Pest Risk Analysis

Biosecurity Risk to New Zealand of

Pinewood Nematode (*Bursaphelenchus xylophilus*)

(Photo. Y. Arakawa)

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Summary

The pinewood nematode (PWN), *Bursaphelenchus xylophilus* (Steiner & Buhrer, 1934), causative agent of Pine wilt disease (PWD) is a major forestry pest in Japan, China, Korea and Europe. *B. xylophilus* is native to North America where it is not known to damage indigenous conifer species. There are 55 confirmed species of *Bursaphelenchus*, of which *B. xylophilus* is considered to be the most significant pest.

Currently seven *Bursaphelenchus* species are classified as unwanted organisms by New Zealand. They are *B. xylophilus, B. eggersi, B. hellenicus, B. leoni, B. mucronatus, B. sexdentati* and *B. teratospicularis.* In 1986 *B. xylophilus* was classified as a very high risk pest by the European Plant Protection Organization. In 1999 PWN was detected in Portugal in association with *Pinus pinaster*. There have also been reports of pathogenic *Bursaphelenchus* spp. in Greece in 2000 and associated with *Pinus* tree death in Australia in 2001.

Concerns within the New Zealand Ministry of Agriculture and Forestry (MAF) over the level of biosecurity risk from PWN have risen with increasing global trade in PWN host material, the rapid spread of pine wilt in East Asia, and recent findings of pathogenicity of other *Bursaphelenchus* spp. It has also become evident that the presence of this disease in New Zealand could impact on our export of New Zealand forest products. This necessitated the completion of a pest risk analysis for *Bursaphelenchus* spp**.** to estimate the likelihood of entry, establishment and spread in New Zealand, and the economic and/or environmental consequences. *B. xylophilus*, the most intensively studied nematode of *Bursaphelenchus* spp has been used as a model species.

The pest risk analysis details available information on the following factors relevant to the biosecurity risk to New Zealand of *Bursaphelenchus xylophilus* and other pathogenic *Bursaphelenchus* spp.:

- The level of pathogenicity of identified *Bursaphelenchus* spp.;
- Potential pathways for the entry of PWN into New Zealand;
- Likelihood of establishment and spread of PWN in New Zealand;
- The potential economic and/or environmental impacts of the establishment of PWN in New Zealand.

Analysis of these risk factors identified the following conditions:

- New Zealand has suitable abiotic (temperature and annual precipitation) requirements for the establishment of PWN, and host species (principally Pine (*Pinus radiata*) and Douglas fir) are distributed throughout New Zealand;
- The establishment of PWN in New Zealand will mainly be dependent on the availability of a suitable insect vector. No species of *Monochamus* beetles are currently established in New Zealand. It is not known to what extent local native and exotic insects could act as a vector of PWN in New Zealand. The most possible candidates are the Cerambycids e.g. *Arhopalus ferus* and *Hexatricha puverulenta* which are both abundant in New Zealand.
- If PWN becomes established in New Zealand it is unlikely to show pine wilt symptoms under current New Zealand climatic conditions.

- The most likely method of establishment of PWN is via *Monochamus* spp. carried by untreated coniferous wood packaging material. The likelihood of this occurring however is currently considered low.

Based on the findings of the risk assessment the following measures were recommended to mitigate the risk of entry and establishment of insect vectors of PWN to prevent the establishment of the nematode in New Zealand.

- Review the import health standard for wood packaging material to impose mandatory treatment for all coniferous wood packaging material before entering New Zealand. Recommended treatments should exceed either fumigation with methyl bromide at 48 g/m^3 for more than 24 continuous hours and at a minimum temperature of 10°C, or heat treatment to a minimum continuous core temperature of 56°C for more than 30 minutes. Currently these treatments are expected to become mandatory for all imported solid wood packaging material entering New Zealand during 2005.
- Ensure the New Zealand border clearance systems for general imported cargo are sufficient to detect any significant increase in the entry of *Monochamus* spp and other insect vectors as hitch-hiker pests.
- Test all species and life-stages of *Monochamus* intercepted at the New Zealand border or post-border for the presence of *Bursaphelenchus* nematodes. The interception data would add significantly to our understanding of the risk these potential vectors pose to New Zealand and could be used to improve the effectiveness of risk management strategies.
- Undertake post-border surveillance for *Monochamus* spp. and PWD in New Zealand to ensure early detection for eradication.
- Include in general surveillance testing for the presence of *Bursaphelenchus* spp in dead or dying coniferous trees with wilt symptoms in high risk areas

The pest risk analysis also identified the following issues that require further research:

- Determine the level of susceptibility of *P. radiata* and native conifer species to PWN.
- Determine the likely vector status of candidate insects established in or native to New Zealand.

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1. INTRODUCTION

This pest risk analysis has been carried out in accordance with Forest Biosecurity Risk Analysis Handbook (Draft 2003) which is based on the International Plant Protection Convention (IPPC) International Standard for Phytosanitary Measures 11 (2001) (ISPM 11).

MAF Forest Biosecurity carries out risk analyses to assist with determining appropriate risk management measures for forest produce pathways and forestry pests. This pest risk analysis investigates the following factors associated with the phytosanitary risk of *Bursaphelenchus xylophilus* -pinewood nematode (PWN) and other pathogenic *Bursaphelenchus* spp to New Zealand:

- The level of pathogenicity of *Bursaphelenchus* species to host plants.
- The potential pathways for the entry of PWN into New Zealand.
- The likelihood of establishment and spread of PWN in New Zealand.
- The level of the potential economic and environmental impacts from the introduction and/or establishment of PWN in New Zealand.
- The phytosanitary measures required to reduce to an acceptable level the risks associated with the potential establishment of PWN in New Zealand

1.1. Definitions

Entry (of a pest)

Movement of a pest into an area where it is not yet present, or present but not widely distributed and being officially controlled (FAO 1990).

Establishment (of a pest)
Perpetuation, for the foreseeable future, of a pest within an area after entry (IPPC 1997).

Environment (Biosecurity Act (1993)

Includes:
a) Ecosystems and their constituent parts, including people and their communities; and
b) All natural and physical resources; and
c) Amenity values; and
d) The aesthetic, cultural, economic, and social conditions that affect or are affected by any matter referred to in paragraphs (a) to (c) of this definition.

Import Health Standard

A document issued under section 22 of the Biosecurity Act 1993 by the Director General of MAF, specifying the requirements to be met for the effective management of risks, associated with the importation of risk goods before those goods may be imported, moved from a biosecurity control area or a transitional facility, or given a biosecurity clearance.

For other definitions see:

- Secretariat of the International Plant Protection Convention, International Standards for Phytosanitary Measures, Glossary of Phytosanitary Terms. 2001.
- Biosecurity Act (1993)

2. THE PEST RISK ANALYSIS PROCESS

The procedures used to develop this pest risk analysis are described in detail in the Forest Biosecurity Risk Analysis Handbook. The process of risk analysis follows three main stages:

1) Hazard Identification
2) Risk Assessment
3) Risk Management

The risk associated with the introduction of an exotic forest pest is a sum of the likelihood of pest being introduced and establishing in an area, and the potential consequences or impacts that may occur as a result of the pest's introduction and establishment.

A qualitative risk assessment (very high, high, medium, low or negligible) has been performed for each risk element: likelihood of introduction; entry potential; establishment potential; spread potential; and consequences of introduction and establishment, based on available pest information. In order to assess the potential economic and environmental importance of the unwanted impact of PWN, the information obtained from areas where the pest occurs naturally or has been introduced were compared with similar conditions in New Zealand. The conclusions from the pest risk assessment were used to identify possible risk management strategies to manage the identified risks to an appropriate level.

3. INITIATION OF THE PEST RISK ANALYSIS PROCESS

3.1. Initiation event

The pinewood nematode, *B. xylophilus*, causative agent of pine wilt disease, is a major forestry pest in East Asia and Europe. There are about 55 described *Bursaphelenchus* species and of those *B. xylophilus* is considered to be the most significant disease agent. Currently, seven *Bursaphelenchus* species, including *B. xylophilus*, are classified as unwanted organisms by New Zealand Ministry of Agriculture and Forestry (MAF) due to their potential biosecurity risk to plantation and indigenous forests.

During the last 15 years several countries have reported an increasing number of interceptions of *B. xylophilus* and other *Bursaphelenchus* species and their insect vectors, *Monochamus* spp., in pine chips, unseasoned lumber and wood packaging materials. An analysis of the biosecurity risks of these nematode species on New Zealand is therefore required because:

- Global trade in host material has increased;
- The potential risks of establishment of insect vectors of *Bursaphelenchus* spp. in New Zealand was unknown;
- PWN has recently been found in Portugal;

- Other previously unidentified pathogenic *Bursaphelenchus* spp. have been discovered in Greece;
- Tree deaths associated with *Bursaphelenchus* spp. have recently been recorded in Australia; and
- Recent research findings associated with the development and implementation of ISPM 15, the international guidelines for the trade in wood packaging material.

Due to the limited availability of biological information about most of the *Bursaphelenchus* species, *Bursaphelenchus xylophilus*, the most intensively studied nematode of the genus *Bursaphelenchus*, is used as a model for this risk analysis.

3.2. Previous detections of Bursaphelenchus *spp. in New Zealand*

To date no species of *Bursaphelenchus* have been detected in New Zealand or intercepted on goods imported into New Zealand (MAF Quancargo 2004, MAF Bugs 1999, MAF PPIN 2004). This in part is not surprising as these nematodes are microscopic and as such, would only be detected on host products if symptoms were evident or microscopic examinations were undertaken. As nematodes are not routinely tested for at the New Zealand border, the lack of records is not unexpected. However, *Monochamus* beetles, the insect vector of *B. xylophilus*, have been intercepted on imported goods in New Zealand on numerous occasions. The interception records of these vector insects have been summarised in Appendix 1.

