

Pest Risk Analysis (PRA) for the territories of the European Union (as PRA area) on *Bursaphelenchus xylophilus* and its vectors in the genus *Monochamus*

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An analysis of the risk to the countries of the European Union from a possible introduction of *Bursaphelenchus xylophilus* and its vectors in the genus *Monochamus* is performed by assembling relevant biological, climatic and commercial information. The risks presented by different trade pathways are assessed and phytosanitary measures to reduce the risks are proposed. The conclusion of the PRA, based on EPPO PRA guideline no. 1, is that these pests present a serious risk to European coniferous forests.

Introduction

A Pest Risk Analysis (PRA) for the territories of the European Union (as PRA area) was carried out on *Bursaphelenchus xylophilus* and its vectors in the genus *Monochamus*, on the basis of information compiled in the format of EPPO PRA Guideline no. 1 'Check-list of information required for PRA' (OEPP/EPPO, 1993) (Part A). A detailed analysis was made of the risks presented by different trade pathways (Part B) and of the phytosanitary measures to reduce these risks (Part C). The conclusion of the PRA is then presented in Part D.

PART A. INFORMATION FOR PRA

Section 1. The organism

1.1 Name and taxonomic position

Bursaphelenchus xylophilus

Name: *Bursaphelenchus xylophilus* (Steiner & Buhner) Nickle

Synonyms: *Aphelenchoides xylophilus* Steiner & Buhner

Bursaphelenchus lignicolus Mamiya & Kiyohara

Taxonomic position: Nematoda: Aphelenchoididae

Common names: pine wood nematode, pine wilt disease (English);
nématode du bois de pin, nématode des pins, dépérissement des pins (French);
Kiefernholznematode, Kiefernwelke (German).

Notes on taxonomy and nomenclature: *B. xylophilus* was first described in the USA as *Aphelenchoides xylophilus* (Steiner & Buhrer, 1934). Later it was described again, as *Bursaphelenchus lignicolus*, when recognized to be the causal agent of pine wilt disease in Japan (Mamiya & Kiyohara, 1972). The synonymy was recognized in 1981 (Nickle *et al.*, 1981). A very similar but non-pathogenic species, *B. mucronatus*, was described by Mamiya & Enda (1979), differing morphologically only in minor respects from *B. xylophilus* and most obviously by the presence in the female of a caudal mucro (finger-like projection) in the former species which is absent in the latter. However, populations of *B. xylophilus* were subsequently discovered in the USA which also carried a mucro on the tail (Wingfield *et al.*, 1983). There has thus been much discussion about the taxonomic relationships between these two species, and also with *B. fraudulentus* Rühm, a nematode first described in Germany and also known to occur in Austria and Hungary (Schauer-Blume, 1987; Tomiczek, 1988; Schauer-Blume & Sturhan, 1989). Biochemical studies of several populations of these nematodes have clearly confirmed the distinctness of the three species (Webster *et al.*, 1990; Abad *et al.*, 1991; Tares *et al.*, 1992; Harmey & Harmey, 1993).

B. xylophilus is a native of North America, whereas the other two are Palaearctic species, one colonizing coniferous trees, the other deciduous. *B. xylophilus* found in Japan and other Asian countries is known to be an introduction from North America. Another apparently closely related species, *Bursaphelenchus kolymensis* Korenchenko, has been described from *Larix* from the far east of Russia (Korenchenko, 1980); this nematode has not been studied extensively but is possibly synonymous with *B. mucronatus* (Magnusson & Kulinich, unpubl.). *B. xylophilus* has been shown to be a heterogeneous species in its biology, pathogenicity, morphology and genetics (Bolla *et al.*, 1986; Kiyohara & Bolla, 1990; Tares *et al.*, 1992), whereas *B. mucronatus* is not so; the latter species does, however, appear to separate into an east Asian 'race' and another race throughout the rest of the palaeartic region (Riga *et al.*, 1992). *B. mucronatus* is present in Canada, in Quebec province (Harmey & Harmey, 1993).

Monochamus spp.

Name: *Monochamus* spp.

Taxonomic position: Insecta: Coleoptera: Cerambycidae

Common names: sawyers, pine sawyers, longhorn beetles

Notes on taxonomy and nomenclature: species of *Monochamus* from conifer trees are the only important known vectors of *B. xylophilus* and, of these, *M. alternatus* is the major vector in Japan, whereas *M. carolinensis* is the major vector in North America. Other less efficient *Monochamus* vectors have been recorded in Japan and North America (Table 1) (Mamiya, 1972; Luzzi & Tarjan, 1982; Luzzi *et al.*, 1984; Kobayashi *et al.*, 1984; Linit, 1988, 1989).

Other genera of the Cerambycidae (e.g. *Acalolepta*, *Acanthocinus*, *Amniscus*, *Arhopalus*, *Aseum*, *Corymbia*, *Neacanthocinus*, *Rhagium*, *Spondylis*, *Uraecha*, *Xylotrechus*) and other Coleoptera (e.g. *Chrysobothris*, *Hylobius*, *Pissodes*) have been found to carry nematodes in or on their bodies but there is no evidence that they have any role as vectors in nature (Mamiya & Enda, 1972; Mamiya, 1976a; Linit *et al.*, 1983).

1.2 Relationship with known quarantine pests

Bursaphelenchus xylophilus

B. xylophilus is related to *Rhadinaphelenchus* (= *Bursaphelenchus*) *cocophilus* (Cobb) J.B. Goodey, the red ring nematode, which is a quarantine pest for the Asia and Pacific Plant Protection Commission (APPPC), the Caribbean Plant Protection Commission (CPPC), the Inter-African Phytosanitary Council (IAPSC) and the South Pacific Commission (SPC). *R.*

cocophilus is a serious pest of coconut and other palm species throughout Central America, the Caribbean and parts of South America. Like *B. xylophilus*, it has a beetle vector, in this case the palm weevil *Rhynchophorus palmarum*, which carries it to new infection sites; it can also infect palm trees via the damaged or senescent roots if infected wood is placed in the soil (Griffith & Koshy, 1990).

Monochamus spp.

There is no close relationship of *Monochamus* spp. with other quarantine pests.

1.3 Methods of identification for inspection purposes

Bursaphelenchus xylophilus

When examined with a high-power microscope, *B. xylophilus* shows the general characters of *Bursaphelenchus* spp.: lips high and offset; stylet with weakly developed basal knobs; median bulb well developed; dorsal oesophageal gland opening inside median bulb. In the female, the post-uterine sac is long. In the male, the tail has short, terminal caudal alae and the spicules are well developed, with a prominent rostrum (Mamiya & Kiyohara, 1972; Nickle *et al.*, 1981).

B. xylophilus can be distinguished from other *Bursaphelenchus* spp. by the presence of the following three characters: in the male the spicules are flattened into a disc at their distal extremity; in the female, the anterior vulval lip is a distinct overlapping flap; the tail tip is rounded in nearly all individuals. This last character can be used to separate most populations of *B. xylophilus* from *B. mucronatus*, in which the female has a mucronate tail tip; however, as mentioned in section 1.1, some populations of *B. xylophilus* also display this feature (Wingfield *et al.*, 1983) but *B. mucronatus* females never have round tails. The morphological similarity of these two species makes correct identification by microscopic examination very difficult. However, the origin of the population (i.e. North America, Europe, Asia) can, to a certain extent, provide a useful clue to the identity.

DNA fingerprinting and PCR techniques can be used to distinguish *B. xylophilus* from related *Bursaphelenchus* spp. and also between strains of *B. xylophilus* itself (Harmey & Harmey, 1993). Abad *et al.* (1991) provided a microsatellite dot-blot technique which identifies *B. xylophilus* specifically. However, the techniques are available only to specialist laboratories and have not yet been developed sufficiently to be a reliable tool for routine diagnosis.

Monochamus spp.

Larvae display the same facial features as other cerambycids. The body is elongate, soft, with ten abdominal segments, including the anal mamilla. In *Monochamus*, the length of the head is distinctly greater than the width. All the abdominal tergites are simple, without spines nor sclerified plates. The larva is apodous.

Adult *Monochamus* are 15–30 mm long. Characteristics of the genus are: forehead transversal or sub-square; antennal protrusions very high, close together and separated by a deep hollow; antennae slender, very long for the male, distinctly longer than body for the female, the third segment being at least twice the length of the scape; pronotum slightly convex, with a broad collar margin and a broad basilar margin marked by a groove, each side with a large lateral submedian conical protuberance; anterior coxal cavities opening rearwards; prosternal protuberances rounded, lower than coxae; mesosternal process narrow; elytra wider than pronotum at the base, shoulders protuberant, apices obsoletely truncated; legs thin and elongate, especially in males where the anterior tibia is arched and tarsi bordered by hairs.

Table 1. *Monochamus* species from coniferous trees, known to be vectors of *Bursaphelenchus xylophilus* or considered to be potential vectorsEspèces de *Monochamus* inféodées aux conifères, reconnues comme vecteurs de *B. xylophilus* ou considérées comme vecteurs potentiels

<i>Monochamus</i> species	Geographical distribution	Main hosts	Vector status
North America			
<i>M. carolinensis</i> Olivier	US (eastern half), Canada (east & US border), Mexico (north central)	<i>Pinus</i>	+
<i>M. clamator</i> LeConte	US (west coast), Canada (BC)	<i>Pinus contorta</i>	–
<i>M. marmorator</i> Kirby	US, Canada	<i>Abies, Picea</i>	+
<i>M. mutator</i> LeConte (syn. <i>M. maculosus</i> Haldeman)	US, Canada	<i>Pinus</i>	+
<i>M. notatus</i> (Drury)	US, Canada	<i>Pinus strobus</i>	–
<i>M. obtusus</i> Casey	US (west coast), Canada (BC)	<i>Pinus, Abies, Pseudotsuga</i>	+
<i>M. rubigeneus</i> Bates	US (south), Mexico, Guatemala, Honduras	<i>Pinus</i>	–
<i>M. scutellatus</i> Say ssp. <i>scutellatus</i>	Eastern North America (including parts of Mexico)	<i>Pinus, Picea, Abies, Larix</i>	+
<i>M. scutellatus</i> ssp. <i>oregonensis</i> LeConte	US (west coast), Canada (BC)	<i>Picea</i>	–
<i>M. titillator</i> (Fabricius)	US (centre, east & south-east), Canada (Ontario)	<i>Pinus, Abies, Picea</i>	+
Palaeartic region (overlapping <i>B. xylophilus</i>)			
<i>M. alternatus</i> Hope	Japan, Korea Republic, Taiwan, Hong Kong, Lao, China (Anhui, Guangdong, Hunan, Jiangsu, Shandong, Zhejiang, i.e. east & centre)	<i>Pinus, Cedrus, Abies, Picea, Larix</i>	+
<i>M. grandis</i> Waterhouse	Japan	<i>Abies, Picea</i>	–
<i>M. nitens</i> Bates	Japan	<i>Pinus</i>	+
<i>M. saltuarius</i> Eschscholz	Japan, China (Heilongjiang; NE), Siberia, Lithuania, central and eastern Alps, middle and east Europe and south to Italy	<i>Picea</i>	+
<i>M. subfasciatus</i> Bates	Japan	<i>Ginkgo biloba</i>	–
<i>M. tesserula</i> White	Japan, China	<i>Pinus</i>	–
<i>M. urussovii</i> (Fischer) (syn. <i>M. rosenmuelleri</i> Cederhielm)	Japan, China (Liaoning, Heilongjiang, Inner Mongolia, i.e. NE), Siberia, Russia (Caucasus), Finland, Poland	<i>Abies, Larix, Picea, Pinus</i>	–
Palaeartic region (not overlapping <i>B. xylophilus</i>)			
<i>M. galloprovincialis</i> (Olivier)	Portugal, N. Africa, Italy, France, Greece, Germany, Poland, Sweden, Finland, Russia (European), Siberia	<i>Pinus</i>	–
<i>M. sartor</i> Fabricius	mid-Europe (east France to west Ukraine)	<i>Picea, Pinus</i>	–

Table 1. Continued

<i>Monochamus</i> species	Geographical distribution	Main hosts	Vector status
<i>M. sutor</i> (Linnaeus)	China (Heilongjiang, Liaoning; NE) Siberia, Russia (European), Georgia, the Nordic countries, middle and east Europe; the Pyrenees, Alps	<i>Pinus</i> , <i>Picea</i> , <i>Larix</i>	–

Note: *B. xylophilus* has once been detected in Mexico (Dwinell, 1993); it was presumed to have been transmitted by *Monochamus*, but the species was not identified. Two species of *Monochamus* are reported to occur in Mexico, *M. carolinensis* and *M. rubigenus* (Dillon & Dillon, 1941; Chemsak & Linsley, 1995). Evans *et al.* (1993) mentioned the presence of *M. clamator* and *M. notatus* in Mexico but no confirmation in a published report has been found.

