The underlying resistance mechanisms are largely unknown.	The actual host status remains uncertain for some species such as *Pinus taeda*, *Pseudotsuga menziesii*, and *Picea abies*. The host status of *P. radiata* warrants further examination as this species arguably originates from the native range of the PWN. *Pinus radiata* has shown susceptibility in inoculation trials, but also potential for the development of resistance traits. (Cf. sections 3.3 and 7.6)
PWN evolutionary potential	The spread of the PWN across various continents suggests a high evolutionary potential and a capability to adapt to new environmental conditions. Hybridisation has also been observed in the laboratory and in field conditions, notably between *B. xylophilus* and the lesser-pathogenic species *B. mucronatus*.	If introduced in NZ, *B. xylophilus* spread would not be constrained by environmental conditions. Its potential to be effectively vectored and spread by current bark beetles and woodborers (e.g. *Arhopalus ferus*) is largely unknown. Non-pathogenic *Bursaphelencus* species are already established in NZ pine plantations and are seemingly spread by forest insects. Most of these potential vectors for PWN are colonisers of dead wood material including logs, dead branches, stumps, and the occasional damaged tree. The effective vectoring of PWN, or an emerging hybrid, would rapidly disseminate PWD in NZ. (Cf sections 2.2 and 3.2)

Risk management (Surveillance and detection)		
Early detection of infested trees and stands	Managers require quick and precise methods for detecting trees showing PWD symptoms. At the landscape level, remote sensing has yielded valuable results but tends to over-detect wilting trees, which may not necessarily be associated with PWN infection. At tree level, the ability to sample in the canopy would enhance the effectiveness of PWN detection. Molecular diagnostics also require improvements to be more operational in the field.	Not all infested trees are immediately symptomatic. If relying on symptom detection only, an incursion of PWN may remain undetected in NZ for several years. NZ could also benefit from improvements in detection methods developed globally. Refining the status of tree species as susceptible or tolerant to support existing commercial species surveillance programmes and mapping these host trees in high-entry risk areas could enable more focused and effective surveillance. (Cf. sections 4 and 5.**3**)
Early detection of vectors	Semiochemical-based trapping methods for capturing *Monochamus* can be improved (current pheromone attraction range ≤ 500 m) and other potential vectors not attracted to pheromones (host volatile attraction range typically ≤ 50 m). Other innovative detection methods could be developed (e.g. based on VOCs).	Methods for trapping and checking potential vector beetles for the presence of PWN could be further developed, including optimisation of traps and lures, and their placement. NZ should be implementing a trapping programme for wood and bark boring beetles and providing ongoing support for scientific and diagnostic expertise. (Cf. sections 4 and 7.3)
Risk management (Incursion response and long-term management planning)		
Attempting eradication or longer-term containment operations	Maps of suitable hosts must be available to support delimitation investigations and control efforts. A strategy for leveraging the Allee effect can be used to exploit low population densities for eradication or containment but may require good knowledge of vector population dynamics and dispersal patterns. Direct control methods also need to be understood, including constraints to applications, efficiencies and costs.	NZ should develop a comprehensive mapping programme for pine trees and other exotic conifers in environments likely to be first colonised by wood and bark-boring insects. As the wood packaging material and hitchhiker pathways remain the most likely mode of entry for most species, ports and urban or suburban industrial areas should be primarily targeted. The current availability of phytosanitary products, and control equipment as well as policy, logistical or other constraints to control operations need to be assessed.
Integrated pest management / Efficacy of PWN treatments	Direct control methods for PWN, such as chemicals, natural enemies, and competitors, have been tested in controlled laboratory conditions or on young seedlings. Their application and cost-efficiency in field conditions have yet to be evaluated.	Based on experience overseas, there is no single solution to address the management of PWD. An IPM strategy combining multiple measures and tailored for the specific NZ situation holds the greatest promise if eradication is not successful.
Regulating insect vector populations	Implementing a combination of strategies such as planting resistant pine tree genotypes, mixing host and non-host tree species, and removing vector breeding substrates from forests can form a robust integrated control strategy.	Several strategies can be explored in an IPM approach to prevent beetle outbreaks in at-risk areas. Forest management methods can limit the abundance of breeding material, such as grinding of logging residuals. Diversifying forest composition by mixing conifer species or incorporating deciduous trees could reduce susceptibility to beetle vectors.