4. PEST INFORMATION

4.1. Pest taxonomy

Scientific Name:	*Bursaphelenchus xylophilus* (Steiner & Buhrer, 1934) Nickle
Synonyms:	*Aphelenchoides xylophilus* (Steiner & Buhrer, 1934) *Bursaphelenchus lignicolus* (Mamiya & Kiyohara, 1972)
Class:	Secernentea
Subclass:	Diplogasteria
Order:	Aphelenchida
Suborder:	Aphelenchina
Family:	Aphelenchoididae
Subfamily:	Bursaphelenchinae
Common Name:	pinewood nematode, pine wilt nematode

Bursaphelenchus xylophilus was first described in the USA as *Aphelenchoides xylophilus* (Steiner and Buhrer, 1934), but at that time no correlation was made between *B. xylophilus* and pine wilt disease (PWD). In 1972, after the discovery of the relationship between nematodes and PWD in Japan, the nematode was described as *B. lignicolus* (Mamiya and Kiyohara, 1972). The synonymy between *B. xylophilus* and *B. lignicolus* was recognised in 1981 (Nickle *et al.*, 1981).

4.2. Biological characteristics of Bursaphelenchus xylophilus

4.2.1. Morphology of *Bursaphelenchus xylophilus*

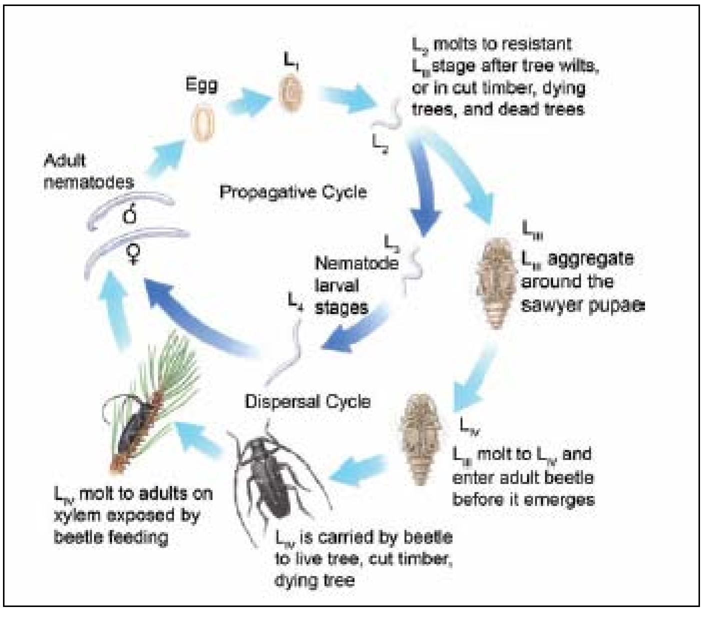
Bursaphelenchus xylophilus is microscopic (less than 1mm long) and the taxonomic features of the *Bursaphelenchus* spp. are consistent with the general characteristics of the order Aphelenchida. The taxonomic features are follows (Hunt, 1993):

- amphidal apertures oval, pore-like dorsosublateral on labial region;
- basal swellings, or knobs, usually weakly developed or entirely absent;
- oesophagus comprising a narrow, cylindrical procorpus, a strongly developed, offset ovoid to rounded rectangular median bulb with cresentic valve plates and well developed oesophageal glands forming a dorsally overlapping lobe;
- all three gland orifi (including the dorsal gland orifice) located within the median bulb;
- anus a broad transverse slit with an overhanging anterior lip;
- vulva posterior at 60-98%;
- genital tract monoprodelphic, usually outstretched;
- sperm large and rounded;
- spicules typically rose thorn shaped with prominent apex and rostrum;
- bursa usually absent (not so in *Bursaphelenchus*);
- usually three pairs of caudal papillae present.

4.2.2. Life cycle of *Bursaphelenchus xylophilus*

The life cycle of *B. xylophilus* (Fig 1) is similar to that of most other *Bursaphelenchus* spp. that have phoretic relationships with forest insect vectors (Fig. 2).

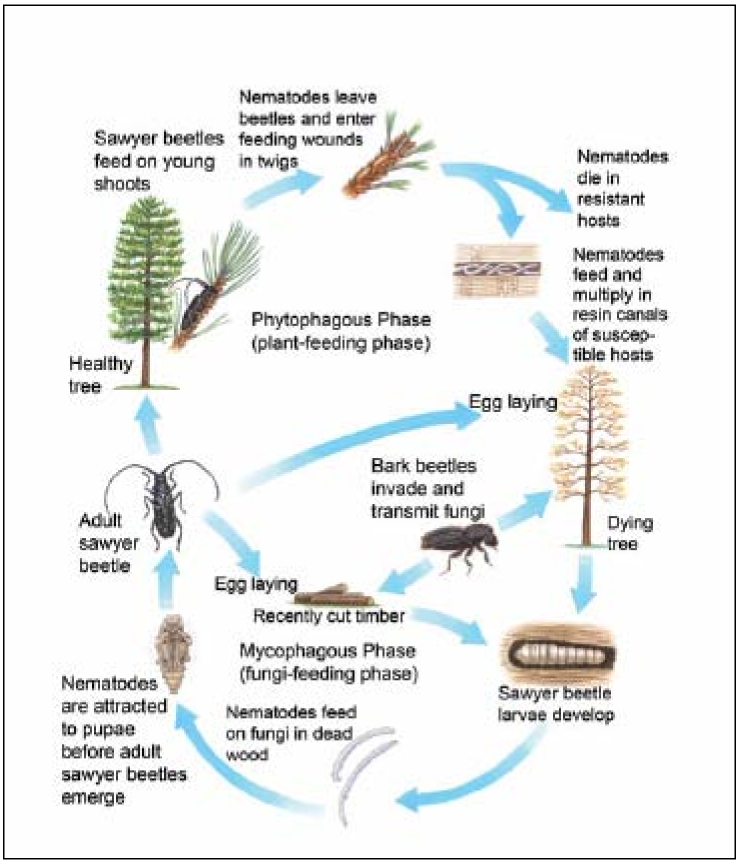
Figure 1. Life cycles of pinewood nematode



(www.na.fs.fed.us/spfo/pubs/howtos/ht_pinewilt/pinewilt).

Two different life cycles exist for *B. xylophilus* and both are well integrated with its insect vector (Coleoptera: Cerambycidae). Dead or dying trees, infected with *B. xylophilus*, harbour millions of the nematodes in their dispersal phase. When adult insect vectors pupate and emerge from wood they carry the fourth-stage nematode larvae (dauer larvae) to new trees. This transmission occurs when the adult insect vectors feed on young shoots (maturation feeding), or during oviposition by an adult females (Fig. 2).

Figure 2. Relationship of pinewood nematode and pine sawyer beetle



(www.na.fs.us/spfo/pubs/howtos/ht_pinewilt/pinewilt.)

Immediately after entering the tree, the juvenile nematodes moult to become adults which then mate and begin egg production. The propagative phase of the life cycle begins with the hatching of the eggs (Fig. 1). The nematode develops through four larval stages and matures to either a female or male form (Fig. 1). Reproduction is amphimictic. Under ideal conditions of suitable temperature (20-25º C) and plentiful food, a generation can be completed in four days (Mamiya, 1984). During the propagative cycle, nematodes may feed on the epithelial cells of the resin ducts (phytophagous) or on the fungi that invade the wood (mycophagous) (Fig. 2). The rapid multiplication of nematodes in the resin canals and their attack of epithelial cells lead to the wilting and subsequent death of the host tree. Usually a susceptible host tree dies 30-40 days after infection. The infected trees may contain millions of nematodes distributed throughout the trunk, branches and roots. The dying trees become attractive to mature insect vectors which oviposit in the bark.

As the tree dies, *B. xylophilus* cease to multiply, and enter into the dispersal phase of the life cycle, with the development of a specialised dispersal third-stage juvenile larva. The development of this specialised dispersal third stage juvenile appears to be triggered when the food resources of a host tree become scarce. The "dispersal" larvae gather in the wood surrounding the pupal chamber of the insect vector, possibly under the influence of substances diffusing from the developing pupae. As the beetle approaches pupation the dispersal third stage larvae develop into the specialised fourth-stage dispersal dauer larvae, which congregate in the pupal chamber until pupation is completed.

Soon after pupation is completed the dauer larvae invade the trachea of the beetle where they become inactive and wait for dispersal of the newly emerging beetle adults (Mamiya, 1984). In the dispersal phase, the specialised dispersal third stage juvenile and the dispersal fourth stage dauer larvae of *B. xylophilus* allow the nematode to resist adverse conditions.

4.3. Other related species of Bursaphelenchus

There are 55 described species of *Bursaphelenchus.* Approximately 75% of them are associated with conifer species (Braasch, 2001). All are primarily mycophagous, and the majority have a phoretic relationship with bark beetles and wood borers (Massey, 1974). Apart from *B. xylophilus* and *B. mucronatus,* very little is known of the pathogenicity of other *Bursaphelenchus* species.

Bursaphelenchus xylophilus has been placed into a group of species based on morphological similarity, including *B. xylophilus, B. mucronatus* and *B. fraudulentus*. The morphological characteristics used to group these species are (Braasch, 2001):

- presence of four lateral incisures,
- one single preanal,
- one pair of adanal,
- and two pairs of postanal caudal papillae,
- in males; characteristic spicules in males;
- and a large vulval flap in females.