1.4 Methods for detection

Bursaphelenchus xylophilus

There is no practical method for detection of *B. xylophilus* during visual inspection of suspect wood. However, the presence of fungal growth (especially blue stain) and grub holes caused by larval boring of *Monochamus* spp. indicate that further investigation may be warranted.

Wood samples should, if possible, be taken from around insect grub holes. Nematodes can be extracted from infested wood using a modified Baermann funnel technique involving immersion of small pieces of wood or wood shavings in water (Wingfield *et al.*, 1982). This technique relies on the fact that living nematodes will migrate from wood into the surrounding water, settle and can be decanted from the bottom of the funnel. It should be noted that failure to recover nematodes by this method does not guarantee freedom from infestation, since the distribution of nematodes in wood depends on the degree of colonization and can be very patchy (Yik & Birchfield, 1981; Warren & Linit, 1992).

Specialist expertise is needed to distinguish *B. xylophilus* from other nematodes usually found within wood, and especially to separate the different species of *Bursaphelenchus* (see also section 1.3).

Monochamus spp.

Presence of flat-headed larval stages under bark and/or presence of oval grub holes can be reliable indicators of the presence of cerambycid beetles. However, the oval holes into the wood are often difficult to detect because they are blocked with wads of wood particles. On round wood, even debarked, small pieces of bark may remain to conceal larvae and their bore holes. Expert taxonomic help is needed to distinguish to genus or species level. Presence of round emergence holes and possibly adult beetles may provide further confirmation of *Monochamus* spp.

Section 2. Biological characteristics of the pest and its vectors

2.1 Life cycle

There is a complex relationship between *B. xylophilus* and its *Monochamus* vectors. This is illustrated in Fig. 1 (adapted from Wingfield, 1987).

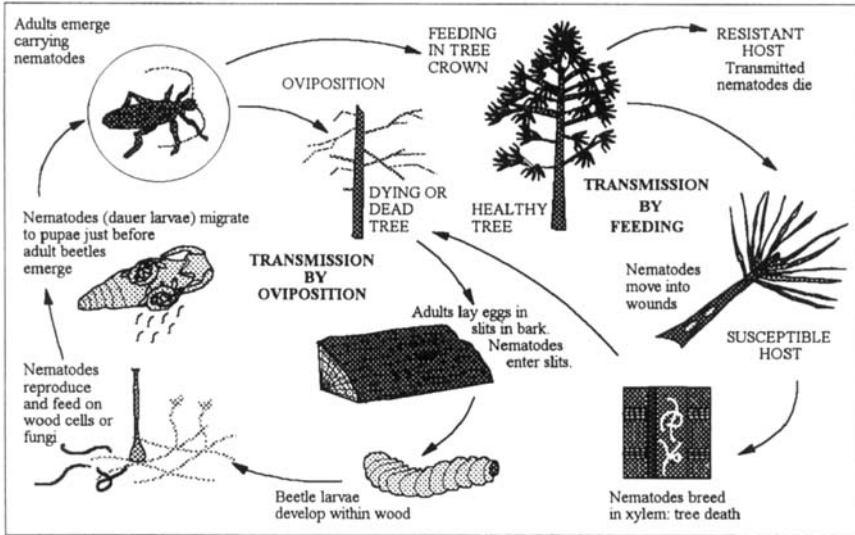


Fig. 1. The relationships between the life cycles of *Bursaphelenchus xylophilus* and its vectors in the genus *Monochamus*.

Relation entre le cycle biologique de *B. xylophilus* et celui de ses vecteurs du genre *Monochamus*.

Bursaphelenchus xylophilus

B. xylophilus has two different modes in its life cycle, a propagative mode and a dispersal mode (Wingfield, 1987). After the juvenile fourth stages (dauer larvae) of the nematodes have been carried from infested wood by a vector, they enter a new tree during the process of egg laying and/or while the adult *Monochamus* spp. feed on young shoots (maturation feeding). Immediately after entering the tree, the juvenile nematodes moult to adults, mate and begin to lay eggs. During this propagative stage of rapid multiplication, the nematode population is composed of males, females and four juvenile stages. The rate of multiplication and the distribution of the nematodes within the tree depend mainly on the susceptibility of the tree species, its condition and, particularly, on temperature (Rutherford & Webster, 1987; Rutherford *et al.*, 1990).

At a certain time after initial invasion, the nematode population ceases to multiply and begins to decline. This may be connected with reduced availability of food, for example when fungi have fully exploited the wood, or may be due to reduced temperatures. When these conditions are present, the population commences the dispersal mode of its life cycle. A different type of third-stage juvenile is produced, termed the dispersal third-stage juvenile (Mamiya, 1983a), although it is more correctly described as a survival stage, being capable of resisting adverse conditions. These juveniles gather in the wood surrounding the pupal chambers of the *Monochamus* vectors, possibly under the influence of substances diffusing from the developing pupae. Close to the time of emergence of the beetle, the nematodes moult into the special dispersal fourth-stage juveniles (dauer larvae), which are closely associated with the presence of the late pupal stage of the vector (Mamiya, 1983b; Linit, 1988), although laboratory experiments have shown that a small proportion of the population may moult to this stage under widely fluctuating temperature conditions (Tomminen *et al.*, 1991). Transfer of the dauer larvae to the vector insects within the pupal chamber is facilitated by the presence of fungi forming long-necked perithecia on which the nematodes gather. When the young adult beetle emerges, it brushes against the perithecial necks, picking up the nematodes which then

settle below the elytra and, in particular, within the tracheae. The immature adult beetle then flies from the wood, carrying nematodes.

Clearly, the propagative mode allows the nematode to reproduce quickly when conditions permit, whereas the dispersal mode facilitates transmission by the vector but also allows long periods of quiescence when unsuitable conditions prevail.

Monochamus spp.

The relationships shown in Fig. 1 differentiate clearly between the feeding and oviposition activities of adult *Monochamus* spp. This is an important distinction since it is possible for nematodes to be transmitted by both routes, the probability of successful nematode breeding depending on other factors such as temperature, tree susceptibility, general tree health, etc. It is therefore convenient to consider the life cycle of the vector by starting with the adult stage.

Adult *Monochamus* spp. emerge from the wood in which they have developed during the spring or early summer, depending on geographical location and on temperature. This period is generally from mid-May (Gardiner, 1970) to mid-June (Li *et al.*, 1986) but can be even later in some parts of their range in Japan (Kanehori *et al.*, 1976). Webb (1909) recorded adult emergence and oviposition of *M. titillator* in early March in Mississippi (US), reflecting the very high average temperatures in this region. Adults fly to the crowns of trees to feed on current or one-year old twigs where they feed on the bark, sometimes causing considerable damage. This feeding activity is carried out by both sexes and helps to sustain the adult during mating and, for the female, oviposition. Females of *M. titillator* feed for about 3 weeks before they commence oviposition (Alya & Hain, 1985). Adults live for considerable periods in the field, for example activity periods, including oviposition, of up to 83 days were recorded for *M. carolinensis* in the USA (Walsh & Linit, 1984). The span of the flight period for populations of *Monochamus* spp. is wide with most reports indicating that activity declines in October. However, Fatzinger (1985) reported that *M. carolinensis* and *M. titillator* adults were caught in traps in all but the coldest months of the year and that there were up to three distinct activity phases.

Mating usually takes place on the trunk of the tree, particularly while the female is chewing an oviposition pit in the bark. At this stage there may be considerable competition for females and, thus, males will fight actively to defend females (Hughes, 1979; Hughes & Hughes, 1987; Edwards & Linit, 1991). Mating takes place frequently during the entire life of the female and is necessary to ensure that all eggs are fertilized. Experiments in which previously inseminated females mated with radiation-sterilized males indicated that infertile eggs were laid, confirming the need for mating immediately prior to egg laying (Hughes & Hughes, 1987).

The critical factor in determining the success or otherwise of *Monochamus* spp. is the ability of adult females to lay eggs under the bark of suitable host-tree species. The period during which bark is suitable for oviposition and successful egg development is relatively short. Felled trees become attractive to ovipositing beetles within 2 h (Wilson, 1961) and remain suitable for between 30 and 80 days depending on temperature, degree of exposure of the surface of the tree and the relative humidity of the immediate environment. However, early work in Canada indicated that several *Monochamus* spp. successfully attacked logs that had been left in the forest from September of the previous year, indicating that bark retains its attractiveness over winter (Morley, 1939). In Europe, studies by Tragardh (1939) on trees that had been damaged in a wind storm on 1 May were not attacked by *M. sutor* when inspected on 8 July, a period of 69 days. A second sample taken on 27 August indicated high levels of successful attack by this beetle, confirming that the bark had remained suitable for at least 70 days and probably considerably longer. In this case some trees with green crowns that had been partially damaged in the storm were attacked even though they were still alive and would normally be expected to resist attack.

The female excavates a conical scar in the bark with the mandibles and deposits an egg by means of the ovipositor. There is usually one egg per scar although many scars are constructed that do not contain eggs. Females live for up to 83 days and continue egg laying until death, laying 40–215 eggs. Walsh & Linit (1985) investigated *M. carolinensis* oviposition biology and showed that the mean lifespan of the adult females was around 62 days, during which they laid a mean of 200 eggs, averaging 0.87 eggs per oviposition scar. Eggs hatch within 4 to 12 days depending on temperature.

The first-stage larva begins feeding on phloem and cambium in the sub-cortical zone. By the third instar, the larva begins to bore into the sapwood forming a gallery composed of an S-shaped horizontal portion perpendicular to the axis of the tree and a vertical portion parallel to the axis. Later larval stages complete the gallery by forming a wide pupal chamber in the vertical portion. *M. alternatus* has four larval instars, whereas 3–8 have been recorded for *M. carolinensis*. Although tunnelling in the wood is a characteristic feature of *Monochamus* spp., the larvae derive little or no nutrition from the wood itself and still need to feed on the cambium to complete development (Webb, 1909). Webb (1909) recommended that *Monochamus* spp. could be managed by debarking logs to kill the larvae and that the procedure was effective up to a week after the larvae first begin to excavate the wood.

The final larval stage plugs the opening of the tunnel with wood borings and begins pupation. The pupal stage lasts for up to 19 days and afterwards the fresh adult begins to chew through the xylem to emerge; between eclosion and emergence there may be an interval of 6–8 days. Any of the life stages (except the pupae) may hibernate and in *M. alternatus* it is usually the last larval stage. Where hibernation does not take place, the complete development from oviposition to adult emergence takes 8–12 weeks.

Life cycle periods are temperature-dependent, ranging from 1 to 2 or more years. *M. alternatus* usually has one generation per year but may require 2 years for development, especially in the cooler areas of northern Japan. In Missouri (US), *M. carolinensis* develops through two partially asynchronous generations per year. For further information on biology see Kobayashi *et al.* (1984) for *M. alternatus*, and Kondo *et al.* (1982) and Linit (1988) for *M. carolinensis*.

2.2 Dissemination and dispersal

Bursaphelenchus xylophilus

Plant-parasitic nematodes are generally restricted to aquatic environments but *B. xylophilus* is capable of moving outside water or water films and is relatively a highly mobile nematode. It is this ability that allows it to move from wood into its vector and from one piece of wood to another; this characteristic is used as a means of collecting *B. xylophilus* from fungal cultures by allowing the nematodes to climb small sticks of wood placed upright in the agar (McGawley *et al.*, 1985). There is experimental evidence (Kiyohara & Tokushige, 1971; Halik & Bergdahl, 1987, 1992) to indicate that *B. xylophilus* can move from pieces of wood in the soil into nearby roots of pine seedlings, particularly if the roots have been damaged. Kiyohara & Togushige (1971) reported that five trees were killed by nematodes from wood disks placed in the soil invading through roots without wounds. The related quarantine pest, *Rhadinaphelenchus cocophilus*, with a similar dependence on a phoretic insect host for dissemination, also demonstrates ability to invade roots from wood particles in the soil (Griffith & Koshy, 1990). Braasch (unpubl. data) found that *B. xylophilus* could transfer from surrounding wood chips into fresh-cut stumps of 3-year-old *Pinus sylvestris*. These means of infestation have not so far been demonstrated in nature.

Transmission by vectors of the genus *Monochamus* is the primary means of movement of *B. xylophilus* between trees. The nematode species is, however, recognized to have two types of transmission to new hosts, either during oviposition of the vector beetles (termed the

'mycophagous' phase by Wingfield (1987)) or during maturation feeding by the vector (the 'phytophagous' phase of Wingfield (1987)). These are illustrated in Fig. 1. Transmission by oviposition is initiated by attraction of *Monochamus* spp. to dying or recently dead trees. This may result in egg laying, during which the nematode is transmitted. Thereafter, the nematodes and beetle larvae independently exploit the food resources of the dead tree for their development, and come into contact again when the nematodes invade the body of the newly formed adult beetle just before it leaves the wood. During the mycophagous phase, the nematodes apparently feed primarily on the hyphae of fungi present in the trees.