Support for vulnerable stakeholders / Acceptability of PWN treatments	Factors that influence stakeholders' perceptions of PWN and influence their decision-making need to be understood. For the most vulnerable stakeholders, their readiness and ability to manage potentially large-scale damage and market closures needs to be assessed. Requirements for targeted support such as public subsidies, stakeholder coordination, and adherence to wood market regulations need to be identified.	If in NZ, PWD would be expected to significantly affect stakeholders in the forest and wood sectors. NZ must identify its champions and also its most vulnerable stakeholders, including in hard-to-reach areas and communities where managing PWN could pose significant social or economic challenges.

9. Recommendations for further readiness work

Preparedness:

- We recommend collaborating with the Ministry for Primary Industries to develop readiness plans and tools to inform and guide response activities in New Zealand, including addressing the activities outlined in Section 8, Risk Management Options (surveillance trapping, selection of detection methods and scaling up for a response, treatments and social licence to operate), and:
- Mapping of *Pinus* species in urban areas is recommended, particularly near points of entry or where wood packaging material may be stored and would potentially offer broader benefits. In Australia, existing mapping of pines helped to hasten efforts to eradicate giant pine scale in Adelaide.
- We recommend exploring eDNA as an effective monitoring and detection tool.

Research:

- Ethanedinitrile (EDN) is a broad-spectrum chemical fumigant with efficacy against pathogens, weeds and nematodes, and is approved by the Environmental Protection Authority for use in New Zealand, under tarpaulins. There is a developing body of research to investigate the effectiveness of EDN, in response to bans on methyl bromide, with promising results for control of PWN and vector (Arbuzova et al., 2020; Lee et al., 2017; Seabright et al., 2020; Stevens et al., 2022). Research could be undertaken in a New Zealand specific context, to test the efficacy of EDN for treatment of infected wood prior to moving it outside of infested areas.
- To assist predictions of the impact of PWD in New Zealand, it would be beneficial to screen New Zealand *Pinus radiata* germplasm for resistance to PWD, as well as test for susceptibility of native conifers (Lawson & Sathyapala, 2008). A protocol was recently developed for the in vitro propagation of *P. massoniana* clones and evaluation of resistance to PWN (Guo et al., 2023).
- The longhorn beetles *Arhopalus ferus*, *Hexatricha pulverulenta* and *Oemona hirta* have been suggested as potential primary vectors of PWN. Tests could be undertaken with *Bursaphelenchus* spp. already present in New Zealand, to assess vector potential.
- Climate modelling could be undertaken to better understand where climate change may cause the greatest impact in New Zealand, for example through increased temperatures or drought conditions. For example, Gruffudd et al. (2019) predict expression in healthy trees in the south of Germany, should PWN enter the country and should there be another hot year like 2003, and also predict expression across almost all of Germany, should the trees be under stress. Likewise, Ikegami and Jenkins (2018) used thermal modelling to predict that susceptible trees

currently outside of the area suitable for development of the disease are at risk in future based on climate projections.

- A stocktake of aerial surveillance techniques available in New Zealand could be undertaken, with consideration of supporting research on presymptomatic detection (e.g., as Scion has done with myrtle rust).

Future proofing:

- To spread the risk and potentially improve the resilience of forests and forest investment, diversifying forest tree species should be considered, along with replacing susceptible tree species or clones with less susceptible or resistant tree species.
- In tandem with the above, a better understanding of the risks of climate change and expected shifts in New Zealand could inform the selection of the right trees for the right places within the country, aiming to minimize tree stress which predisposes the trees to various pests and diseases including PWD.