Bursaphelenchus mucronatus are morphologically very similar to *B. xylophilus* but they are considered to be non-pathogenic or to have markedly reduced virulence in comparison with *B. xylophilus*. The distinguishing feature that differentiates these two species is the shape of the female tail. *B. mucronatus* has a caudal mucron, while populations of *B. xylophilus* have a tail shape variation that causes them to be confused with *B. mucronatus* (Mamiya and Enda, 1979; Braasch, 1996). The taxonomy of *B. mucronatus* is also the subject of some debate. However, DNA studies of these nematodes have clearly confirmed the differences between the three species (Harmey and Harmey, 1993, Kanzaki, N., and Futai, K. 2002).

Bursaphelenchus mucronatus has been reported from Japan, China, Korea, and most of Europe including Russia (Webster, 1999). It has also been reported from Quebec province in Canada (Harmey and Harmey, 1993). Between 1996 and 1998 a survey of *Bursaphelenchus* species associated with conifer trees in Greece revealed five *Bursaphelenchus* species (Skarmoutsos and Michalopoulos, 2000): *B. sexdentati, B. hellenicus, B. leoni, B. eggersi* and *B. tetratospicularis*. The most frequently isolated species from wilting trees was *B. sexdentati* and as such is considered to be a significant contributor to the wilting phenomenon in pine species in Greece. A laboratory trial conducted on pine seedlings to investigate the pathogenicity of *B. sexdentati, B. hellenicus* and *B. leoni* revealed that *B. sexdentati* caused high mortality in *Pinus pinaster, P. nigra* and *P. sylvestris.*

B. hellenicus appeared not to be pathogenic to the species tested (*P. brutia, P. pinaster* and *P. sylvestris*) while *B. leoni* was pathogenic to *P. halepensis* (Skarmoutsos & Michalopoulos, 2000).

Isolation of another closely related species, *B. kolymensis,* from larch, has been reported from the far east of the former USSR. Although this nematode has not been studied extensively it is suspected to be synonymous with *B. mucronatus* (Korenchenko, 1980).

In 2000, an exotic species of *Bursaphelenchus* was found in association with dead and dying *Pinus* spp. in Melbourne, Australia. Later this was identified to be close to *B. hunanensis* (Family Aphelenchoididae). It was suspected that *Arhopalus rusticus*, which has also been found during a survey on several dead trees in Templestowe, Melbourne, may have contributed to the spread of the nematode. It is still not known if *B. hunanensis* is a primary (i.e. causes the death of the tree) or secondary pathogen. The disease appears to be confined to mature pines, which are possibly under stress when infected. The disease is widely distributed across Melbourne (Ridley *et al*, 2001).

Currently *B. mucronatus, B. sexdentati, B. hellenicus, B. leoni, B. eggersi, B. tetratospicularis* and *B. xylophilus* are classified as unwanted organisms in New Zealand.

4.4. A description of Pine Wilt Disease (PWD)

Pine wilt is a severe hypersensitive response caused by the *B. xylophilus* in susceptible *Pinus* trees. The natural response of trees against the movement of PWN results in the blockage of xylem vessels and tracheids, and the release polyphenols, oleoresins and toxins which kill or injure neighbouring cells (Myers, 1988). The rapid dispersal of *B xylophilus* within the tree tissues increases the hypersensitive response. As the infection progresses, the damaged areas coalesce resulting in the death of cambium around the tree e.g. ring-barking the tree.

Trees affected by PWD characteristically exhibit a rapid foliage colour change from green to yellow-green to reddish or brown. In addition to rapid wilting and yellowing of the foliage, another important symptom is reduced resin production. When branches of healthy trees are cut, a thick, sticky resin will be produced at the site of the wound. On a diseased tree, resin may be reduced or completely absent. Branches and twigs become brittle and dry and will break easily. Trees with winter burns (yellow foliage) may have similar symptoms but can be easily distinguished from PWD as they have flexible branches and good resin production. Depending on the environmental conditions and susceptibility of the tree species, the disease may progress rapidly causing the tree to wilt and die within a short period of time (Mamiya**,** 1983).

It has been proposed that PWD is the result of the activities of *B. xylophilus* and a bacterial associate. Seedling pine trees inoculated with:

- aseptic nematodes (Zhao *et al*., 2000a);
- treated with antibiotics and then inoculated with nematodes that still harbour the bacteria (Zhao *et al*., 2000b); and
- Inoculation with bacterial associates of *B. xylophilus* without the nematode (Kawazu *et al*., 1999, Zhao *et al*., 2000a);

did not cause PWD indicating that the presence of both organisms is required to produce symptoms. This research is still in its early stages and further work will be required to determine the precise relationship between *B. xylophilus* and its bacterial associate, and the expression of PWD.

4.5. *Insect vectors* of *Bursaphelenchus xylophilus*

Most *Bursaphelenchus* species have a phoretic relationship with insects, especially Cerambycidae beetles which lay eggs in dying and dead trees. The main vectors of *B. xylophilus* are various species of *Monochamus* (Cerambycidae), commonly known as longhorn beetles or sawyers (Appendix 2, Table 2.). The genus *Monochamus* has about 150 known species distributed across Asia, Africa, Europe and North America. All species are indigenous to temperate regions and attack various species of Pinaceae that are stressed or recently killed by bark beetles, diseases or climatic factors. Adult *Monochamus* species are attracted to recently felled trees, logs or dead or dying trees for breeding. *Monochamus* oviposit and the larvae develop only in trees or in logs with bark. The female beetle gnaws an irregular hole through the bark (oviposition pit) and inserts her eggs. *Monochamus* larvae feed from 1 to 2 months on the cambial layer of the tree. Later, the larva bores into the sapwood, forming an oval entrance hole. The tunnel is usually u-shaped, and the pupal cell is located in the sapwood just beneath the outer bark

Monochamus alternatus is the main vector of *B. xylophilus* in Asia, while *M. carolinensis* is the main vector in North America. The nematode has also been found in association with several other Cerambycids such as: *Arhopalus rusticus*, *Spondylis buprestoides*, *Corymbia succadanea*, *Acalolepta fraudatrix*, *Acanthocinus griseus* and *Uraecha bimaculata* (Mamiya, 1976), one species of Bupresidae and two species of Cucurlionidae. However transmission of PWN was only observed in *M. alternatus*, *M. carolinensis*, *M. mutator*, *M scutellatus* and *M. titillator*. More recently, Evans *et al*. (1996) added *M. saltuarius*, *M. obtusus*, *M. nitens* and *M. marmorator* to the confirmed vector list. The recent discovery of *B. xylophilus* in Portugal also implicates *M. galloprovincialis* as a vector (Sousa *et al*., 2001).

Figure 3. *Monochamus alternatus* female feeding on *Pinus densiflora* twig

(photographed by K. Togashi)

It has also been reported that several beetle species of Cerambycidae may have the potential to provide a phoretic vehicle for *B. xylophilus*. However *Monochamus* spp. are thought to be the only group that could effectively create an epidemic based on their following characteristics:

1. The capability of carrying large populations of *B. xylophilus*. *M. alternatus* has capacity to carry more than 250 000 nematodes per beetle (Mamiya, 1984) compared to 1 per beetle for *Acanthcinus griseus*. *Acanthcinus griseus* is one of the most likely non-*Monochamus*

candidates known to date to provide a phoretic vehicle for *B. xylophilus* (Maehara and Futai, 2002);

2. The ability to create the suitable conditions within the tree for congregation and infestation of *B. xylophilus* e.g. high CO_2, presence of unsaturated fatty acids, oleic acid, and linoleic acid compounds (Mamiya, 1984);
3. The ability to co-vector suitable host fungi for *B. xylophilus* between host trees (not an exclusive function of *Monochamus* spp.).

Table 1. *Monochamus* species from coniferous trees, known to be vectors of *Bursaphelenchus xylophilus* or considered to be potential vectors of *Bursaphelenchus* spp.