Transmission during maturation feeding of the adult *Monochamus* spp. occurs on young shoots, the nematodes leaving the beetle and entering the feeding wounds. This form of transmission is only successful in leading to completion of the life cycle and re-association with the vector if the tree is killed by the nematodes or dies from other causes while the nematodes are still present. Death of the tree from activities of the nematode results from a combination of susceptible tree species and stress conditions (generally high temperatures; see section 8.1). This occurs in Japan and China, and in the case of exotic pines in certain parts of the USA (Wingfield, 1987). When these conditions are absent, the transmitted nematodes are not able to multiply well and are unable to distribute from the maturation feeding site to the rest of the tree. In such cases, minor damage may be caused near the feeding site. Thus, in its native range, *B. xylophilus* relies on the oviposition (mycophagous) cycle to reproduce and disperse. This is the case in North America, presumably reflecting the presence of co-evolved native coniferous species that may have acquired tolerance or resistance to the nematodes. Evidence to support this comes from the development of pine-wilt symptoms when *B. xylophilus* comes into contact with susceptible species (for example *Pinus sylvestris*) under suitable climatic conditions in the southern USA. Although nematodes are not able to overcome tree defences and give rise to tree mortality in native pines, they may remain at the site of maturation feeding in shoots of apparently healthy pines for at least 6 years after entering the tree (Halik & Bergdahl, 1994).

The range of dispersal to new trees is dependent on the behaviour of *Monochamus* spp. in seeking breeding sites and/or in feeding. This, in turn, is dependent on temperature and on the proximity of suitable tree hosts to attract *Monochamus* spp. In general, adult *Monochamus* spp. fly only short distances (tens to hundreds of metres), although there are records of longer distance flight (up to about 3 km). It would appear that dispersal is related to the availability of breeding material. Transmission by oviposition is the most important means of dispersal of the nematode but in Asia, where pine wilt is epidemic, transmission during feeding by adult *Monochamus* spp. is also important.

Some forestry and commercial practices assist dispersal of *B. xylophilus*, for example felling of trees during periods favourable to infestation by the vectors, gathering and storing logs for a period in the forest, similar storage in saw mills situated in forest areas, and transport of infested logs to other areas. In addition, the use of untreated wood products in potentially dangerous situations (e.g. chips or bark as mulch spread in parks, golf courses) could affect multiplication and spread of nematodes and vectors.

Human activity is known to be the principal route for dispersal of *B. xylophilus* over greater distances. The establishment of *B. xylophilus* in Japan is presumed to have resulted from the import of logs at about the turn of the present century (Wingfield *et al.*, 1982). There is strong circumstantial evidence that the jumps in distribution of pine wilt disease into different areas of Japan resulted from the introduction of nematode-infested pine logs from areas where the disease was present (Mamiya, 1983a, 1988). *B. xylophilus* was later introduced to other Asian countries (China, Hong Kong, Taiwan and Republic of Korea), presumably as a result of transport of wood, since the distances involved would probably be too great for *Monochamus* spp. to fly (Ikeda, 1984; Baojun & Qouli, 1989). *B. xylophilus* has been intercepted on a number of occasions during international trade in wood and wood products; for example, different

types of wood (chips, round wood, boards) have been found by European countries to be infested with nematodes after transatlantic transport from Canada or USA (Rautapää, 1986; Schroeder & Magnusson, 1992; Tomminen & Nuorteva, 1992) and/or reported to EPPO over the period 1986/1993.

Monochamus spp.

Monochamus spp. do not seem to share the nematode's ability to establish easily outside their natural ranges, despite their capability of also being transported in traded wood. These insects have been found regularly on imported wood, not only from North America, but also from other parts of Europe (reported to EPPO over the period 1986/1993). They are also regularly intercepted on the pallets, crates or dunnage accompanying other commodities moving in international trade to the USA from other parts of the world (USDA, 1982). However, there are no records of exotic *Monochamus* spp. having become established in new locations. It is possible that the wood that was presumed to have been the cause of the various introductions of *B. xylophilus* (mentioned above) may also have been carrying the vectors and indeed this is the most likely scenario for introduction. The requirement for repeated mating of the female of *Monochamus* spp. in order for her successfully to lay fertile eggs reduces the chances of successful oviposition, but not of successful nematode transmission, in a new location. Females will attempt to lay eggs even when the eggs are likely to prove infertile and thus, as shown by Linit (1988), transmission of nematodes may take place without subsequent development of the vector. In such a situation, the presence of native vectors is essential for further transmission of *B. xylophilus*. Although this provides the most likely explanation for transmission into exotic locations, it is also conceivable that other pathways for the transfer of nematodes to the ecosystem may have occurred.

2.3 Survival under adverse conditions

Both *B. xylophilus* and *Monochamus* spp. have temperature-dependent life cycles and are adapted to survive periods of low temperature (Rutherford & Webster, 1987; Kondo *et al.*, 1982).

Bursaphelenchus xylophilus

In *B. xylophilus*, the 3rd dispersal juvenile stage develops in the population when conditions are no longer suitable for rapid multiplication. This is presumably when the availability of food in the wood, either as plant cells or fungal mycelium, is reduced (at the completion of the first phase of wood decay) and when moisture content falls below a certain threshold. The 3rd dispersal juvenile is more active than other stages; it is characterized by a thick cuticle and contains accumulated lipid stores, permitting it to tolerate adverse conditions such as desiccation, lack of food and low temperatures (Mamiya, 1976b, 1984; Wingfield, 1983, 1987; Bergdahl, 1988).

The 4th dispersal stage ('dauer larva') could also be considered to be a survival stage, allowing it to survive transportation on and within its vector. However, because its very existence is so closely linked to the vector (in nature it develops only on stimulation from the beetle's ecdysis from pupa to adult, and transforms to adult nematode immediately on leaving the vector), its capacity to survive other adverse conditions is probably limited.

Monochamus spp.

Any of the larval stages of *Monochamus* spp. can hibernate. In addition, there appears to be a stage of diapause during larval development which can occur at different stages of the life cycle

(or indeed be absent) depending on which larval stage is present at the onset of winter (Togashi, 1991). Similar prolonged periods of larval development, including two winters within the wood, have been recorded for North American *Monochamus* spp., e.g. *M. scutellatus* (Cerezke, 1975). This may explain the presence of beetle adults that are recorded as flying out of furniture or houses some years after construction.

2.4 Adaptability

Bursaphelenchus xylophilus

B. xylophilus is an organism with remarkable flexibility in its biology. It has demonstrated this flexibility in exploiting new situations in Japan, China, etc., where it was able to enter the ecosystem in an area far outside its normal geographic range, adapting to different tree-host species, different vector species and possibly different climatic conditions. Since *B. xylophilus* is not known to damage indigenous tree species within its native range in North America, it has, apparently, also been able to exploit an altogether different *modus vivendi* in relation to exotic tree species (both in North America and in other parts of the world) by invading and killing healthy trees through the feeding wounds caused by its vector. *B. xylophilus* has the capability to utilize different forms of nutrition and can survive on the cells of living trees, on the mycelia of a number of fungal species (Kobayashi *et al.*, 1974, 1975; Fukushige, 1991) and on yeast associated with the vector beetle (Ogura & Tamura, 1989).

DNA profiles indicate considerable plasticity in the nematode genome. Populations from different parts of North America and from different host species show small differences in morphology, particularly in relation to the shape of the female tail and in pathogenicity towards exotic tree species. Populations maintained as laboratory cultures sometimes appear to change with time in their pathogenicity and their biochemical composition (Bolla *et al.*, 1986; Kiyohara & Bolla, 1990). The species has also been shown experimentally to be able to interbreed, to a greater or lesser extent, with different populations of *B. mucronatus* (De Guiran & Boulbria, 1986; De Guiran & Bruguier, 1989; Riga *et al.*, 1992; Bolla & Boschert, 1993; Braasch, 1994), although it should be noted that this phenomenon has not been reported to occur in nature in the Asian countries where the species co-habit (Evans *et al.*, 1993).

Monochamus spp.

Monochamus spp. do not demonstrate particular adaptability. There are no records of changes in behaviour by extension of geographic or host range. Although subspecies of *Monochamus* spp., such as *M. scutellatus* subsp. *oregonensis* and *M. scutellatus* subsp. *scutellatus* are described, it is not clear whether these are purely named from geographic separation or whether there is genetic incompatibility.

Section 3. Geographical distribution of the pest

3.1 Present occurrence in PRA area

Bursaphelenchus xylophilus

B. xylophilus is not known to occur in the PRA area (territories of the European Union). Nematological surveys have been conducted in recent years in a number of EU countries: France, The Netherlands, Germany, Austria, Italy, Sweden, Finland and UK (Baujard *et al.*, 1979; Weischer, 1983; Bongers, 1985; De Guiran & Boulbria, 1986; Magnusson & Schroeder, 1989; Schauer-Blume & Sturhan, 1989; Tomminen *et al.*, 1989; Tomminen, 1990; Braasch,

1991; Palmisano *et al.*, 1992) without ever finding *B. xylophilus*. Many of these surveys detected related species of *Bursaphelenchus*: *B. mucronatus* in France, Germany, Italy, Sweden and Finland, *B. fraudulentus* in Germany and Austria. Surveys conducted in other European countries likewise failed to detect *B. xylophilus* but showed the presence of other *Bursaphelenchus* spp.: *B. mucronatus* in Norway, Bulgaria and Russia (McNamara & Støen, 1988; Kulinich & Kolossova, 1995), and *B. kolymensis* in Russia (Korenchenko, 1980), although it is suspected that the latter species may be a synonym of *B. mucronatus* (see also section 1.1). The finding of *B. mucronatus* in Primorskiy Territory in the Russian Far East and interceptions of the nematode in wood imported from Siberia into other European countries (Braasch, 1991; Kulinich & Kolossova, 1993, 1995; Anon., 1994) demonstrates the distribution of this species across the whole of the northern Palaearctic region, through Korea (Choi & Moon, 1989; Lee *et al.*, 1990), China (Jiang, 1988; Baojun & Qouli, 1989) and into Japan (Mamiya & Enda, 1979). *B. mucronatus* is also present in Quebec province, CA (Harmey & Harmey, 1993).

Monochamus spp.

Native European *Monochamus* spp. are present in all parts of the PRA area except UK and Ireland (see also Table 1). In areas adjoining the PRA area, *Monochamus* spp. appear to be present in European, west Asian and some North African countries; they are not known to occur in Cyprus.

3.2 World distribution of the pest

Bursaphelenchus xylophilus

The world distribution of *B. xylophilus* is shown in Fig. 2. *B. xylophilus* is widespread in Canada and USA and there is a single report of its presence in Mexico (Dwinell, 1993). Further details

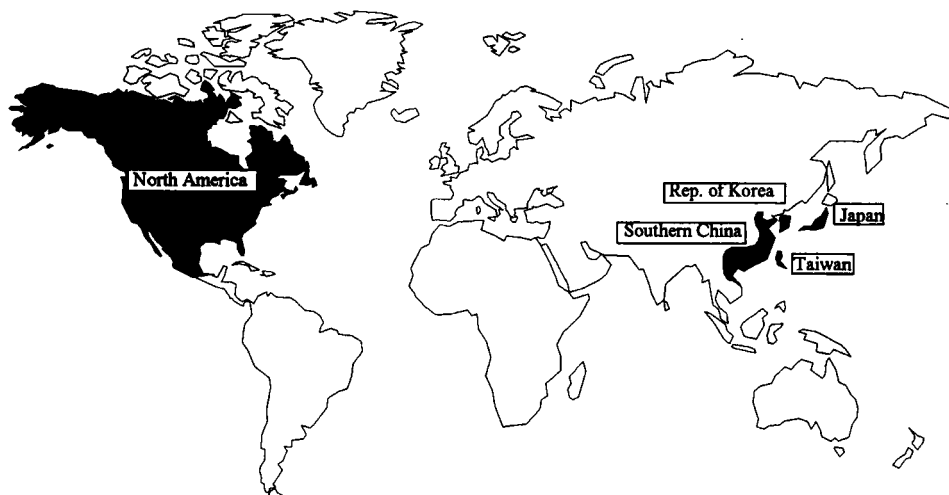


Fig. 2. The known world distribution of *Bursaphelenchus xylophilus*.
Répartition mondiale connue de *B. xylophilus*.

on distribution in North America are given in EPPO/CABI (1996), from which it appears that *B. xylophilus* occurs in practically all states/provinces of Canada and USA where pine forests exist. The situation is not quite clear in the far north. In Japan, *B. xylophilus* is now widespread in three of the four main islands, Kyushu, Shikoku and Honshu, but has not yet reached Hokkaido (Rutherford *et al.*, 1990; Evans *et al.*, 1993). It has spread into China, Korea and Taiwan during the past 25 years and is thought to have reached these locations from Japan (Mamiya, 1988). In all these new areas, *B. xylophilus* has become associated with *M. alternatus* as principal vector. In China, it is restricted to the provinces of Jiangsu, Anhui, Guangdong, Shandong and Zhejiang, and in the Republic of Korea to the province of Pusan in the south-east (Fig. 3). Pine wilt symptoms are evident in all these locations.