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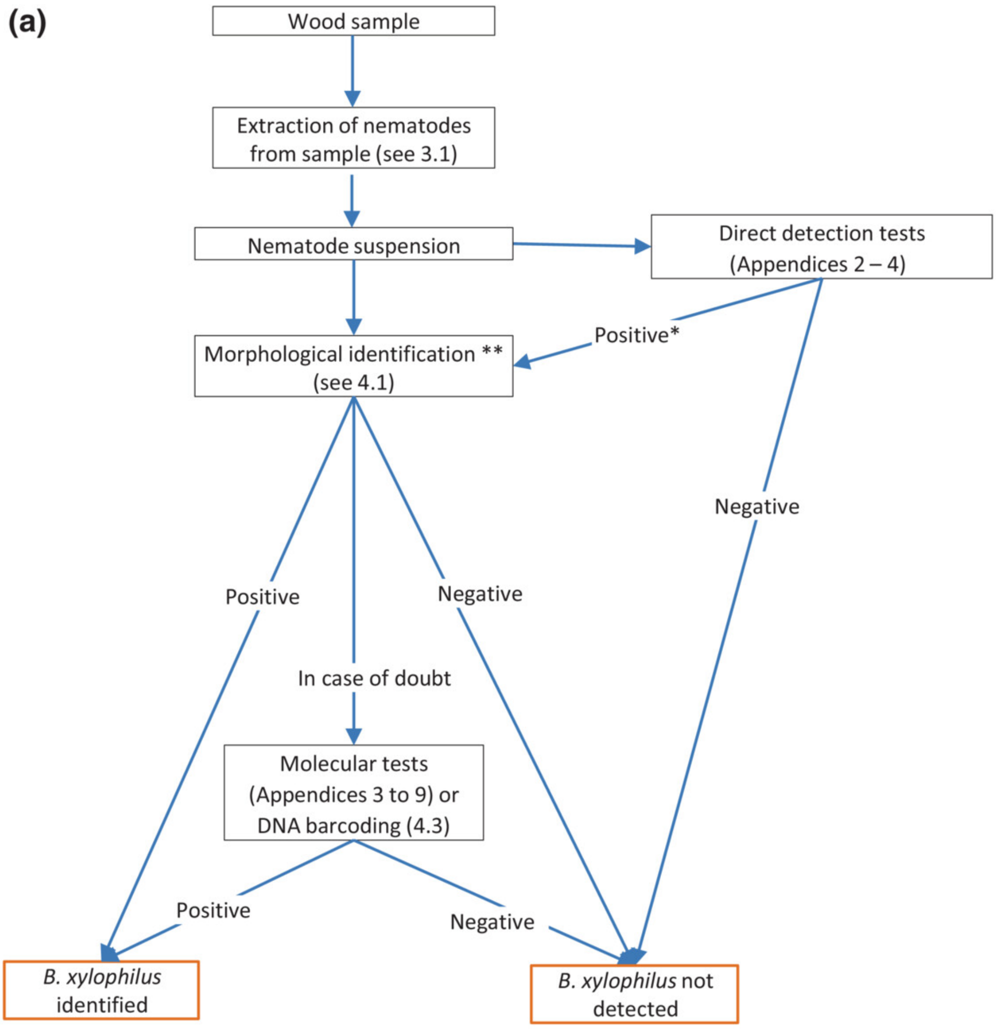