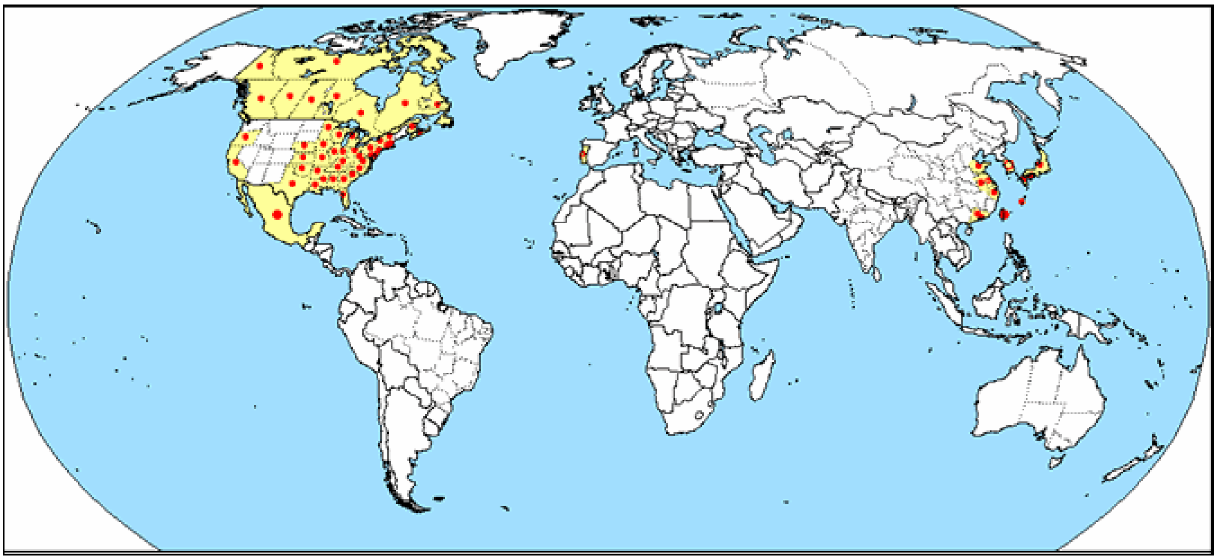
***Monochamus* species**	**Geographical distribution**	**Main hosts**	**Vector status**
North America			
M. carolinensis Olivier	USA (eastern half), Canada (east & US border), Mexico(north central)	*Pinus*	+
M. clamator LeConte	USA (west coast), Canada (British Columbia)	*Pinus contorta*	-
M. marmorator Kirby	USA, Canada	*Abies*, *Picea*	+
M. mutator LeConte (syn. *M. maculosus* Haldeman)	USA, Canada	*Pinus*	+
M. notatus (Drury)	USA, Canada	*Pinus strobus*	-
M. obtusus Casey	USA (west coast), Canada (British Columbia)	*Pinus*, *Abies*, *Pseudotsuga*	+
M. rubigeneus Bates	USA (south), Mexico, Guatemala, Honduras	*Pinus*	-
M. scutellatus Say subsp. *scutellatus*	Eastern North America (including parts of Mexico)	*Pinus*, *Picea*, *Abies*, *Larix*	+
M. scutellatus subsp. *oregonensis* LeConte	USA (west coast), Canada (British Columbia)	*Picea*	-
M. titillator (Fabricius)	USA (centre, east & south-east), Canada (Ontario)	*Pinus*, *Abies*, *Picea*	+
Palaearctic region			
M. alternatus Hope	Japan, Korea Republic, Taiwan, Hong Kong, Lao, China (Anhui, Guangdong, Hunan, Jiangsu, Shandong, Zhejiang, i.e. east & centre)	*Pinus*, *Cedrus*, *Abies*, *Picea*, *Larix*	+
M. nitens Bates	Japan	*Pinus*	+
M. saltuarius Eschscholz	Japan, China (Heilongjiang; NE) Siberia, Lithuania, central & eastern Alps, central & eastern Europe and south to Italy	*Picea*	+
M. tesserula White	Japan, China	*Pinus*	-
M. urussovii (Fischer) (syn. *M. rosenmuelleri* Cederhielm)	Japan, China (Liaoning, Heilongjiang, Neimenggu; i.e. NE) Siberia, Russia (Caucasus), Finland, Poland	*Abies*, *Larix*, *Picea*, *Pinus*	-
M. galloprovincialis (Olivier)	Portugal, North Africa, Italy, France, Greece, Germany, Poland, Sweden, Finland, Russia (European), Siberia	*Pinus*	+
M. sartor Fabricius	Central Europe (eastern France to western Ukraine)	*Picea*, *Pinus*	-
M. sutor (Linnaeus)	China (Heilongjiang, Liaoning; NE), Siberia, Russia (European), Georgia, the Nordic countries, central & eastern Europe, the Pyrenees, Alps	*Pinus*, *Picea*, *Larix*	-

(EPPO pest data sheet, Skarmoutsos and Michalopoulos, 2000**)**

4.6. Geographical distribution of Bursaphelenchus xylophilus

Bursaphelenchus xylophilus, a native of North America, is widespread in Canada and USA. It is suspected to have arrived in Japan in infected timber from the United States at the beginning of the twentieth century. Although PWD was identified in Japan in 1905, the pathogenicity of *B. xylophilus* was not detected until 1971. In recent years, infections have been discovered in Korea, in mainland and off shore islands of China, in Taiwan and in Portugal (Mota *et al*., 1999; Skarmoutsos and Michalopoulos, *2000*). The current geographical distribution of *B. xylophilus* is shown in Figure 4.

Figure 4. Current confirmed distribution of *Bursaphelenchus xylophilus*



(www.eppo.org/QUARANTINE/nematodes/Bursaphelenchus_xylophilus/BURSXY_map)

Asia:	Japan (Honshu, Kyushu, Ryukyu Archipelago, Shikoku), China (restricted distribution - Anhui, Guangdong, Jiangsu, Shandong, Zhejiang; Zhang and Huang, 1990), Republic of Korea (restricted distribution -province of Pusan), Taiwan, Hong Kong.
North America:	Widespread in the USA (40 states), Canada (Alberta, British Columbia, Manitoba, New Brunswick, Ontario, Quebec, Saskatchewan), and Mexico*(*Dwinell, 1993).
Europe:	Restricted distribution in Portugal.
South America:	Not reported.
Africa:	No confirmed reports

4.7. PWN host range and susceptibility

Bursaphelenchus xylophilus is primarily a fungal-feeding nematode (mycophagous) but may also feed on plant cells (phytophagous). The full range of fungi that can be utilised has not been determined, but the following are known to be good hosts: *Ceratocystis* spp., *Ophiostoma minus* (bluestain fungus), *Botrytis cinerea* (grey mould), *Ceratostomella ips* (blue stain fungus), *Colletotrichum* sp., *Fusarium* spp., *Macrophoma* sp., *Monochaetia* sp., *Nigrospora* sp., *Pestalotia* spp., *Rhizosphaera* spp., *Sordaria* sp. and *Trichoderma* sp. (Dropkin *et al*, 1981; Maehara & Futai, 2000; Ye *et al*, 1993).

Most *Bursaphelenchus* spp. are restricted to conifer species, but being a "host" for *B. xylophilus* does not necessarily mean that the nematode is feeding on the tissues of the tree. The nematodes may live on fungi resident within the tree tissues and therefore not all trees "infested" with PWN go on to develop symptoms of PWD. Trees that are not susceptible to PWD may still become infected with *B. xylophilus* and remain free of symptoms for a number of years while containing live nematodes. In some less sensitive pine species, *B. xylophilus* may be tolerated without causing PWD. Other resistant trees may successfully limit nematode spread close to the site of original infection. Such trees can still act as reservoirs for the nematode, even though they do not show any symptoms of disease.

The susceptibility or tolerance/resistance of tree species to PWN varies (Table 2, Table 3). There are many contradictions in the literature in relation to the susceptibility of various pine species to *B. xylophilus* (Dropkin *et al.*, 1981; Furuno, 1982; Kondo *et al.*, 1982; Wingfield *et al.*, 1984). In some cases susceptibility seems to depend on the age of the inoculated trees (Bain & Hosking, 1988), or the conditions under which the trials were conducted. Sutherland *et al*. (1991) demonstrated that normally fairly resistant larches, such as *Larix laricina* and *L. occidentalis*, suffered 90% mortality when infected at high temperatures.

Table 2. Susceptibility of various *Pinus* species to *B. xylophilus*

Resistant	Intermediate	Susceptible
Pinus clausa	*Pinus banksiana* (Jack pine)	*Pinus ayacahuite*
Pinus elliottii (slash pine)	*Pinus bungean*	*Pinus densiflora* (Japanese red pine)
Pinus fenzeliana	*Pinus caribaea**	*Pinus kesiya (= P. khasya)*
Pinus morrisonicola	*Pinus contorta*	*Pinus koraiensis*
Pinus rigida	*Pinus cooperi*	*Pinus leiophylla*
Pinus taiwanensis	*Pinus echinata* (shortleaf pine)*	*Pinus luchuensis*
Pinus virginiana	*Pinus engelmannii*	*Pinus mugo*
	Pinus halepensis subsp. *halepensis*	*Pinus muricata*
	Pinus halepensis subsp. *brutia*	*Pinus nigra*
	Pinus jeffreyi	*Pinus. pinaster*
	Pinus lambertiana	*Pinus sylvestris*
	*Pinus massoniana**	*Pinus thunbergii* (Japanese black pine)
	Pinus monticola	
	Pinus montezumae var. hartwegii	
	Pinus oocarpa	
	Pinus palustris (longleaf pine)*	
	Pinus patula	
	Pinus pinea	
	Pinus ponderosa (Ponderosa pine)	
	Pinus pentaphylla	
	*Pinus pungens**	

Resistant	Intermediate	Susceptible
	Pinus radiata (Monterey pine)*	
	Pinus rudis	
	Pinus resinosa (red pine)	
	Pinus stobiformis	
	Pinus strobus *	
	Pinus tabulaeformis	
	Pinus taeda (loblolly pine)*	
	Pinus wallichiana (= *P. excelsa, P. griffithii)*	
	Pinus yunanensis	

- **indicates the equivocal status of the host by different authors.**

(Dropkin et al., 1981; Evans et al., 1996; Li & Wang, 1997; Yang & Wang, 1989; Chang & Lu, 1996,; Mamiya, 1976; Furuno, 1982)

Table 3. Other conifer hosts of *B. xylophilus*

Abies ambilis	*Larix decidua*	*Picea abies*
Abies balsamea	*Larix kaempferi*	*Picea englemannii*
Abies firma	*Larix laricina*	*Picea canadensis*
Abies grandis	*Larix occidentalis*	*Picea glauca*
Abies sachalinensis	*Pseudotsuga menziesii*	*Picea jezoensis*
Cedrus atlantica	*Chamaecyparis nootkatensis*	*Picea mariana*
Cedrus deodara		*Picea pungens*
		Picea rubens
		Picea stichensis

(Evans et al., 1996)

Wingfield *et al* (1984) demonstrated that seedling plants of *Pinus* species were highly susceptible to *B. xylophilus*. Inoculation studies conducted on mature *Pinus banksiana, P. resinosa* and *P. nigra* in a forest situation failed to kill the trees or produce any detectable damage. However all seedlings of the same pine species inoculated with *B. xylophilus*, under greenhouse conditions, were killed (Wingfield *et al* (1984)).