There is a record of the presence of *B. xylophilus* in dying pines in Nigeria (Khan & Gbadegesin, 1991) but this has not been confirmed by specialist taxonomists. Similarly, the report by Harrachi *et al.* (1990) of the presence of a *Bursaphelenchus* sp. in wilted *Pinus halepensis* in Morocco, needs more precise identification.

Monochamus spp.

Monochamus spp. on conifers are more widely distributed than *B. xylophilus*, having representatives of the genus virtually throughout the northern hemisphere with the exception of UK and Ireland.

3.3 Area of origin and history of spread

B. xylophilus is native to North America and is thought to have been carried to Japan at the beginning of the twentieth century on timber exports (Nickle *et al.*, 1981; Mamiya, 1983b; Malek & Appleby, 1984a; Wingfield *et al.*, 1982). The DNA profiles of the Japanese and some of the USA races of *B. xylophilus* are identical, which supports this hypothesis (Tares *et al.*, 1992). Movement within Asia is also thought to have been via timber exports and internal movement of timber (see also section 2.2).

3.4 Overlap of world distribution of the pests with that of major hosts

The principal hosts for *B. xylophilus* are in the genus *Pinus*, although the nematode has been found in a number of other conifer genera (see section 4). *Pinus* spp. have a worldwide distribution both as native and, increasingly, as exotic plantation trees for afforestation and reforestation but, for the moment, *B. xylophilus* only overlaps with its potential hosts in North America and in a few Asian countries. There is therefore a considerable potential for further spread. Similarly, for *Monochamus* spp., coniferous hosts have only been exploited so far in the nearctic and palaeartic forest regions.

Section 4. Host plants of the pest

4.1 Host plants reported in areas where the pest now occurs

Assessment of world literature and direct contact with researchers in Japan and elsewhere provides the list of susceptible and resistant tree species in Table 2. This includes information on the relative degree of susceptibility within the genus *Pinus* and also in other species of conifer.

It is not always clear from the literature whether records quoted refer to seedling trees under laboratory or nursery conditions or to more mature trees. Since it is known that seedling trees (either maintained or not under controlled environmental conditions) demonstrate a much higher susceptibility to invasion by *B. xylophilus* and especially to artificial inoculation by the

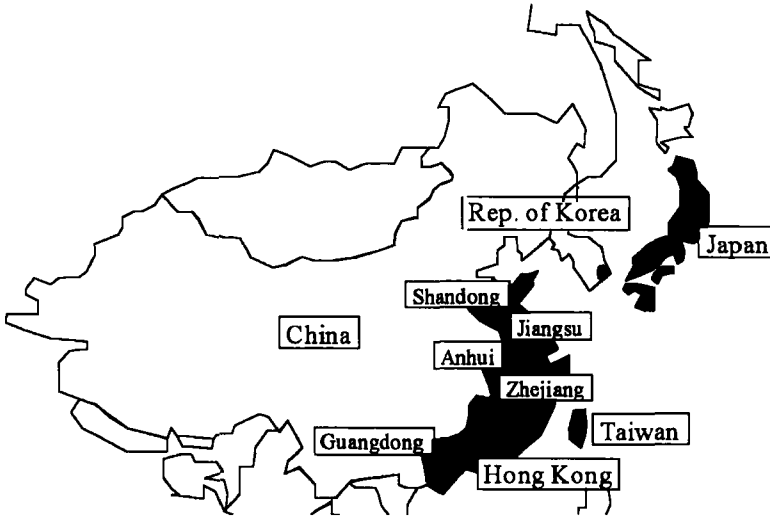


Fig. 3. The distribution of *Bursaphelenchus xylophilus* in Asia.
Répartition de *B. xylophilus* en Asie.

nematode, the lists in Table 2 should be viewed with considerable caution. In addition, different authors report different states of susceptibility/resistance for the same species, e.g. *P. taeda* (Mamiya, 1976b; Malek & Appleby, 1984b; Luzzi *et al.*, 1984). In fact, of the tree species in Table 2, the only species that have been reported to succumb to pine wilt disease as mature trees in the field are *P. sylvestris*, *P. thunbergii*, *P. luchuensis*, *P. densiflora* and *P. nigra*. This fact perhaps represents a more realistic indication of the truly susceptible species. Furthermore, it can be stated that only those species of *Pinus* that have not co-evolved with *B. xylophilus* are included here (Evans *et al.*, 1993; USDA, 1991).

Susceptibility to pine wilt disease should be clearly distinguished from the ability of *B. xylophilus* to colonize dead trees during the mycophagous phase of its life cycle. A much wider range of tree species can be colonized in the latter state than can be killed by the disease, and perhaps wider also than the range suitable for breeding by *Monochamus* spp. This aspect of the host/parasite relationship is covered in section 7.5 (Specific pathways for the pest from infested host plants in its country of origin to susceptible host plants in the PRA area).

4.2 Host plants growing in PRA area

The proportion of the territories of some EU countries occupied by forests and by coniferous forests in 1990 is shown in Table 3. Of the species concerned, *Pinus* spp. form by far the greatest proportion. In France, for example, there are 540,000 ha of *Abies*, 750,000 ha of *Picea* and 3,100,000 ha of *Pinus*, of which 1 million ha are found in the Landes department (in the south-west), producing 8 million m³ of wood. In northern areas of the EU the areas covered by conifers are greater than in other parts of the Union. In Sweden, *Pinus* and *Picea* forests cover 11,840,000 and 11,066,000 ha respectively. In Finland, *Pinus* dominates, extending over 17,025,000 ha, while *Picea* covers 6,710,000 ha.

The main European forest species of *Pinus* is *P. sylvestris*, which has been found very susceptible to *B. xylophilus* under both natural and artificial conditions (Table 2). This species has a very extensive natural distribution throughout the continent (Fig. 4), predominantly in the north but also extending well into the Mediterranean area. In addition, *P. sylvestris* has been widely planted as a forest tree in northwestern countries and elsewhere, outside its natural

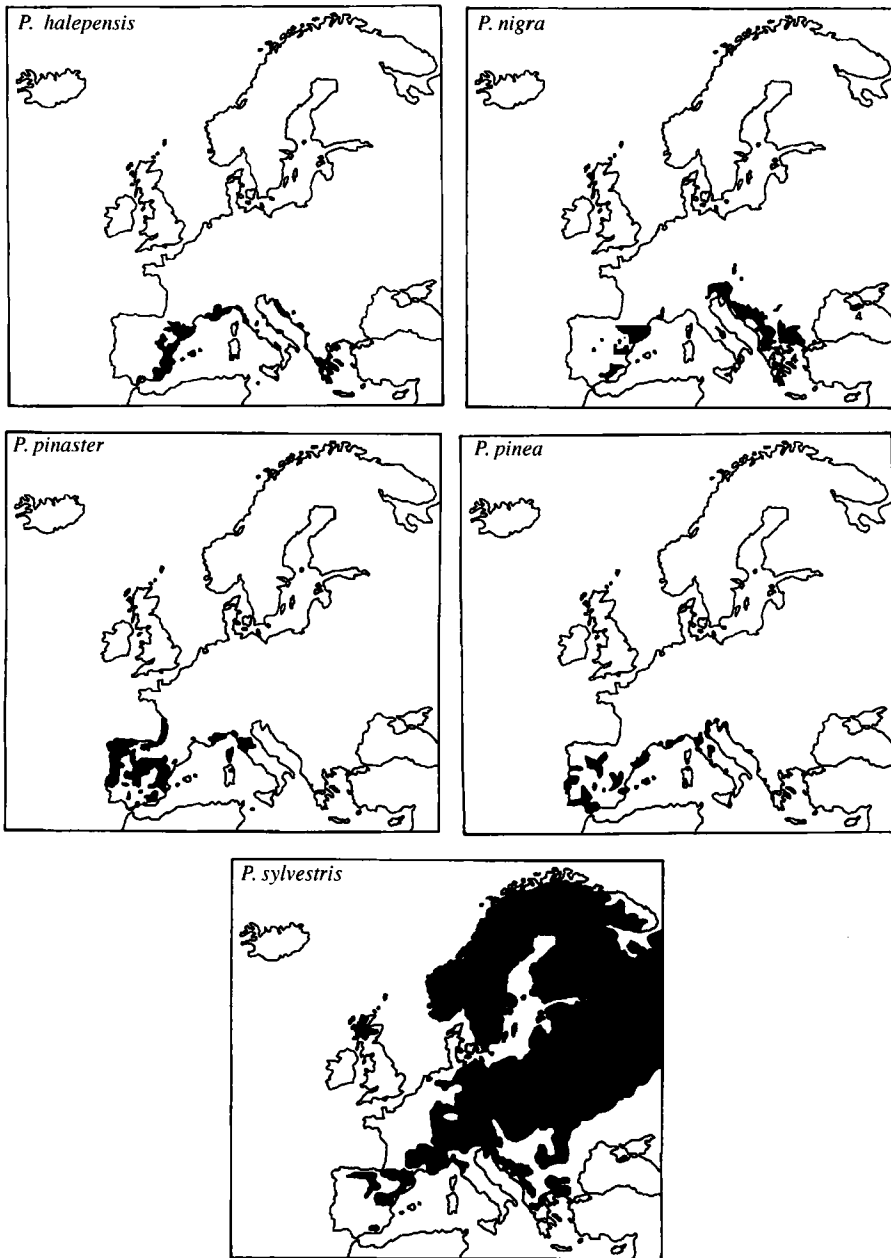


Fig. 4. The natural distribution of the major *Pinus* spp. in Europe (adapted from Polunin & Walters, 1985). Répartition naturelle des principales espèces de *Pinus* en Europe.

Table 2. (a) Susceptibility of various *Pinus* species to *Bursaphelenchus xylophilus* (data refer to evidence of reproduction of the nematode in living trees). (b) Crosses between *Pinus* spp. found resistant to *Bursaphelenchus xylophilus*. (c) Conifer species other than *Pinus* recorded as hosts of *Bursaphelenchus xylophilus*

(a) Sensibilité de certaines espèces de *Pinus* à *B. xylophilus* (indications expérimentales de l'intensité de reproduction chez des arbres vivants). (b) Croisements entre *Pinus* trouvés résistants à *B. xylophilus*. (c) Conifères autres que *Pinus* signalés comme hôtes de *B. xylophilus*

(a) Susceptibility of *Pinus* spp.

Resistant	Intermediate	Susceptible
North American species (Canada and USA)		
<i>P. clausa</i>	<i>P. banksiana</i>	
<i>P. elliotii</i>	<i>P. contorta</i>	
<i>P. rigida</i>	<i>P. echinata</i>	
<i>P. virginiana</i>	<i>P. engelmannii</i>	
	<i>P. jeffreyi</i>	
	<i>P. lambertiana</i>	
	<i>P. monticola</i>	
	<i>P. palustris</i>	
	<i>P. ponderosa</i>	
	<i>P. pungens</i>	
	<i>P. radiata</i>	
	<i>P. resinosa</i>	
	<i>P. strobus</i>	
	<i>P. taeda</i>	
Central American species (including Mexico)		
	<i>P. caribaea</i>	<i>P. ayacahuite</i>
	<i>P. montezumae</i> var. <i>hartwegii</i>	<i>P. leiophylla</i>
	<i>P. oocarpa</i>	<i>P. muricata</i>
	<i>P. cooperi</i>	
	<i>P. patula</i>	
	<i>P. rudis</i>	
	<i>P. strobiformis</i>	
Euromediterranean species		
	<i>P. halepensis</i> subsp. <i>halepensis</i>	<i>P. mugo</i>
	<i>P. halepensis</i> subsp. <i>brutia</i>	<i>P. nigra</i>
	<i>P. pinea</i>	<i>P. pinaster</i>
		<i>P. sylvestris</i>
Asian species		
<i>P. fenzeliana</i>	<i>P. bungeana</i>	<i>P. densiflora</i>
<i>P. morrisonicola</i>	<i>P. massoniana</i>	<i>P. kesiya</i> (= <i>P. khasya</i>)
<i>P. taiwanensis</i>	<i>P. pentaphylla</i>	<i>P. koraiensis</i>
	<i>P. tabulaeformis</i>	<i>P. luchuensis</i>
	<i>P. wallichiana</i> (= <i>P. excelsa</i> ,	<i>P. thunbergii</i>
	<i>P. griffithii</i>)	
	<i>P. yunnanensis</i>	

Table 2. Continued

(b) Resistant hybrids

P. densiflora × *P. nigra* subsp. *laricio*
P. densiflora × *P. mugo*
P. rigida × *P. taeda*
P. thunbergii × *P. tabulaeformis*
P. thunbergii × *P. massoniana*
P. thunbergii × *P. nigra* subsp. *laricio*

(c) Hosts in other genera

North American	Euromediterranean	Asian
<i>Abies amabilis</i>	<i>Cedrus atlantica</i>	<i>Abies firma</i>
<i>Abies balsamea</i>	<i>Cedrus deodara</i>	<i>Abies sachalinensis</i>
<i>Abies grandis</i>	<i>Larix decidua</i>	<i>Larix kaempferi</i> (= <i>L. leptolepis</i>)
<i>Chamaecyparis nootkatensis</i>	<i>Picea abies</i>	<i>Picea jezoensis</i>
<i>Larix laricina</i>		
<i>Larix occidentalis</i>		
<i>Picea engelmannii</i>		
<i>Picea glauca</i>		
<i>Picea mariana</i>		
<i>Picea pungens</i>		
<i>Picea rubens</i>		
<i>Picea sitchensis</i>		
<i>Pseudotsuga menziesii</i>		

range. Of the more southern *Pinus* spp. with relevance to commercial forestry and the environment, *P. nigra*, *P. pinaster* and *P. pinea* appear in Table 2 as susceptible species, and *P. halepensis* as having moderate resistance; their natural distribution is also shown in Fig. 4. *P. nigra* has been widely planted throughout Europe. The other native European species, *P. mugo*, *P. cembra* and *P. uncinata* have limited distributions at higher altitudes, mainly in the Alps; the former species is considered as highly susceptible and no information is available on the susceptibility of the others.