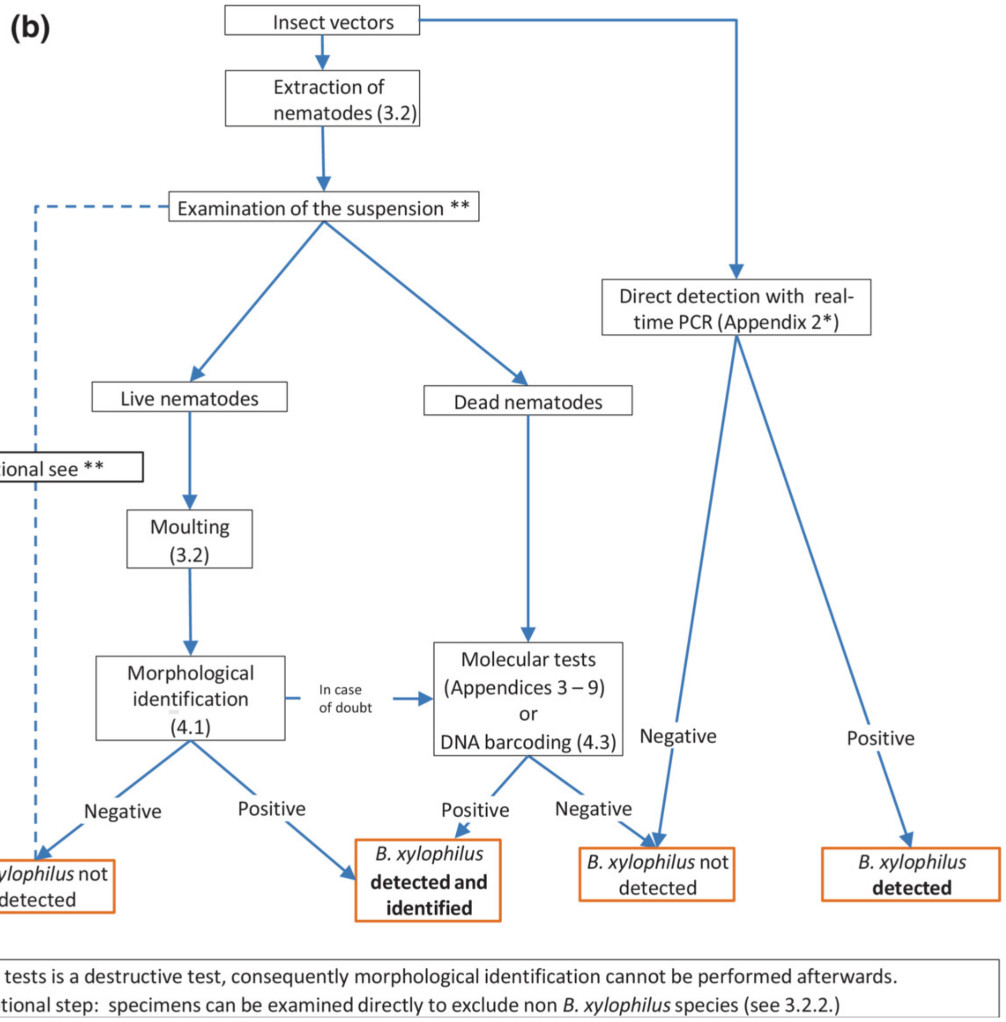
Appendix A