Pinus radiata is a species with an equivocal status as a host for *B. xylophilus*. Some researchers classified *P. radiata* as a susceptible host while others as a resistant host (Dropkin *et al*., 1981; Mamiya, 1984). Bain and Hosking (1988) studied *P. radiata* in its natural habitat (California), where both *B. xylophilus* and its vectors are endemic. They found no instances of infection despite a targeted search. Furuno *et al* (1993) reported approximately 80% mortality of *P. radiata* to PWD in Japan in an experiment conducted from 1960 to 1990. They tested the susceptibility of exotic pine species to PWD in areas that contain a number of different pine species more than 20 years of age and grown under conditions of natural infection of PWN. The results indicated that most of the pine species native to Eastern North America, such as *P. clausa, P. echinata, P. elliottii, P. taeda, P. virginiana*, and *P. pungens,* were resistant, and pine species native to Europe, such as *P. nigra, P. pinaster, P. pinea and P. sylvestris,* were highly susceptible to PWD.

More recently, *P. radiata* was classified as an intermediate host in the Pest Risk Analysis conducted for the European Union (Evans *et al*., 1996). In its native range in North America, PWN mainly depends on the mycophagous cycle for reproduction and dispersal and has not been found to damage indigenous conifer species. . In California where both *B. xylophilus* and its vectors

(*Monochmaus carolinensis*) are endemic *P. radiata* has not been affected by PWN. This may explain the presence of co-evolved native tree species resistant or tolerant to the PWN in North America. This is further supported by the fact that PWD develops in susceptible exotic species under suitable climatic conditions in North America (*Pinus sylvestris*), Japan and other countries. Experiments conducted in Japan on mature *P. radiata* trees, where a different insect vector (*M. alternatus*) of PWN occurs, recorded tree mortality rates as high as 60%. A similar situation could be expected in New Zealand where radiata pine and Douglas fir are exotic and a suitable native insect vector or established *Monochamaus* vector may be present. This pest risk analysis for New Zealand therefore considers *Pinus radiata* as an intermediate host for PWN. In North America, Douglas fir is regard as resistant to pine wilt but the trees could harbour PWN and are therefore considered to be a host species.

5. PEST SIGNIFICANCE

Pest significance is a measure of the potential impact the pest may have in this instance on the New Zealand economy and environment should the pest become fully established in New Zealand.

5.1. Potential economic impact of PWN

The potential economic impact of a pest is a measure of the economic importance the pest could have on economic or commercial activities in an area should the pest be introduced or become established in that area.

5.1.1. Economic impact in offshore countries

Pine wilt disease causes significant economic loss in some parts of the world. In southern Japan, where it was first recorded in 1905 (Yano, 1913), the disease initially caused losses of as much as one million m^3 of wood production per year. Destroying infected trees as a control measures brought this figure below half a million m^3 of wood production per year. During the Second World War when the control measures were abandoned, the disease spread throughout Japan to all but the most northern island of Hokkaido. In 1979, a recorded loss of 2.4 million m^3 wood, (approximately 1% of the growing stock pine in Japan) was reported. In 2000, the infested area was estimated to be 580 000 ha or 28% of Japan's total of 2.1 million ha of pine forest. Today PWD is widespread in Japanese red pine (*P. densiflora*) and Japanese black pine (*P. thunbergii*). *B. xylophilus* is considered to be the most serious forestry pest in Japan, with losses of nearly one million m^3 pine wood annually (Mamiya 2004). In 1986 the annual budget for control measures of PWN was $US 50 million, of which 55% was used for aerial spraying, 8% for ground spraying, and the residue for removal or salvage of dead trees.

In 1979 *B. xylophilus* was identified in the USA in association with dead *P. sylvestris* (Malek & Appleby, 1984). While *B. xylophilus* is widespread in natural coniferous forests in North America, significant losses are seldom recorded. Most tree deaths attributed to the PWD in North America are in exotic species located man-made forest ecosystems such as ornamental conifer plantings, wind-breaks, or Christmas tree plantations. The greatest economic impact from the PWN in North America resulted from the restrictions enacted between 1972 and 1989 on the movement of untreated wood chips and timber. It is also estimated that the economic losses from restrictions placed on green lumber exports to Europe during the 1990s was around $100 million annually (Dwinell, 1997).

5.1.2. Potential economic impact in New Zealand

The forestry sector plays an increasingly important role in New Zealand's economy. If *B. xylophilus* became established in New Zealand, the spread of PWD has the potential to have a major impact on New Zealand's forestry based industries. Ninety five percent of commercial forestry in New Zealand is based on plantations of *Pinus radiata*. This industry accounted for approximately three billion New Zealand dollars of exports in 2003, a domestic market of an equivalent size, and the second largest employer in New Zealand.` If biotic and abiotic factors are as favourable to *B. xylophilus* in New Zealand as they are in Japan, the mortality rate could be as high as 60%. The economic impact in New Zealand under those circumstances would cause a direct loss in productivity of plantation forests, and would create difficulties in exporting logs and timber, especially to countries which already have quarantine barriers against *B. xylophilus*.

Because of the devastating impact PWD has had on native conifer forests in Japan, China, Korea, and Taiwan, untreated wood from areas where the PWN was known to occur was banned in the European Union and China. Extra heat-treatment for untreated lumber is required by these importers to ensure the wood products are free of living nematodes and their beetle vectors. Likewise, if PWN become established in New Zealand, countries, importing unprocessed *P. radiata* (e.g. logs, timber, wood chips) from New Zealand would require, as a phytosanitary measure, the wood to be heat treated before export. The countries most likely to require this extra heat treatment would be those that do not have, or have restricted distributions of *B. xylophilus*, and have significant areas of natural and/or plantation forests of susceptible *Pinus* species. Currently such countries would include Australia, China and Korea. The extra cost of heat-treating unprocessed wood products such as logs would substantially reduce or eliminate the profitability of those products. It is unlikely that any added value would be gained from such a treatment in logs.

5.2. Potential environmental impact of PWN

The potential environmental impact of a pest that becomes established in an area would depend on the ability of the pest to cause such direct or indirect impacts as ecosystem destabilization, reduction in biodiversity, reduction or elimination of species, and non target effects of control measures.

5.2.1. Environmental impact in offshore countries

In Japan, China and Korea rapid spread of pine wilt has destroyed large areas of pine forests causing significant changes in the local ecosystem. On Ogasawara Island (Japan) damage to pine forests has destroyed almost all of the *P. luchuensis* trees on the Island. Extensive tree loses due to PWN had severe impact on erosion control, sand stabilisation, wind protection, and maintenance of aesthetic value. Most pine forests impacted by PWD have seen the Pinus species within replaced with evergreen broad leaved trees. This has reduced the species richness and resulted in changes to the associated flora and fauna in the forest. Pines have high aesthetic value in countries such as China, Japan and Korea and large losses of trees have a sociologically impact.

5.2.2. Potential environmental impact in New Zealand

The most aesthetically and culturally significant native conifer species in New Zealand belong to the Araucariaceae and Podocapaceae families. There are 17 species of these families in New Zealand. It is not known if New Zealand's endemic conifer species are susceptible to PWN infection, or if *B. xylophilus* would have any impact on the native nematode populations in New Zealand. However, PWD could have a direct impact on susceptible exotic species such as fir

(*Abies*), spruce (*Picea*), and larch (*Larix*) grown in urban areas for recreational and amenity value. High tree mortality of pine and Douglas-fir (*Pseudotsuga menziesii*) plantations and the large scale removal of diseased trees as control measures would increase erosion and result in micro–climate modification of affected areas. Ultimately, adverse impacts on native flora and fauna habitats, indigenous ecosystems and biodiversity could ensue.

6. LIKELIHOOD OF ENTRY OF PWN AND VECTORS

The most likely pathway for the introduction of pinewood nematode into New Zealand is through the import of untreated forest products produced from host tree species harvested from PWN infested forests. Examples of such forest products currently imported into New Zealand include:

- Solid wood packaging material.
- Wood chips.
- Sawn wood.
- Nursery stock.
- Logs, poles, piles, rounds and sleepers ("logs").
- Seeds and cones.

The sections 6.1 to 6.7 provide an assessment for each pathway of the likelihood of entry and transfer of PWN with or without its insect vector to hosts in New Zealand.

6.1. Assessment of coniferous solid wood packaging material

Wood packaging material includes all wood and wood products used in supporting, protecting or carrying a commodity such as dunnage, crates, fillets, spacers, pallets, drums, reels, packing cases, box pallets and gluts. Approximately 50% of all loaded shipping containers entering New Zealand contain wood packaging material. In the USA around 50% of solid wood packaging material is manufactured from hard wood (non-host) species.

Generally, unprocessed raw wood used in packaging comes from very low quality trees with no immediate commercial value. There is an increased likelihood of the use of dead or dying coniferous trees in the production of inexpensive dunnage and other packaging material. These are more likely to harbour PWN and insect vectors carrying the PWN larvae.

Around 95% of the *Monochamus* spp. (vectors of PWN) intercepted on material imported into New Zealand were on wood packaging material (see Appendix 1). The likelihood of entry of adult beetles which carry dauer larvae of PWN is very high when imports are from countries where PWN is present and widespread. Currently, the majority of imported wood packaging material arrives in New Zealand in containers imported from Australia and countries in South East Asia. Projected, future trade patterns indicate increases in the importation of wooden packaging material from China, Hong Kong and Taiwan. It is possible, however, that much of this wood packaging material could originate from North America.

Current New Zealand import requirements for wooden packaging material do not require mandatory treatment. Regulatory measures are limited to visual inspection of the product upon arrival. The treatment options while not being mandatory include:

- Fumigation with methyl bromide or sulphuryl fluoride at 80 g/m^3 for more than 24 continuous hours, at a minimum temperature of 10°C.

- Fumigation with phosphine at 1.41 g/m^3 minimum atmospheric concentration for more than 72 hours, at a minimum temperature of 10°C and a maximum temperature of 30°C.
- Heat treatment to a minimum continuous core temperature of 70°C for more than four hours.
- Chemical preservation as in Table 4.
- ISPM 15 treatment options e.g. heat treatment to a minimum continuous core temperature of 56°C for more than three hours or methyl bromide fumigation at 45 g/m^3 for more than 16 continuous hours, and at a minimum temperature of 10°C.