Of the other native European coniferous species, *Picea abies* and *Larix decidua* have very wide distributions as important forest trees and both are susceptible species. Many of the other host-plant species in Table 2 are found artificially planted in the EU, especially the North American species. This reflects the increased interest in exotic conifers for both commercial forestry and for ornamental use. Clearly, the extent of availability of any given species will depend on its commercial potential. However, various *Picea*, *Pinus* and *Abies* spp. native to North America are grown extensively, and increasingly, in the EU; many are known hosts for *B. xylophilus*.

4.3 Nature of the host range

Information on host range of *B. xylophilus* has been gained from both field observation of mature trees and from inoculation trials conducted either in the laboratory and glasshouse using seedling trees or in the field. Evidence for extensive mortality in the field comes only from the genus *Pinus* in Asia.

Monochamus spp. are more cosmopolitan than *B. xylophilus* in their host ranges. They are known to colonize virtually all conifer genera, with the exception of *Thuja plicata*.

Table 3. Statistics on land areas, forested areas and proportion of coniferous forests in various EU Member States and Norway

Surfaces totales, surface forestières et part de forêts de conifères dans les Etats membres de l'UE et de la Norvège

Member State	Total surface area (thousand ha)	Forested area (thousand ha)	% coniferous forest
Belgium	3051	600	47
France	54900	15136	36
Germany	35633	7200	69
Greece	13200	5750	19
Ireland	8400	400	90
Italy	30125	6400	25
Netherlands	3370	350	65
Portugal	9117	3000	46
Spain	50500	12500	48
UK	23900	2200	73
Sweden	41078	28950	79
Denmark	4307	500	62
Finland	33811	26354	90
Norway	30881	7036	23
Total Nordic countries	110077	62840	57
Total for EU and Norway	414232	146319	46

Section 5. Potential of the pest for establishment in the PRA area

5.1 Climatic conditions for pest development

The development of both *B. xylophilus* and *Monochamus* spp. is strongly dependent on temperature. In the laboratory, *B. xylophilus* can be maintained on fungal cultures. It reproduces in 12 days at 15°C, 6 days at 20°C and 3 days at 30°C; at higher temperatures, the rate of development slows again. Dwinell (1986) reported optimal temperatures for nematode development as high as 35–40°C in piles of wood chips. Egg laying starts on the 4th day after hatching, and the eggs hatch in 26–32 h at 25°C. The temperature threshold for development on *Botrytis cinerea* is 9.5°C (Mamiya, 1975; Tomminen, 1993). Populations from different climatic regions show differences in developmental rates and temperature optima (Rutherford & Webster, 1987).

Data from North America and Japan indicate that tree mortality arising from expression of pine wilt disease is largely determined by summer-temperature isotherms. In particular, no pine wilt disease has been recorded where mean daily summer temperatures are 20°C or below. In Japan, epidemic wilt conditions require higher temperatures than these, being recorded only where mean August temperatures exceed 24°C (Fig. 5, Table 4). Confirmation of the very important role of high temperature in wilt development comes from the decline in wilt with increasing altitude in Japan, despite the presence of both *B. xylophilus* and *Monochamus* spp. (Rutherford *et al.*, 1990).

Two factors, moisture deficit and high temperatures, have been consistently associated with the expression of pine wilt disease in Japan and in parts of the USA (Rutherford & Webster, 1987). These exert their influence by accelerating the development of both the nematode and its vector insects in susceptible tree species. There has been relatively little study of other abiotic factors on the possible expression of the disease. It would appear likely that any factor causing



Fig. 5. The locations of the temperature records in Table 4.
Localisation des enregistrements de températures du tableau 4.

stress of the tree would exacerbate the effects of the nematode but the evidence for this is equivocal. For example, Bolla & Fitzsimmons (1988) tested the effects of simulated acid rain on *B. xylophilus*-inoculated *Pinus strobus*, *P. sylvestris* and *P. nigra* seedlings. Surprisingly, exposure to acid rain reduced susceptibility to the nematode. This was thought to be a result of increased oleoresin production which slowed nematode reproduction even in *P. sylvestris*. However, this tolerance to the nematode was lost within 60 days of treatment with acid rain. When seedlings of the three *Pinus* species were treated with the toxic chemicals copper sulphate or lead acetate, the plants succumbed rapidly to *B. xylophilus*. The authors concluded that pollutants could give rise to increased sensitivity to the nematode (Huber *et al.*, 1989).

Although not definitive, these experiments and the general observation that stressed trees are more liable to succumb to *B. xylophilus* than healthy trees indicate that atmospheric pollution and other forms of biotic and abiotic stress are likely to add to the risks from the nematode when susceptible tree species and sufficiently high temperatures are present.

Table 4. Mean temperatures at 9 representative locations in Japan (see Fig. 4 for map)
Températures moyennes à 9 sites représentatifs au Japon (voir fig. 4)

	April	July	October	January
Sapporo	6.4	20.2	10.8	-4.6
Sendai	10.0	22.0	14.4	1.0
Tokyo	14.1	25.2	17.6	5.2
Nagoya	13.8	25.8	17.0	3.7
Kyoto	13.9	26.5	17.1	4.0
Hiroshima	13.4	25.7	17.0	4.0
Takamatsu	13.5	26.3	17.2	4.8
Fukuoko	14.6	26.9	18.2	5.8
Kagoshima	16.4	27.4	20.0	7.2

5.2 Data on climatic conditions in PRA area

The main climatic zones related to potential for tree growth as defined by Kreysa & Last (1988) are shown in Fig. 6. The main ecological and silvicultural characteristics of each zone are summarized in Table 5. Fig. 7 shows the August isotherms of 15, 20 and 25°C.

Availability of suitable tree species and almost universal presence of *Monochamus* spp. in most EU Member States indicates that *B. xylophilus* could establish and spread. Average summer temperatures in the Mediterranean and, to a lesser extent, in the continental dry region of Fig. 6 are high enough to support pine wilt in susceptible trees.

There is increasing evidence that the climate of Europe is in the process of changing, the predictions from models being regarded with greater confidence as they are refined. Table 6 summarizes the expected change during the next 40 years or so, until 2030. These are regarded as conservative predictions but would still have the effect of moving the area of risk of expression of pine wilt disease further northwards in the European Union.

Section 6. Control of the pest

6.1 Control measures in regular use

In North America, no active control measures are taken even in the USA where there is some mortality to exotic pines. Apart from general forest hygiene to prevent timber degradation from

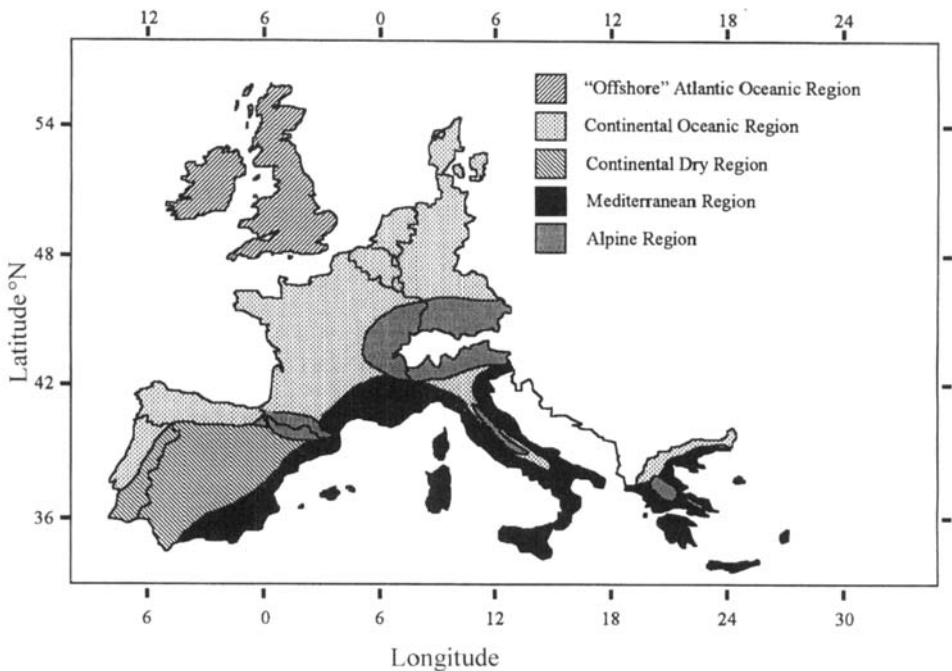


Fig. 6. The distribution of transboundary climatic regions within the EU Member States (re-drawn from Kreysa & Last, 1988).

Répartition des régions climatiques chevauchant les frontières de plusieurs Etats membres de l'UE.

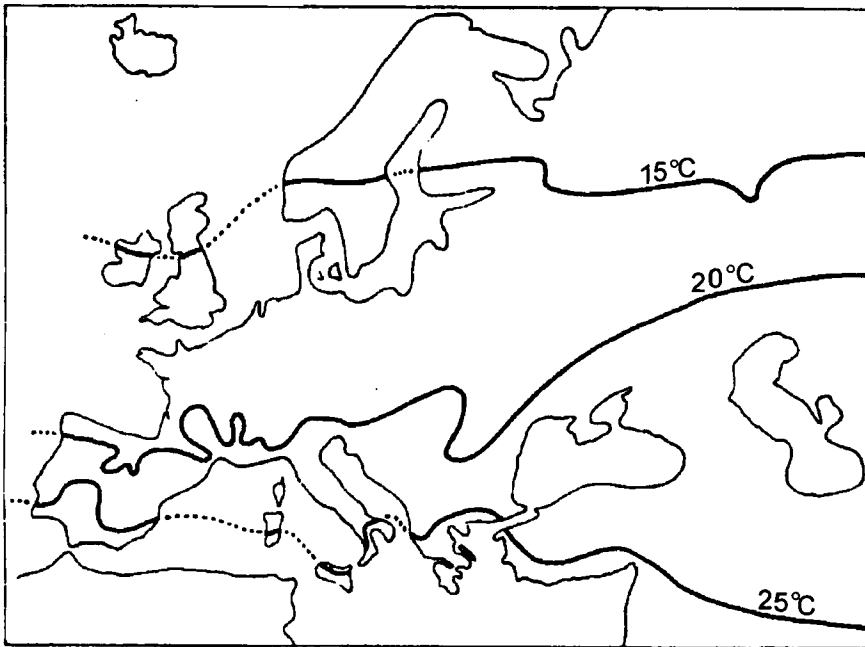


Fig. 7. Mean August isotherms in Europe (from De Guiran & Boulbria, 1986).
Isothermes moyens du mois d'août en Europe.

Table 5. Climatic regions within the European Union in relation to tree growth characteristics (based on Kreysa & Last, 1988)

Régions climatiques de l'Union européenne par rapport aux caractéristiques de la végétation forestière (selon Kreysa & Last, 1988)

Climatic region	Tree growth characteristics
Offshore Atlantic oceanic region	Trees grow very quickly. Main problems arise from wind damage, heavy rainfall and instability from shallow soils.
Continental oceanic region	Trees grow well, although slower than in offshore Atlantic oceanic region. High quality forests and continuing new planting. Eastern part of region suffers from 'forest decline'.
Continental dry region	Trees grow slowly. Main purpose is erosion control. Fire is a major problem.
Mediterranean region	Trees grow slowly. Fire is the most serious problem but erosion control is also important. Decline of <i>Pinus</i> is evident in the south east of the region and insect attacks (e.g. <i>Thaumetopaea pityocampa</i>) are frequent.
Alpine region	Severe climate and rocky terrain results in poor growth and makes commercial operations difficult. Soil erosion protection is a major function of forestry.