Flow diagrams for the extraction and identification of *Bursaphelenchus xylophilus*.



* after a positive real-time PCR or LAMP test the extracted nematodes not used for these tests (or the nematodes obtained after a second extraction from the same or a duplicate sample) are used for morphological identification.
** Identification can be performed based on morphology however considerable experience in nematode taxonomy, is required. In case of doubt, a molecular test should be performed. When only juveniles are present, they should be cultured on *Botrytis cinerea* (syn. *Botryotinia fuckeliana*).

Figure A1. Flow diagram for the extraction and identification of *Bursaphelenchus xylophilus* from wood samples (borrowed from EPPO (2023b)).



* This tests is a destructive test, consequently morphological identification cannot be performed afterwards.

** Optional step: specimens can be examined directly to exclude non *B. xylophilus* species (see 3.2.2.)

Figure A2. Flow diagram for the extraction and identification of *Bursaphelenchus xylophilus* from insect vectors (borrowed from EPPO (2023b)).

Appendix B

Summary of the incursion of *Bursaphelenchus hunanensis* in Melbourne, Australia

The successful response to an incursion of *Bursaphelenchus hunanensis* in Australia offers some insights into eradication as a viable strategy. Although there are other cases to learn from, such as in Europe, Australia bears greater similarity in that no *Monochamus* spp. are already present. This exotic nematode, never previously found outside of China, was isolated from a dying *Pinus halepensis* near the main port in Melbourne in 2000 (Smith et al., 2008). Of note is that the initial diagnostics only showed secondary fungal infection, but further diagnostics work was deemed warranted because of the high volume of international cargo passing through the port, and the large area of plantation forests surrounding the city (Hodda et al., 2008). An eradication campaign was initiated, identifying and sampling rapidly dying *Pinus* spp. within the city and across the state of Victoria, with 35 out of 450 trees testing positive for the nematode, and a further 50 sampled trees from across the country testing negative (Smith et al., 2008). Positive trees were removed under quarantine conditions and disposed of by burning or deep burial. Trees that died and tested positive for the nematode were generally older (~40 years) and were also infected by other pathogens and affected by abiotic factors such as drought and high salinity. After four years no more positive trees were detected, yet *Pinus* spp. continued to die across Melbourne, and these deaths were attributed to extended drought conditions (Smith et al., 2008). The positive trees were found in a plume shape in the direction of prevailing winds from the port (Figure B1), and it has been surmised that the nematode entered the port and spread by a single flight of vectors which failed to establish, or by an inefficient vector that could not transmit sufficient numbers of nematodes. Although *Arhopalus rusticus* was found within several of the dead trees, and some of these were infected with *B. hunanensis*, *A. rusticus* is thought to be an unlikely vector as it does not typically feed on healthy trees (Smith et al., 2008). Hodda et al. (2008) details the response stages and lessons learned.

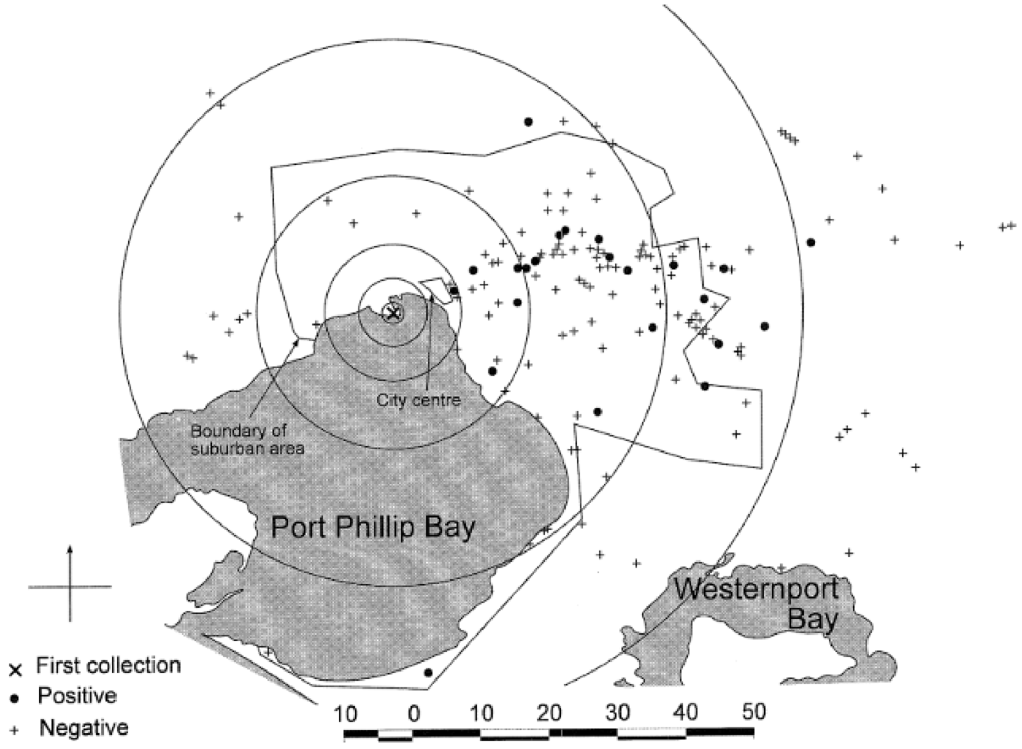


Figure B1 Trees sampled for nematodes around Melbourne. Concentric circles or arcs correspond to distances of 1, 5, 10, 20, 40 and 60 km from the first tree. Figure borrowed from Hodda et al. (2008).