Table 4. Chemical preservation methods specified in New Zealand's import health standards for wood packaging, sawn wood and logs.

Chemical	Minimum Retention
Boron compounds *(insecticidal and limited fungicidal protection)*	0.1% Boric Acid equivalent minimum loading in the sapwood core
Copper azole *(insecticidal & fungicidal protection)*	0.27% mass/mass OR 1.35 kg/m^3 in softwood timbers, 2.7 kg/m^3 in hardwood timbers.
Copper Chrome Arsenic (CCA) *(insecticidal & fungicidal protection)*	0.27% mass/mass OR 3 kg/m^3 minimum preservative retention
Arsenic*(insecticidal protection only)*	0.04% minimum preservation loading in sapwood core
Permethrin *(insecticidal protection only)*	Minimum retention of not less than 0.06% mass/mass

Experimental evidence has shown that 40g/m^3 methyl bromide for 24 h is the minimum requirement to achieve 100% mortality of PWN in wood (Soma *et al.*, 2001). This brings into question the effectiveness of ISPM 15 fumigation level of methyl bromide (45 g/m^3 for more than 16 continuous hours, at a minimum temperature of 10°C) on *B. xylophilus*.

With no requirement for mandatory treatment, coniferous wood packaging material has the potential to serve as a pathway for the introduction of PWN and its insect vectors into New Zealand.

6.2. Assessment of wood chips

Wood chips, with a high moisture content and suitable temperature, can act as an excellent substrate for the reproduction and survival of PWN (Dwinell 1986; Kinn 1986; Halik and Bergdhal, 1992). Wood chips could transfer PWN to its host trees or insect vectors, as wood chips are frequently used as mulch material for growing plants. Halik and Bergdhal (1992) reported the transmission of PWN from woodchips to susceptible trees when chips are buried among wounded and unwounded tree roots.

Currently, all wood chips, sawdust, wood shavings and wood wool imported into New Zealand require mandatory treatment by either:

- fumigation with methyl bromide or sulphuryl fluoride at 80 g/m^3 for minimum of 24 continuous hours, and at a minimum temperature of 10°C or
- heat treatment for more than 4 h at a minimum continuous core temperature of 70°C.

Under these phytosanitary measures, the likelihood of PWN or its insect vectors entering New Zealand through this pathway is negligible.

6.3. Assessment of poles, piles, rounds and sleepers

Poles, piles, rounds, and sleepers (logs) include any wood pieces larger that 300 mm in minimum thickness (cross-section). *Monochamus* spp. preferentially oviposit in the bark of freshly cut trees and trees damaged by other biotic and abiotic factors (Fig. 2). The removal of bark from harvested trees reduces survival but, if the larvae enter the wood before the debarking process, they could develop to the adult stage. Logs from areas known to have PWN may be colonized by the PWN and *Monochamus* spp. Logs imported into New Zealand are required to be debarked, but for many of the host species none of the chemical/heat or fumigation treatments are mandatory. For logs from *Pinus* species and Douglas fir a high temperature (70^0C for 4 hours) heat treatment is required. Upon arrival, only 10% of untreated logs are visually inspected for pest and diseases. Visual inspection of logs is very unlikely to detect microscopic PWN however larvae of *Monochamus* spp. could be found as entry holes would be present. Logs therefore could act as a pathway for entry of PWN, but are much less likely to be an entry pathway for *Monochamus* spp.

6.4. Assessment of sawn wood

Sawn wood is defined here as wood sawn longitudinally, with or without its natural rounded surface, without bark and no larger than 300 mm in thickness. The probability of *Monochamus* spp. and PWN being present in sawn wood is similar to that in untreated logs. Although the processing method of sawing could reduce the *Monochamus* spp. survival, some larvae could survive the process and continue to live in the sawn wood.

The majority of the wood from conifer tree species imported into New Zealand originates from USA and Canada (Table 4), where *B. xylophilus* and *Monochamus* spp. are endemic. However, *Thuja plicata,* the main species of sawn wood (comprising 95% of imported soft wood) is not a host of either *B. xylophilus* or *Monochamus* spp. (Evans *et al.*, 1996).

All sawn timber arriving in New Zealand undergoes a 100% inspection of the exterior of each stack of sawn wood and a 10% piece by piece (board by board) inspection of each lot. PWN are microscopic and undetectable by visual inspection and currently inspection for signs of *Monochamus* boring (grub holes with distinct shape) is the only indication for the presence of PWN in the wood. Although PWN could be present in the wood with no grub holes, the likelihood of entry and establishment is high if entered with an insect vector

Table 5. Import volumes of sawn wood in 2003

Type of sawn wood	Volume (m^3)	Main country/area of origin
Western red cedar (*Thuja plicata*)	30,000	Canada, USA
Tropical Hardwoods	1000	Asia& Pacific
Eucalyptus	1500	Australia
Other assorted timbers	2500	Europe, Asia

(MAF Forestry Trade statistics 2004)

New Zealand's current import requirements for sawn timber of *Pinus* species originating from areas considered by the MAF not to be free of *Fusarium circinatum* or Pitch Canker disease require mandatory heat treatment to 70°C (core temperature) for 4 hours. Taking into account the current phytosanitary measures, the overall likelihood of entry of *Monochamus* spp. and PWN by this pathway is considered negligible.

6.5. Assessment of nursery stock

During maturation feeding of *Monochamus* spp., *B. xylophilus* can enter healthy conifer plants. Some trees remain asymptomatic for several years while *B. xylophilus* survive and reproduce in the tree (Halik & Bergdhal, 1994). Asymptomatic, infected nursery stock is a potentially high risk pathway for PWN to enter New Zealand. Currently, the importation of *Pinus* spp. and *Pseudotsuga menziesii* nursery stock into New Zealand is prohibited due to the risk of importation of pitch canker disease. However the risk remains with the importation of nursery stock of other non-prohibited host trees such as Spruce, Cedar, Larch and Fir from countries known to have PWN. Presently there is no specific inspection for PWN but all nursery stock is required to undergo a period in post entry quarantine prior to their release. Nursery stock of all tree species is required to be grown in soil-free media or treated with fenamiphos (a nematicide) prior to export to New Zealand. If the infested nursery stock has not shown wilt symptoms during the required period in the quarantine facility, and subsequently planted in close proximity to susceptible trees, there is a small risk of transfer of the nematode to new trees. The current trade volumes for Spruce, Cedar, Larch and Fir are very small and restricted to 1-2 consignments per year. Taking into account current phytosanitary measures on host materials the likelihood of entry via this pathway is considered negligible.

6.6. Assessment of seeds and cones

There is no documented evidence of *B. xylophilus* being carried in seeds and cones of conifers. Other nematodes of the family Aphelenchoididae however, can live in coniferous seeds (Evans *et al.,* 1996). Although there is no specific testing procedure for PWN, all imported cones and coniferous seeds coming into New Zealand are required to go through stringent quarantine procedures before biosecurity clearance is given.

- Pines cones imported for decorations must be heat-treated (70°C (core temperature) for 4 hours) or contain no seed and have been completely covered in lacquer or a thick paint or varnish layer.
- *Pinus* or *Pseudotsuga menziesii* seeds for sowing imported from countries not considered by MAF to be free of pitch canker such as the USA, European Union and countries of South East Asia are requiring going in to a stringent high security

quarantine facility on arrival in New Zealand. This includes all the countries known to have PWN except Canada.

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If the current phytosanitary measures remain the same the likelihood of entry of *B. xylophilus* by this pathway is considered negligible.

6.7. Entry of a Monochamus species as a hitch hiker

In June 2004, a post border interception of male and female *Monochamus alternatus* associated with cardboard packaging was reported. However, no dauer larvae of PWN were found in the beetles. This has demonstrated that *Monochamus* spp. could enter into New Zealand as a hitch hiker in non-forest products and potentially transmit PWN into susceptible host material.

7. LIKELIHOOD OF ESTABLISHMENT OF PWN

7.1. Epidemiology

Bursaphelenchus xylophilus cannot infect a tree that is not damaged or in some way stressed. As shown in Figure 2 infection can occur through bark scaring or through leaf and twig damage caused when the insect vector feeds on the phloem of young twigs of susceptible live trees (primary transmission) or when the female beetles lay eggs (oviposition) in freshly cut timber or dying trees (secondary transmission). In their natural habitat, nematodes are transferred to damaged trees along with a suitable wood degrading fungus by an insect vector, during oviposition. Under these conditions, *B. xylophilus* is mostly restricted to damaged and dying trees, where it performs a role as a saprophyte or saprophytic associate. North American *Pinus* trees in their natural range are rarely adversely affected. In the Japanese environment, *B. xylophilus* encountered a new species of beetle vector, *Monochamus alternatus*. This vector has a slightly different behaviour to that of the North American species. Between emergence as an adult and oviposition, *M. alternatus* visits healthy pines to feed on young leaves and twigs. Such feeding is necessary for maturation of the beetles. It is at this time that *B. xylophilus* is able to infect healthy trees and it becomes a pathogen rather than a saprophyte.

Once a tree has succumbed to PWD, it is then a suitable candidate for inoculation with wood decaying fungi and eggs of subsequent generations of *Monochamus* beetles. The beetles are then able to increase at a greater than normal rate, due to removal of the limiting factor of suitable hosts for its young, cyclically increasing the epidemic (Mamiya, 1984)**.**

7.2. Survival ability of PWN

The dispersal third stage juvenile PWN larvae are resistant to temperature extremes, desiccation and food shortage and can survive for considerable periods within the dead tree. The dispersal fourth stage dauer larvae are equipped with a sticky protective coating that appears to facilitate attachment to, migration on, and exit from the beetle vector. This larval stage can survive for periods of at least a week on the surface of pines before successfully entering through wounds.