Table 6. Predicted climate change parameters for western Europe to the year 2030, according to the Hadley Centre transient climate change experiment (Hadley Centre, 1992)
 Evolution prévue des paramètres climatiques de l'Europe occidentale jusqu'en 2030, selon l'expérience du Hadley Centre sur la modification passagère du climat

Parameter	Prediction for year 2030
Temperature	Warming minimum near Atlantic (0.2°C per decade), maximum in eastern Europe (0.35°C per decade). Decrease in very cold winter spells and increase in numbers of hot summers. Marked reduction in frosts in Atlantic offshore.
Precipitation	General increase between 20% and 40% in central and northern Europe. Iberian Peninsula will have decrease in rainfall, while south of Europe will have drier summers.
Soil moisture	Little change in winter likely except in Iberian Peninsula where there will be drying. In summer, southern Europe will become drier and could extend the limits of this climate zone quite far north, including much of UK.
Storminess	Storm tracks in North Atlantic could extend eastwards.

the activities of *Monochamus* spp., there is no means of restricting dispersal of either *B. xylophilus* or *Monochamus* spp. vectors within North America.

In Japan, control measures involving aerial application of insecticides against adult *Monochamus* spp. are carried out in areas with high-value trees. However, these measures are, at best, only partially successful. Rapid removal of trees killed by pine wilt disease is practised in public parks, etc. but, with the decline in the labour force in forestry, this effective method of restricting breeding of the *Monochamus* spp. is no longer practised on a commercial scale. Nematicides have also been used but with limited success and potential dangers from phytotoxicity (Takase *et al.*, 1982; Matsuura, 1984; Shoji, 1985). Breeding programmes for resistance to *B. xylophilus* have shown some success, particularly the cross *P. thunbergii* × *massoniana* (Ishii *et al.*, 1982; Furuno & Futai, 1986). Attempts to use biological control agents such as parasitoids and pathogens against *Monochamus* spp. have, so far, proved unsuccessful (Nagase *et al.*, 1982; Ogawa, 1988; Matsuda *et al.*, 1989).

Control measures in China have been concerned mainly with removal of infested material and restriction of movement of infested wood into nematode-free areas (Tang *et al.*, 1989). In one instance, the authorities felled a band of pine 80 km long by 4 km wide in order to prevent natural dispersal of *Monochamus* spp. and *B. xylophilus* into pest-free areas.

So, the only practical control measure in the forest is a high level of hygiene, removing dead or dying trees in order to limit breeding possibilities for *Monochamus* spp.; particularly important is the rapid removal of infested wood before vector insects can emerge carrying the nematode.

Treatment of consignments against both *B. xylophilus* and *Monochamus* spp. can be achieved successfully by heat-treating infested wood, either for the minimum time or within a kiln-drying schedule that achieves the same temperature (Evertsen *et al.*, 1991), or by using fumigation with pesticides. These measures are of value in preventing long-distance movement of the pest with timber commodities.

6.2 Records of eradication of the pest

There is no record of any successful eradication programme from a region, with the possible exception of the Chinese mass felling and removal programme mentioned above.

Section 7. Transport of the pest

7.1 Method of natural spread elsewhere in the world

Natural spread occurs through the flight activity of *Monochamus* spp. This is generally restricted to relatively short distances of less than 5 km within forest blocks. However, there is evidence that dispersal between forest blocks can take place, indicating that natural spread can occur over quite large distances provided that the distance between forest blocks is within the immediate flying range of adult *Monochamus* spp. There is no other means of natural spread, although on a very local basis there is some evidence from Japanese research that spread of *B. xylophilus* between trees can occur when the trees are joined by root grafts (Evans *et al.*, 1993). Trees left after felling of neighbouring trees commonly develop root grafts with the remaining stumps, which may grow actively for 30–40 years (Gleason & Fulling, 1966). This phenomenon is known to occur in at least 58 species of conifer, among which are 26 species of pine (Gleason & Fulling, 1966). Root grafting is important for within-stand spread of pathogens like *Armillaria mellea*, *Phellinus chrysoloma*, *Ophiostoma ulmi* and *Heterobasidion annosum* (Gleason & Fulling, 1966; Johansson & Unestam, 1988). *B. xylophilus* can be present in the root system of diseased trees, and root grafts could therefore allow it to spread from infested stumps to living trees within forest stands. Clearly this would not apply to between-forest dispersal.

7.2 Pattern of international trade in the major host plants of the pest

International trade in wood and wood products forms a major proportion of the export trade for Canada, USA and Japan. In particular, there is a very long history of trade in wood between North America and Europe, especially to the UK. In common with all internationally traded commodities, this trade is dependent on supply and demand, on international currency exchange rates and on competition with domestic EU and European markets (Fig. 8). North

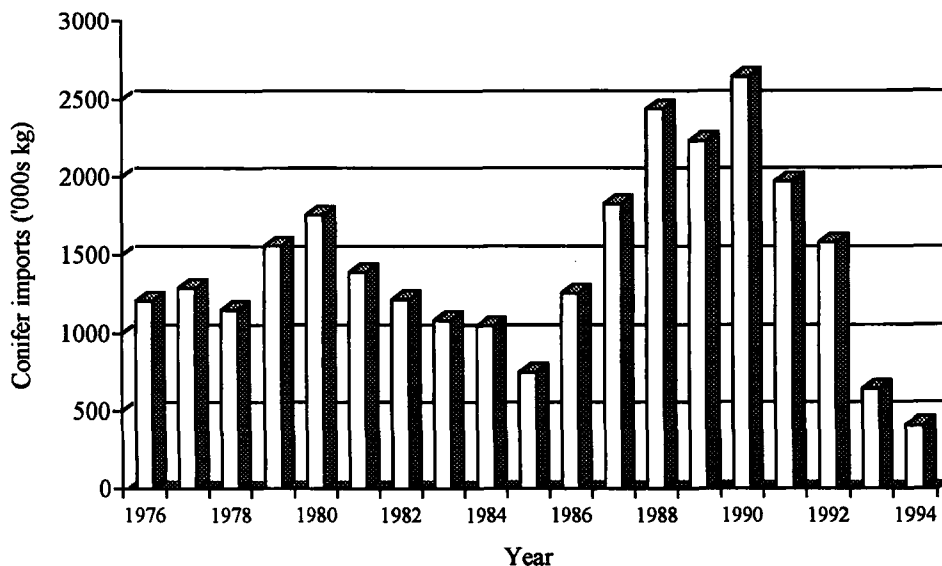


Fig. 8. Quantities of wood (all categories) exported from Canada to the EU from 1976 to 1994. Quantités de bois (toutes catégories) exporté du Canada vers l'UE de 1976 à 1994.

Table 7. Quantities and percentages of coniferous wood by commodity class imported from Canada to the EU for two representative years

Quantités et pourcentages du bois de conifères, par catégorie de marchandise, importés du Canada vers l'Union européenne pendant deux années représentatives

	1990 (year of maximum volume imported)		1994 (first 6 months)	
	tonne	%	tonne	%
Fuel wood	125	0.005	34	0.012
Chips or particles	14	0.001	0	0.000
Wood waste or scrap	873	0.033	86	0.030
Telegraph poles	46	0.002	0	0.000
Wood in the rough — treated	517	0.020	200	0.069
Wood in the rough — untreated	77592	2.936	1034	0.359
Split poles or stakes	0	0.000	0	0.000
Sawn wood >6 mm	2550979	96.535	285721	99.133
Wood products	11476	0.434	1081	0.375
Packaging wood (not associated with other products)	926	0.035	63	0.022
	2642548	100.000	288219	100.000

American exports to Asia are also substantial and are, of course, the route by which *B. xylophilus* was probably introduced into that region.

Despite more than 200 years of transport of wood to Great Britain from Canada, there has not been any introduction of either *Monochamus* spp. or of *B. xylophilus*. This is very likely due to the fact that the British Isles have been, up to very recently, virtually devoid of coniferous forests. *P. sylvestris* is native only to parts of Scotland in the north of Great Britain, whereas most trade imports were received in ports in the south. This situation has now changed so that the islands of Great Britain and Ireland are quite widely afforested with coniferous species. In addition, the risk of introduction of forest pests has become considerably greater with the great increase of international trade in wood in recent years.

Analysis of these timber imports by commodity class is summarized in Table 7 which includes both the peak year and the 1994 partial data from Fig. 8. Trade is dominated by sawn wood and, to a very much lesser extent, wood in the rough (including roundwood). Wood chips represented a very small proportion even during the peak year of export but did not feature at all during 1994. Wood as packaging is also important but, because it tends to have little or no further commercial value, this material is produced from the cheapest grades of timber. Waste wood and scrap includes dunnage (loose packaging material used to support shipments of other products in transit) and tends to be produced from the lowest quality of timber and, consequently, presents the highest risks of being produced from salvage material that is likely to be infested by *B. xylophilus* and/or *Monochamus* spp. Volumes of this commodity are very small but, because of their association with commodities other than timber, are easily overlooked as a potential source of exotic pests.

7.3 Records of interceptions of the pest

B. xylophilus has been intercepted in shipments of wood and/or chips to Finland, Norway, Sweden and France (Rautapää, 1986; Magnusson & Schroeder, 1989; also reported to EPPO

over the period 1986/1993). It is known to survive in wood for at least one year and could therefore survive shipment to Europe from any source.

Monochamus spp., North American, European and Asian (Siberian), have been intercepted in timber shipments to EU Member States (reported to EPPO over the period 1986/1993). In particular, inspectors in the UK have found larval, pupal and adult *Monochamus* spp. within wood and in association with it. However, there is no indication that any *Monochamus* spp. have established in new locations following transportation.

7.4 Records of movements of the pest not associated with host plants

Apart from occasional transfer of the organisms for scientific purposes, there are no records of movements of either *B. xylophilus* or of *Monochamus* spp. not associated with wood or wood products.

7.5 Specific pathways for movement of the pest to the PRA area

Potential pathways for introduction and establishment of *B. xylophilus* to the forests of the European Union include those where both the nematode and its *Monochamus* spp. vectors are present and also those where the nematode might be present without its vector. The biological characteristics of the two organisms have been described in section 2. In both native North American and exotic locations, *B. xylophilus* is dispersed naturally by *Monochamus* spp., but it is possible for nematodes to be introduced to trees without successful vector breeding and subsequent availability for transportation to other locations. Consideration of pathways must therefore include the possibility that wood entering the sequence in the forest may have either or both *B. xylophilus* and *Monochamus* spp. present. It is also important to include all possible pathways, even though many of these may pose no actual risk of successful transportation and establishment. Such an approach makes for completeness and also allows classification of risks for different pathways. Because of the complexity of this approach, its results are presented separately in Part B, under the title 'Pathway analysis'.

Section 8. Economic impact of the pest

8.1 Type of damage

B. xylophilus can, given the correct combination of susceptible tree species and climatic conditions, result in rapid tree mortality. This results from nematode invasion of the resin ducts, where they feed on the epithelial cells and probably also on the enveloping sheath of parenchymatous cells (Mamiya, 1976a). Since death of ray and axial parenchyma cells occurs prior to the decrease in oleoresin production, and prior to nematode population build-up (Mamiya, 1976a, 1983a), there is a suspicion that chemicals/toxins could be involved in the pathogenesis (Oku *et al.*, 1979; Mamiya, 1982). Benzoic acid and β -hydroxycarvotanacetone, isolated from pine trees naturally infested by *B. xylophilus*, were reported to cause typical wilt symptoms when applied to 3-year-old seedlings of *Pinus densiflora* (Ueda *et al.*, 1984). Similar observations have been made on *P. sylvestris* (Shaheen *et al.*, 1984). When the tree is very susceptible and the temperature is high enough, the symptoms can develop very rapidly (6–8 weeks) and the whole tree dies in the same year. Under cooler climatic conditions bordering the regions of epidemic pine wilt, a tree may die more slowly and can survive to the second year after attack before succumbing (Mamiya, 1983a). If the tree is less susceptible or if the temperature is much lower, localized development and mortality of only part of the tree may be evident (Wingfield *et al.*, 1986; Bedker *et al.*, 1987). In these circumstances, the nematode

may survive for considerable periods of time without giving rise to overt symptoms (Halik & Bergdahl, 1994). If the situation does not change to lower tree resistance, the nematode may eventually die out in the tree. However, Furuno & Futai (1983) observed that, in trees which did not die as a result of inoculation of *B. xylophilus*, the growth rate, in terms of elongation and diameter growth, was much reduced in the 3 years following inoculation.