Additionally *B. xylophilus* can remain viable in cut stumps and cut timber for 2 years. Non-susceptible trees can harbour the nematode for 6 years. In some cases, nematodes can remain viable in susceptible trees for 11 years without causing PWD symptoms (Bergdahl & Halik, 1999).

Specialised fourth stage dispersal dauer larvae can survive for the life of the vector beetle, which may be 4 months (Mamiya, 1984).

Survival in soil is less than 72 h (Mamiya & Shoji, 1989). However, if fragments of wood are present survival can be extended to 12 weeks (Halik & Bergdahl, 1992).

Bursaphelenchus xylophilus is endemic in the southern states of Canada, possibly up to 60^{o} latitude, but PWD is rarely expressed above 40^{o} latitude. Temperature seems to be more limiting to the pathogenicity of the disease than the survival of the nematode. The geographic range of vector beetles will also affect the survival of *B. xylophilus* because without a vector the nematode would not survive.

7.3. Likelihood of transfer of PWN to a suitable host

As shown in Fig 2, transmission of *B. xylophilus* to new host trees is primarily mediated by the vector *Monochamus* spp. during their maturation feeding or oviposition (Fig 2.). Generally, adult *Monochamus* spp. have a short dispersal range of a few hundred metres, but they can fly up to 3 km depending on the availability of trees on which they feed on and oviposit (Kobayashi *et al*., 1984). If *B. xylophilus* enters New Zealand with *Monochamus* spp. in wood packaging for instance, and is transferred to an area close to a forest or a saw mill, there is a high likelihood the nematode will be transferred to a host tree.

Absence of the *Monochamus* spp. and other likely insect vectors of PWN in New Zealand reduce the probability of direct transfer of *B. xylophilus* to the host trees. Experimental evidence has shown that *B. xylophilus* is capable of moving to host trees in the absence of specific insect vectors (Dwinell, 1986; Evans *et al.*, 1996). In untreated wood, such as wood packaging, sawn wood, poles and piles, *B. xylophilus* could survive for long periods after entry and move into non-specific vectors, roots of susceptible trees or into fresh cut stumps. Halik and Bergdahl (1992) indicated that the PWN can move from pieces of wood in the soil into nearby roots of pine seedlings, especially following root damage. It is also not possible to exclude the possibility of transfer of *B. xylophilus* to host trees by a non-specific, native insect vector.

7.4. Availability of host plants in New Zealand

Forests cover 8.0 million hectares, or 29 percent, of New Zealand's land area. Of this, 6.4 million hectares are indigenous and 1.6 million hectares are planted forests. *Pinus radiata*, which is moderate susceptible to PWD, occupies 89% (1.6 m ha) of this and Douglas fir (also a host of PWN) occupies about 6% (109,075 ha). The 5% remainder comprises other softwood and hardwood species. Other susceptible PWN conifer species, such as spruce, fir and larch are mainly grown in urban gardens and have limited distribution in New Zealand.

The major tree species in the indigenous forests are kauri (Araucariaceae), beech (Nothofagaceae), rimu (Podocarpaceae) and tawa (Cupressaceae). Native conifers consist of mainly Podocarpaceae, and Araucariaceae. There have been no reports of susceptibility of these two conifer families to *B. xylophilus*.

7.5 Likelihood of establishment of PWN in New Zealand

Successful establishment of PWN in Japan, China and other East Asian countries illustrates that the organism is able to establish outside its natural range. Establishment of *B. xylophilus* and its vector *Monochamus* spp. is dependent on to some degree on the climate, or more specifically the temperature, in the target area. Usually development and reproduction of *B. xylophilus* occurs between 15-30ºC. However development of nematodes has been reported between 35-40ºC in piles of wood chips (Dwinell 1986).

In Japan PWN has been recorded where average mean summer temperatures range between 10-12 ºC. In New Zealand, except for high altitude areas and some southern regions, all areas have average daily summer temperatures 19-20ºC, and are therefore suitable for the establishment of *B. xylophilus* provided that host trees are available (Knight, 2003).

As discussed in section 6, *B. xylophilus* is most likely to enter with vector insects through wood packaging, logs and sawn wood pathways. If *B. xylophilus* and insect vector-infested wood material were introduced to areas where susceptible trees were present and climatic conditions are favorable, PWN might be able to establish. In the presence of an already established suitable insect vector, the likelihood of establishment of *B. xylophilus* in New Zealand would be high.

If introduced without an insect vector, or with no insect vector already established, *B. xylophilus* could still establish itself in a suitable host by means of non-vector transfers. The likelihood of establishment however, would be low. In this case, the long-term survival of *B. xylophilus* depends on finding a native or established vector.

As mentioned in section 4.5, the most effective vectors of *B. xylophilus* are *Monochamus* spp. *Monochamus* spp. are not known to occur in New Zealand (Bain & Hosking, 1988; PPIN 2004). Observations on the behaviour of *Monochamus* spp. indicate that members of this genus are poor colonisers of new territories, possibly due to the need for regular and frequent mating for the production of viable eggs (Evans *et al*., 1996). This requires individuals of both sexes to be introduced at the same time and to remain in regular contact throughout the egg laying period. The likelihood of *Monochamus* spp. becoming established in New Zealand under current circumstances is low.

Several beetle species have the potential to provide a phoretic vehicle for *B. xylophilus* (Mamiya, 1984). According to Hosking (1989), New Zealand does not have any suitable native vector species. However possible transmission of *B. xylophilus* by native New Zealand insect species has not been studied extensively. Hence it is not known to what extent local native and exotic insects could act as a vector of PWN in New Zealand. The most possible candidates are the Cerambycids e.g. *Hexatricha puverulenta* and *Arhopalus ferus*, which are both abundant in New Zealand. The risk of PWN becoming established in New Zealand appears to be dependent therefore, upon the establishment of *Monochamus* spp. or other suitable insect vectors, either prior to, or in conjunction with, the introduction of *B. xylophilus*.

Since 1961, 38 *Monochamus* spp. interceptions were recorded on wood produce imported into New Zealand. It is apparent that no *Monochamus* spp. are known to have established in NZ over the last 50 years and insect vectors of *Bursaphlenchus* spp. are not known to occur in New Zealand. It is therefore considered that the likelihood of establishment of *B. xylophilus* and its insect vector, *Monochamus* spp., in New Zealand from imported forest produce under the current import measures is low.

7.6 Likelihood of manifestation of PWD in New Zealand

Manifestation of PWD from a PWN infestation is strongly reliant on average daily summer temperature (18-20ºC) and annual precipitation (< 600 mm) levels in the target area. In Japan and some parts of North America, disease develops more rapidly, and is more severe, at high temperatures (>20ºC) and under drought conditions. In the Island of Honshu, Japan PWD has been recorded in areas where the mean annual temperature is above 10-14ºC. In areas with temperatures within 10-14ºC, development of pine wilt was slow and very often damage was limited to few branches of a tree (Ikeda *et al*., 1990; Shimizu, 1987; Xu *et al*., 1996).

The pest risk analysis conducted for the European Union identified that pine forests with host species are vulnerable to PWD if the temperature is more than 20^{o}C for at least 8 weeks for *Pinus* spp. most intensively studied (Evans *et al.,* 1996). According to Braasch and Enzian (2004), the potential development of PWD would be extremely high when long term mean summer temperature is more than 25ºC and long term annual precipitation is less than 600 mm. The potential development of PWD would be low at temperatures less then 18 ºC and annual precipitation more than 600 mm.

In New Zealand the mean summer temperature across the country does not exceed 19 ºC. This includes areas with annual precipitation of less than 600 mm (Appendix 2). Only in the most northern areas of the North Island do mean February (the warmest month) temperatures exceed 20^{0}C (20.3ºC). However as this particular area has an annual precipitation higher than 600mm (Appendix 2) it is unlikely to show extensive pine wilt symptoms in the event of establishment of PWN.

While there may be areas in New Zealand where the disease may not be expressed, the presence of viable populations of *B. xylophilus* could still act as reservoirs for providing fresh infection sources to susceptible areas if a suitable insect vector is available.

8 LIKELIHOOD OF SPREAD OF PWN

By far the most common method of transmitting *Bursaphelenchus* spp. from one tree to another is by vectors, is this instance wood boring insects. *Monochamus* spp. (Pine sawyers) are the main vectors of PWN in East Asia, North America and Europe. For the nematode to spread, infected wood should generally be in a form that is attractive to vector beetles, such as logs or cut wood. Within an area of infection, spread is generally a result of the activities of a beetle vector. However, long distance spread can occur through the movement of infested wood and subsequent feeding on the infested wood by vectors. Infection to previously uninfected cut logs and wood stock piled in collection areas is known to occur by beetle vectors attracted to damaged wood (Wingfield, 1983). Peeling cut logs is considered to be an effective method of preventing post harvest infection by beetle vectors (Lan *et al*., 1999), due to the inability of *Monochamus* spp. to oviposit or feed on debarked logs (Wu *et al*., 2000). Typically, expanding distribution within countries is mediated by human activities, shipments of infected wood and, to a lesser extent, infected vectors.

Susceptible coniferous trees can be infected with *B. xylophilus* through a variety of other means;

- wounds in the roots and lower trunk when in contact with infested material e.g. chipped wood or garden mulch (*B. xylophilus* has been observed to move from infected wood waste mulch into the wounds of lower stems and roots of living trees; Braasch, 1996);
- between trees that have become root grafted (common in nature; Evans *et al*., 1996).