Direct tree mortality is most likely to occur in the more southern Member States of the PRA area (but see also section 5). Losses could be direct, through lower timber production, premature felling, degradation of wood, or indirect by increasing erosion and fire risks where trees are normally grown for non-market benefits. Environment-chamber simulation of the typical daily fluctuations in temperature that would be achieved in an exceptionally hot Swedish summer indicates that tree death could also occur occasionally in the north of the region during prolonged periods of high temperatures, drought or other stress factors (Magnusson, 1992). Water stress has been demonstrated to influence the incidence and severity of pine wilt disease which appears to be more frequent and destructive on dry or exposed sites (Suzuki & Kiyohara, 1978). Soil water potential must apparently drop below field capacity for pine wilt to occur (Ikeda *et al.*, 1990). The possible influence of low soil moisture levels on the threshold temperature for pine wilt expression is unknown (Magnusson, 1986).

8.2 Recorded economic impact

Mortality from *B. xylophilus* in Japan has been substantial, particularly in the period immediately after the Second World War when over a million m³ of pine were lost each year. This figure was reduced substantially when forest hygiene programmes were introduced during the 1950s. However, the rising cost of labour and the declining labour force in forestry has reduced the sanitation programme so that losses are again at a very high level, reaching close to 2.5 million m³ per year (Fig. 9).

So far, losses in China have not been high but this may be a result of the very active forest hygiene programme that has been in place since *B. xylophilus* was first discovered in 1982. The

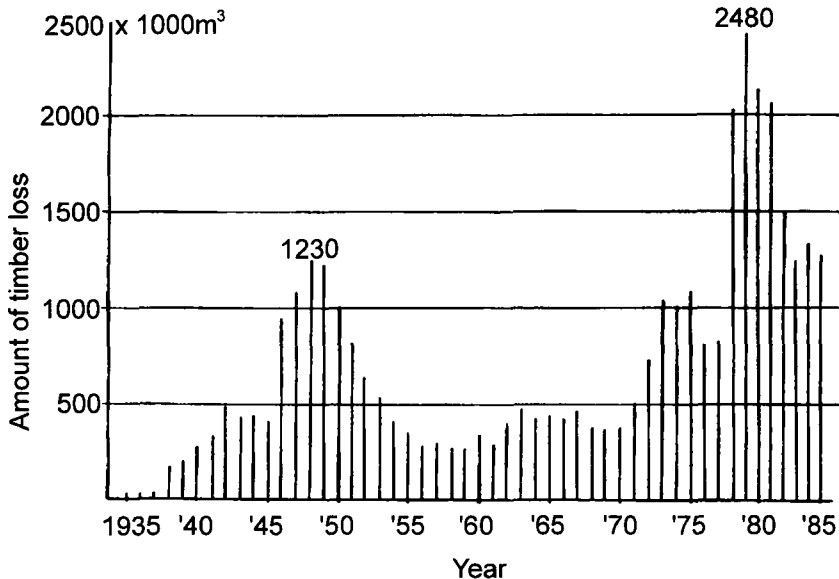


Fig. 9. Annual loss (m³ of wood) of pine trees caused by pine wilt disease in Japan (Mamiya, 1988). Pertes annuelles (en m³ de bois) dues au dépérissement des pins au Japon (selon Mamiya, 1988).

total area with evidence of infestation totals around 30,000 ha with low numbers of trees killed. However, it is difficult to determine the true mortality rate because of the rapid felling regimes that are employed to deal with symptoms of wilt.

Mortality of pines in the southern areas of the USA has been low and restricted to exotic pines grown 'off-site' and therefore under stress.

8.3 Estimated effect of the presence of the pest on exported commodities

Canada and the USA have been major providers of green and, to a lesser extent, dried wood to the EU. Patterns of trade are subject to both market conditions and phytosanitary requirements. Introduction of heat treatment has reduced the numbers of mills capable of meeting EU phytosanitary requirements and volumes of trade to Europe have declined. However, this is compounded by higher currency exchange prices and a resurgence in the domestic US market that has diverted wood production to the domestic North American market. In addition, new suppliers in Scandinavia, the Baltic states and Russia have stepped up the availability of green wood to cater for the continuing demand for this product within the EU. There is therefore no consumer shortage of green wood in the EU, even though the proportion of that wood from North America has declined dramatically (Table 7).

Conditions for export have become more stringent under the EU heat-treatment regime. This has added investment costs to some mills and production costs to those mills participating in the scheme. Some value added to the wood is gained when heating facilities are used to kiln-dry the wood but, if the wood is shipped green, there is no added value to partially cover the cost of heating. Thus, heat-treated wood has to compete with green-wood prices in a global market place and may be at a price disadvantage.

If *B. xylophilus* established in a relatively restricted part of the EU, it is likely that internal plant quarantine measures would have to be introduced under Protected Zone schemes. These would add costs to both the producers and, ultimately, to the consumers. There would also be restrictions on trade to other non-EU countries, within and beyond Europe.

8.4 Costs and side effects of control measures

These are not relevant, as no practical control measures for large-scale forest use are known (see section 6).

PART B. PATHWAY ANALYSIS

A schematic representation of potential pathways for introduction and establishment of *Bursaphelenchus xylophilus* to the forests of the European Union is shown in Fig. 10. This makes the assumption that wood is derived from infested forests and is then processed for possible export to the EU. Each pathway is examined to assess whether there is any likelihood of survival and transfer of *B. xylophilus* and/or *Monochamus* spp. to forests in Europe and is then linked to volumes of trade.

Based on the biology of *Monochamus* spp. in particular, each pathway in Fig. 10 includes an indication of whether it is capable of supporting both the vector and *B. xylophilus*. Although adult *Monochamus* spp. could potentially enter on any commodity ('hitch-hiking'), the ability of the vector to survive and emerge at the end of the pathway is in general dependent on continuation of larval development within wood through to pupation and adult emergence. Thus, in general, the pathways that receive processing in sawmills can be regarded as incapable of supporting *Monochamus* spp. but potentially capable of supporting *B. xylophilus*.

Assessment of the various pathways in Fig. 10 considers initially the intrinsic or innate risk from the commodity, essentially addressing the question of the likelihood of *B. xylophilus* or *Monochamus* spp. being in the commodity and surviving long enough to reach Europe and to

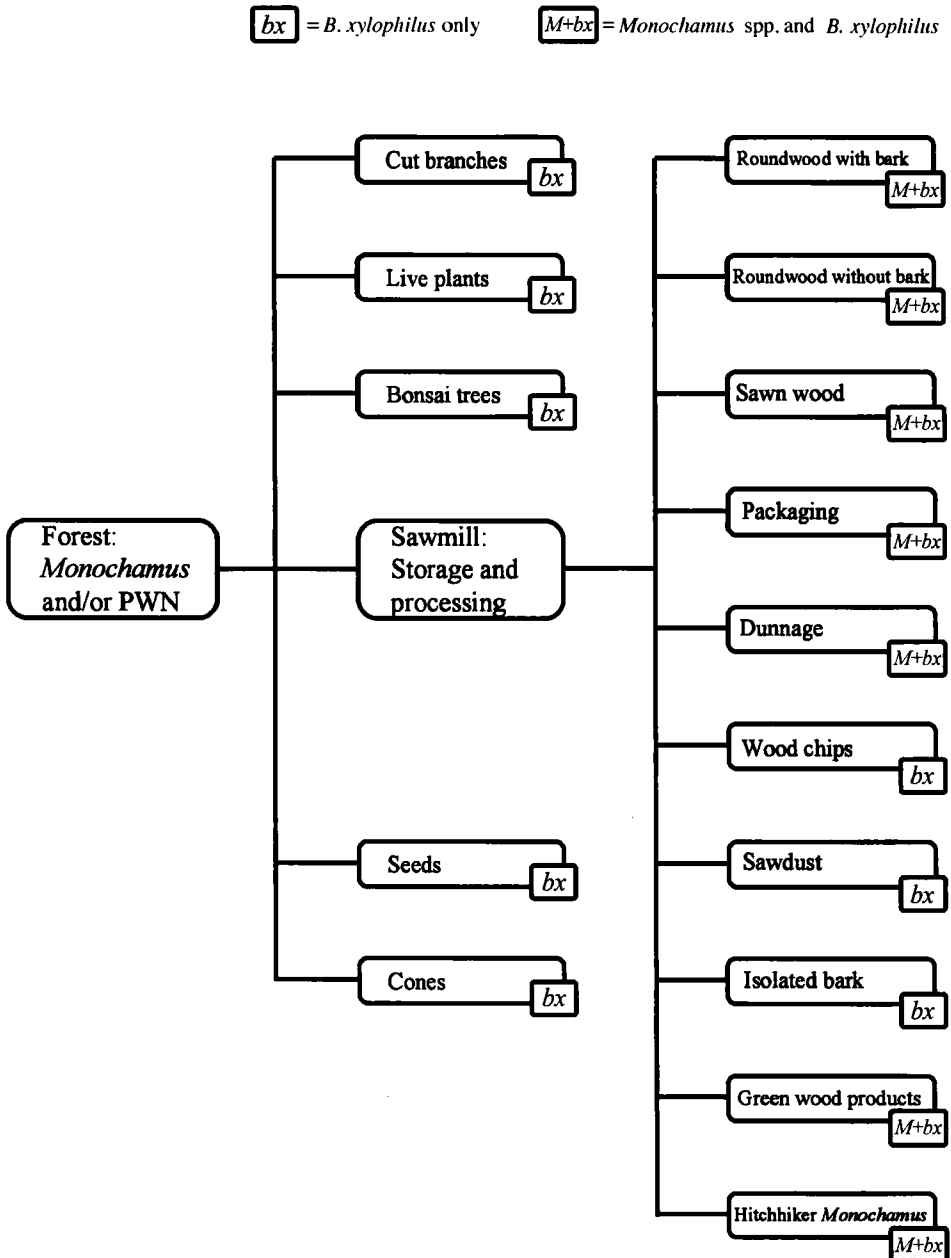


Fig. 10. Possible pathways for transfer of *Monochamus* spp. and/or *Bursaphelenchus xylophilus* to Europe. Filières du transfert éventuel de *Monochamus* spp. ou de *B. xylophilus* vers l'Europe.

be transferred successfully to trees after arrival. The discussion refers only to comparison between different pathways and not, at this stage, to finite probability of introduction and establishment. Comment is made on the volume of trade for each pathway and, in particular, on whether this affects the overall risk from that pathway.

Individual pathways

Cut branches

Cut branches, by virtue of thin bark and small diameter wood, are unable to support *Monochamus* spp. breeding and therefore this is not a viable pathway for the vector.

It is possible that *Monochamus* spp. could perform their maturation feeding on branches before they are cut and, thus, introduce *B. xylophilus* into the branches. It is known from various studies, notably the recent work of Halik & Bergdahl (1994), that nematodes can live and reproduce for considerable periods in branches of living trees, even when the tree is generally regarded as resistant to pine wilt. Branches will rapidly lose any resistance arising from oleoresin pressure and nematodes could reproduce on any fungi present.

Overall, cut branches represent a suitable resource for extended nematode breeding but the length of time that nematodes are likely to survive will depend on the size of the branch. This pathway therefore represents very low innate risk.

There is, in fact, no international trade in this commodity which is prohibited for given conifer species under EU legislation (on account of quarantine pests other than *B. xylophilus*). The effective risk is therefore zero under present regulations.

Plants for planting

Living plants imported for planting are, with the exception of specialist trade in relatively large specimen trees, generally small and are thus, for the same reasons as cut branches, very unlikely to be able to support *Monochamus* spp.

Maturation feeding by *Monochamus* spp. could introduce *B. xylophilus* into conifer plants. Survival and reproduction is possible and, given the right combination of tree species (see section 4.1) and temperature, death of the tree from pine wilt could result. Extrapolating from Halik & Bergdahl's (1994) results with mature pines, it is reasonable to assume that nematode survival and reproduction could be expected for several years if the tree did not die from pine wilt. However, the risks of transfer to new locations remains low because of the probability that only healthy plants would be selected for transportation and any that were likely to succumb to pine wilt disease would do so quickly after inoculation by *Monochamus* spp. If the plants reached Europe and still contained nematodes, the risk of transfer into the general forest ecosystem depends on survival and reproduction of *B. xylophilus* in the tree and for the tree to become suitable for *Monochamus* spp. breeding. If the tree dies quickly after being planted it would still be too small to support the vector. Longer survival of the tree would be accompanied by reduction of *B. xylophilus* populations to zero. It is possible that trees which have received nematode inoculum and which are symptomless may be exported. The likelihood that living trees will be planted in proximity to existing trees in Europe adds a further possible pathway by potentially introducing nematodes within the symptomless imported tree (see section 2.2).

This pathway represents low innate risk.

There is, in fact, no international trade in this commodity which is prohibited for certain conifer species under EU legislation (on account of quarantine pests other than *B. xylophilus*). The effective risk is therefore zero under present regulations.