If *B. xylophilus* were to establish in New Zealand, it could be expected to spread rapidly throughout the country where host trees are existing but only if a suitable insect vector is available. Other non-vector means of spread would be isolated localised occurrences as insect vectors are essential for the dispersal of the nematode.

9 SUMMARY OF RISK ASSESSMENT

Bursaphelenchus xylophilus could enter New Zealand on infected wood packaging, sawn wood, logs and nursery stock imported from countries where the nematode is known to be present. Except for the wood packaging pathway, the likelihood of entry of *B. xylophilus* with its insect vector into New Zealand by other pathways is considered negligible

It is evident from the biology of the PWN that long term establishment and further spread of *B. xylophilus* in New Zealand requires a suitable insect vector. None of the reported insect vectors of the Cerambycidae genera have been recorded as established in New Zealand. As the *Monochamus* spp. are poor colonisers of new territories, the likelihood of *Monochamus* spp. becoming established in New Zealand is considered low with the current phytosanitary measures in place for imported host material. If established by other non-vector methods however, *B. xylophilus* would only cause isolated occurrences of PWD.

Under current phytosanitary measure for imported forest produce, and in the absence of an established insect vector, the likelihood of the establishment of B. xylophilus and its insect vectors, Monochamus spp. from imported forest produce, is low.

Given the likelihood of the insect vectors of PWN establishing in New Zealand would increase over time and with increasing trade, it will be necessary to maintain at least the current or equivalent level of protection afforded by the current phytosanitary measures to ensure the likelihood of establishment remains low.

Bark beetles already present in New Zealand could possibly act as primary vectors of *Bursaphelenchus* spp. The capability of these native insects to become primary vectors however, is still unknown and further research would be required to clarify the risk of transmission of *Bursaphelenchus* spp. by bark beetles in New Zealand.

Fumigation with methyl bromide or sulphuryl fluoride at 80 g/m3 for more than 24 continuous hours at a minimum temperature of 10^{o}C, Fumigation with phosphine at 1.41 g/m3 minimum atmospheric concentration for more than 72 hours, at a minimum temperature of 10^{o}C and a maximum temperature of 30^{o}C, Heat treatment to a mimimum continuous core temperature of 70^{o}C for more than four hours, Chemical preservation as in Table 4 or ISPM 15 treatment option heat treatment to a minimum continuous core temperature of 56^{o}C for more than three hours.

Table 6. Summary of likelihood of entry, establishment and spread of *B. xylophilus* in New Zealand by different pathways under current import regulations[1]

Pathway	Volume	Likelihood of entry on pathway	Likelihood of establishment	Likelihood of spread
Nursery stock	2 per year (~ 50 plants)	Low – currently importation of host material is limited	Negligible	Negligible
Wooden packaging material	200,000 m^3/year	High- if sourced from regions where *B. xylophilus* occurs.	Low[2]	Negligible
Sawn wood (Other conifers except *Thuja plicata*)	272 m^3/year	Low - if sourced from regions where *B. xylophilus* occurs	Negligible	Negligible
Logs (poles, piles, rounds and sleepers*)*	25 m^3/year	Low - if sourced from regions where *B. xylophilus* occurs	Negligible	Negligible
Wood chips	10 tonnes/year	Negligible - all wood chips require mandatory treatment	Negligible	Negligible
Seeds and cones of conifers	20 kg/year	Low	Negligible	Negligible

Note 1: This assumes there are no insect vectors established in New Zealand.
Note 2: This is the most likely entry pathway for an insect vector of PWN.

10. RISK MANAGEMENT

It is evident that the establishment of PWN in New Zealand is mainly dependent on the availability of a suitable insect vector. Therefore the primary focus of any further measures for possible pathways of entry is to mitigate the risk of entry of insect vectors of PWN in to New Zealand to an appropriate level.

10.1 Recommendations for reducing the risks of introducing PWN and Monochamus spp.

This Pest Risk Analysis recommends considering the following measures to reduce the risks of introducing *B. xylophilus* and *Monochamus* spp.

- Review the import health standard for wood packaging material to impose mandatory treatment for all coniferous wood packaging material before entering New Zealand. Recommended treatments should exceed either fumigation with methyl bromide at 48 g/m^3 for more than 24 continuous hours and at a minimum temperature of 10°C, or heat treatment to a minimum continuous core temperature of 56°C for more than 30 minutes. Currently these treatments are expected to become mandatory for all imported solid wood packaging material entering New Zealand during 2005.
- Ensure the New Zealand border clearance systems for general imported cargo are sufficient to detect any significant increase in the entry of *Monochamus* spp and other insect vectors as hitch-hiker pests.
- Test all species and life-stages of *Monochamus* intercepted at the New Zealand border or post-border for the presence of *Bursaphelenchus* nematodes. The interception data would add significantly to our understanding of the risk these potential vectors pose to New Zealand and could be used to improve the effectiveness of risk management strategies.

- Undertake post-border surveillance for PWD and *Monochamus* spp. in New Zealand to ensure early detection for eradication.
- Include in general surveillance testing for the presence of *Bursaphelenchus* spp in dead or dying coniferous trees with wilt symptoms in high risk areas.

Beetles of *Monochamus* spp. could fly up to 3 km to find a host tree for the maturation feeding or oviposition, therefore surveillance for *Monochamus* spp. in high risk areas such as ports, sites processing containers, timber yards, and parks and nurseries, is considered essential for the early identification of the presence of pathogenic *Bursaphelenchus* spp. in New Zealand. The surveillance for *Monochamus* spp. could be included in a wood boring bark beetle surveillance system for high risk sites. Early detection of *Monochamus* spp. is indispensable in preventing the establishment of PWN in New Zealand. The pest risk analysis recognises that wood boring bark beetle surveillance system for high risk sites is to be reinstated.

10.2 Suggestions for further research

This pest risk analysis identified several gaps in the available scientific information with regard to establishment of PWN in New Zealand. The following research topics are recommended for consideration for funding:

- Determine the level of susceptibility of *P. radiata* and native conifer species to PWN.
- Determine the likely vector status of candidate insects established in or native to New Zealand.

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Appendices

Appendix 1. Recorded Monochamus spp. interceptions from various countries since 1961

Summary of *Monochamus* spp. interceptions since 1961						
***Monochamus* species**	**Life stage**	**Alive(A) / Dead (D)**	**Country**	**Host Genus**	**Commodity Type**	**Date**
Monochamus spp.	L	A	Canada	Unspecified	Dunnage	25-Oct-61
Monochamus spp.	P	A	China	*Pinus*	Casewood	15-May-87
Monochamus spp.	L	D	China	Softwood	Casewood	22-May-90
Monochamus spp.	L	A	China	Softwood	Casewood	10-Jan-91
Monochamus spp.	L	A	China	*Picea*	Casewood	12-Oct-93
Monochamus spp.	L	A	China	Hard wood	Casewood	29-Mar-94
Monochamus sutor	L	A	Europe	Softwood	Dunnage	19-Feb-94
Monochamus spp.	L	A	Hong Kong	Softwood	Casewood	28-Jun-93
Monochamus spp.	L	A	Hong Kong	*Cryptomeria japonica*	Casewood	12-Nov-93
Monochamus sutor	L	A	Italy	*Pinus*	Casewood	20-Mar-73
Monochamus spp.	L	A	Italy	*Pinus*	Dunnage	17-Nov-94
Monochamus sutor	A	A	Japan	*Pinus*	Casewood	23-Mar-88
Monochamus titillator	A	A	North America	*Pinus*	Casewood	20-Mar-70
Monochamus titillator	L	A	North America	*Picea*	Casewood	21-Feb-78
Monochamus titillator	L	A	North America	*Pinus*	Casewood	1-Mar-79
Monochamus sutor	A	A	Russia	*Pinus*	Casewood	30-Nov-89
Monochamus sutor	A	A	Russia	Softwood	Dunnage	18-Jan-91
Monochamus spp.	L	A	Russia	*Pinus*	Dunnage	26-Jan-94
Monochamus spp.	L	A	Russia	*Pinus*	Dunnage	2-May-94
Monochamus sutor	L	A	Spain	*Pinus*	Dunnage	17-Nov-94
Monochamus sutor	A	D	UK	*Pinus*	Pallet	1-Jul-87
Monochamus sutor	L	A	UK	*Pinus*	Pallet	1-Jul-87
Monochamus spp.	L	A	UK	Softwood	Dunnage	22-Feb-90
Monochamus spp.	L	D	Unknown	Softwood	Casewood	14-Feb-92
Monochamus titillator	LP	A	USA	*Pinus*	Dunnage	18-Apr-74
Monochamus titillator	A	D	USA	*Pinus*	Casewood	16-Jan-75
Monochamus titillator	A	D	USA	Softwood	Pallet	7-Jun-78
Monochamus titillator	A	A	USA	*Pinus*	Dunnage	23-Jul-79
Monochamus titillator	L	A	USA	*Pinus*	Dunnage	5-Sep-83
Monochamus spp.	L	A	USA	*Pinus*	Casewood	8-Mar-88
Monochamus spp.	L	A	USA	*Pinus*	Casewood	4-Oct-95
Monochamus sutor	L	A	USSR	*Pinus*	Bearer	5-Mar-69
Monochamus sutor	A	A	USSR	Unspecified	Z	7-Apr-81
Monochamus sutor	L	A	USSR	*Picea*	Dunnage	16-Oct-87
Monochamus sutor	L	A	USSR	*Pinus*	Dunnage	16-Oct-87
Monochamus sutor	L	A	USSR	*Pinus*	Dunnage	16-Oct-87
Monochamus spp	L (male & female)	A	S Korea		Cardboard Packaging	11-June-04

(MAF QuanCargo 2004 and MAF Bugs data base 1999)

Appendix 2. Map of long term means of February temperature and means of annual rainfall in New Zealand