Bonsai trees

Bonsais are extremely specialized forms of 'plants for planting' and the same constraints apply, with the additional factor that the trees are kept for long periods before being transported, during which time any nematodes present are likely to have killed susceptible trees or to have declined in resistant trees. Transfer of *B. xylophilus* from a living Bonsai to surrounding trees is

not possible because they would not support vector breeding. The likelihood of transfer by other means is effectively zero.

The risks from this pathway are insignificant.

Trade in Bonsais is small relative to other categories of wood.

Seeds and cones

These can be dealt with together even though the cone may be a more likely commodity to harbour nematodes. Size alone rules out the possibility of vector carriage.

There is no evidence to suggest that *B. xylophilus* could be found in seeds or cones, although it is known that other nematodes of the family Aphelenchoididae can live in coniferous seeds (Braasch, pers. obs.). Potential transfer to Europe could occur if *B. xylophilus* was present because the cones could contain fungal growth similar to that noted in chip piles. However, it seems extremely unlikely that transport from the commodities to susceptible trees could occur in the absence of a vector final pathway.

The risks from this pathway are unknown but are likely to be small.

Roundwood with bark

The presence of both *Monochamus* spp. and *B. xylophilus* is highly likely in any roundwood that is harvested from areas containing the organisms.

Monochamus spp. will be present if trees were weakened sufficiently to enable successful oviposition and larval development; this applies to freshly cut trees and to trees damaged by other biotic and abiotic factors. This is extremely likely in pine-wilt areas where there is a superabundance of breeding material. In non pine-wilt areas, the likelihood of *Monochamus* spp. presence is dependent on other factors, such as tree harvesting programmes, fire, drought, wind throw and snow break, that would weaken trees. The continuing presence of bark provides the fullest opportunity for larvae of *Monochamus* spp. to complete development and emerge as adults. The duration of the life cycle is such that emergence in Europe following even an extended period in transportation is probable. There is no evidence for establishment of *Monochamus* spp. in exotic locations; one reason for this could be that females must be mated repeatedly in order to lay fertile eggs. Synchrony of emergence of both males and females of the same species is therefore a necessity for establishment of exotic *Monochamus* spp. in Europe.

Introduction of *B. xylophilus* into a tree can be by maturation feeding or by oviposition. In both cases survival of the nematode is possible but, following transmission by feeding, the likelihood of spread of the nematode into the trunk of the tree depends on the susceptibility of the tree, infestation being restricted to the branches in native North American *Pinus* spp. (Wingfield & Blanchette, 1984; Bedker, 1987; Bedker *et al.*, 1987; Tamura *et al.*, 1988). If the nematode is present in the trunk, it is capable of surviving and reproducing for at least one year and can thus be expected to be present on arrival in Europe. Any *Monochamus* spp. present in the logs could, thus, emerge carrying *B. xylophilus* and could transfer the nematode to European trees. The presence of bark, provided it is still suitable for oviposition, represents an increased risk because native *Monochamus* spp. might be attracted to the tree and lay eggs. Completion of development of these European *Monochamus* spp. would represent a very high risk since there would be every opportunity for further transfer to native trees.

If no *Monochamus* spp. are present in the imported wood, the nematode could only be transferred to trees by physical contact with suitable breeding substrates in Europe. This would depend on the end use of the commodity. In the case of roundwood with bark, it is possible that the wood would be square-sawn, the outer rounded wood with bark being regarded as low value wood for burning, chipping or carcassing. Such wood and the sawdust produced on cutting would contain nematodes which could survive for a considerable period of time. The

presence and dissemination of the material in the PRA area would present a constant risk of coming into contact with native trees and/or vectors (see section 2.2).

The possibilities of direct transfer of *B. xylophilus* from wood to host plants in the receiving country have been considered by some authors (Dwinell, 1986, 1990; Magnusson, 1986; NAPPO, 1986; Dwinell & Nickle, 1989). The characteristics of *B. xylophilus* (its mobility, resistance to adverse conditions, large numbers in infested wood, relationships with vectors, possibility of non-specific transmission, ability to colonize different types of wood and other substrates) indicate that, under a suitable set of circumstances, the nematodes could be introduced to the PRA area by means of wood infested only with nematodes (i.e. without the vector). Such an event has, of course, not been demonstrated in nature, even in the known areas of *B. xylophilus* distribution, but there exists experimental evidence for all the necessary steps. Any nematodes successfully introduced in this way would, however, have to become associated with a *Monochamus* species in the PRA area to ensure continued survival and dispersal. For this reason, it is virtually impossible that *B. xylophilus* could be successfully established by this means in areas where *Monochamus* spp. are absent.

Information from the outbreak of pine wilt disease in Japan shows that major jumps in the distribution of the disease have been associated with carriage of *B. xylophilus*-infested roundwood, presumably with bark, into previously uninfested areas (Mamiya, 1988).

This pathway, by providing a number of different potential ways of final transfer to EU forests, represents a very high innate risk of transfer and establishment.

There is, in fact, no international trade in this commodity from countries having *B. xylophilus* (on account of quarantine pests other than *B. xylophilus*). The effective risk is therefore zero under present regulations.

Roundwood without bark

Initial probability of either or both *Monochamus* spp. and *B. xylophilus* being present is the same as roundwood with bark. The process of debarking, however, will tend to reduce the survival of any *Monochamus* spp. present but is less likely to affect any *B. xylophilus* already introduced into the wood. Debarking within 7 days of the entry of larvae of *Monochamus* spp. into the wood was regarded by Webb (1909) as a good control measure against these beetles. This reflected the physical removal of the larvae and also the reduction in food for any that had already entered the wood. Once inside the wood, the larvae are protected from physical damage during the debarking process, but may still be at a disadvantage because they require to feed on the cambium virtually until pupation. However, it can be assumed that any larvae large enough to enter the wood completely and to develop the U-shaped tunnel characteristic of *Monochamus* spp. have the potential to complete development to the adult stage.

Transfer of *B. xylophilus* to EU forests at the end of the pathway is likely if *Monochamus* spp. are already present in the wood, for the same reasons as described for *Roundwood with bark*. If *Monochamus* spp. are absent, attraction of native European *Monochamus* spp. to the wood is not possible because of the absence of bark. The potential for non-vector transfer is similar to that of roundwood with bark and depends on the end use of the commodity. Any piece of wood, off-cut or sawdust coming into contact with a standing tree, cut stump or recently felled tree in the PRA area presents a risk of nematodes transferring by active movement.

This pathway represents a high risk of successful vector transfer but, because native European *Monochamus* spp. cannot add to this risk, is of a lower overall risk than *Roundwood with bark*. The risk still remains high.

Volumes of trade in this commodity are variable and relatively low (Table 7). However, the 1990 peak of 77 592 tonnes represents a large number of individual pieces of wood which, combined with the high innate risk in this category, still poses a significant threat.

Sawn wood

The likelihood of either *Monochamus* spp. or *B. xylophilus* being present is identical to the previous category during the early stages of processing, prior to the wood being sawn. The process of sawing reduces the survival of populations of *Monochamus* spp. present because some of the individuals will be killed during sawing and others will be discarded with the rounded outer portion of the stem. Even those that survive the sawing process may be more vulnerable as a result of greater exposure to adverse conditions, such as surface drying of the wood and partial exposure to surface damage. The overall likelihood of *Monochamus* spp. survival to complete the pathway is therefore lower than that for *Roundwood without bark* but, because trees tend to receive multiple attacks in the field, not all larvae are likely to die and thus there is still a high probability of survival of some *Monochamus* in at least one of the pieces cut from an infested trunk. This is evidenced by the discovery of *Monochamus* spp. in wood imported to Europe under the previous Mill Certificate of Debarking and Grubhole Control programme. Green wood will remain suitable for completion of the larval development of *Monochamus* spp. during the duration of any transfer to Europe.

The probability of survival and transfer of *B. xylophilus* in the absence of *Monochamus* spp. is identical to that for *Roundwood without bark* and depends on the end use of the commodity. Any piece of wood, off-cut or sawdust coming into contact with a standing tree, cut stump or recently felled tree in the PRA area presents a risk of nematodes transferring by active movement.

This pathway represents a high risk of successful transfer, but is lower than that for *Roundwood without bark* by virtue of the lowered probability of *Monochamus* spp. being present. When volumes of trade are taken into account (Table 7), the risk becomes highly significant so that even a very small initial infestation level multiplies up to a large number of infested pieces of wood.

Packaging

The term *Packaging* is used here to cover a number of commodity classes that are linked by having the same innate likelihood of successful transfer of *Monochamus* spp. and/or *B. xylophilus*. It includes packing cases, crates, drums, pallets, box pallets and other load boards as described in EU Council Directive 77/93/EEC. This broad category therefore includes all sawn wood that is used to package other commodities and is subject to a similar innate likelihood of having infestation by *Monochamus* spp. or *B. xylophilus* as *Sawn wood* itself.

However, there are two factors that affect the overall likelihood of presence of the organisms. Firstly, the wood will tend to be derived from lower-quality trees reflecting the lower value of the final product. This will increase the likelihood of infested trees being used for processing. Secondly, because some of the wood will be very thin, for example in drums or for the sides of pallets or crates, these components will have a much lower probability of *Monochamus* spp. being present. It is not known if these factors cancel each other out, so it is reasonable to assume that the overall risk from *Monochamus* spp. is similar to that for *Sawn wood*. Any residual risk remaining after processing in the mill will be the same as for *Sawn wood*.

A further factor in this pathway is the fact that the end product has a limited life within its commodity class. For example, boxes and pallets may be used a number of times but are subject to damage in transit and use and are therefore discarded frequently at the point of end use. Indeed, a British company has recently invested in machinery specifically to chip discarded pallets and other packaging wood (Anon., 1995). These chips are then sold on for pulp or for horticultural use as a mulch. Clearly, the presence of *B. xylophilus* in any of the packaging material entering an agglomerate of chips would be able to reproduce

and could potentially contaminate much of the chip pile. The same transfer possibilities as for *Wood chips* then apply.

This pathway represents a high risk of successful transfer, and is similar to that for *Sawn wood*. Volumes of wood in this category are low but, because the end use of the wood is so variable, the risk of transfer remains relatively high.

Dunnage

Dunnage, spacers and bearers combine to form a category of sawn wood that is used to support commodities physically during transportation. They can be associated with any commodity, not just wood and wood products. Dunnage tends to have little or no value as a traded commodity and, thus, is often produced from the lowest quality of wood, particularly from wood salvaged after damage in the forest. The initial probability of infestation by either *Monochamus* spp. or *B. xylophilus* is therefore higher than for all other categories of wood. The process of cutting the wood for use as dunnage will tend to reduce slightly the likelihood of *Monochamus* spp. being present but, because there is no standard size and the wood is often used for a single specific purpose, it may remain as roundwood. The fate of dunnage once it has been used for its original purpose is highly variable and is not under any form of control. It therefore poses a high phytosanitary risk.

Innate probability of successful transfer through the entire pathway is therefore higher than for *Roundwood without bark*; both *Monochamus* spp. and *B. xylophilus* would survive readily and emergence with the vector could result in transmission to European conifers. Wood without the vector would be subject to the same constraints of non-vector transfer that applies to all nematode-infested wood.

There are no reliable statistics on volumes of dunnage but each ship arriving in Europe could potentially carry wood infested with both *B. xylophilus* and *Monochamus* spp.

Wood chips

The process of chipping wood will kill the majority of *Monochamus* spp. that may be present in the wood. Any vectors that survive the initial chipping process will be unlikely to complete development because the wood will tend to be too small to support the full larval and pupal gallery. There is therefore very low risk of vector transfer with wood chips.

There is ample evidence that *B. xylophilus* reproduces successfully in wood chip piles and could be present in larger numbers at the end of transportation than at the start (Dwinell, 1986; Kinn, 1986; Tomminen *et al.*, 1991; Halik & Bergdahl, 1992; Panesar *et al.*, 1994). Wood chips therefore represent a significant inoculum source for potential transfer to European forests. Dauer juveniles have been demonstrated to occur in wood chips (Tomminen *et al.*, 1991) and to increase as a response to abrupt changes in temperatures (Tomminen & Akar, 1990). In addition, the presence of the cerambycids *M. galloprovincialis* and *Acanthocinus aedilis* in pine chips infested with *B. mucronatus* resulted in formation of dauer larvae, and, in the case of *M. galloprovincialis*, successful invasion of the beetles (Tomminen, 1992). Entry of *B. xylophilus* dauer juveniles to tracheae of *M. scutellatus* and to the non-specific potential vector cerambycids *Rhagium inquisitor* and *Asemum striatum* and the weevil *Hyllobius pales* artificially trapped in infested chips was demonstrated by Tomminen & Akar (1990). Attraction of *Hylastes brunneus* (Coleoptera: Scolytidae) and *Hyllobius abietis* to heaps of sawdust has been noted and, occasionally, thousands of individuals may be present (Lekander, 1965). As with sawdust, freshly cut timber and pulpwood stacks also attract *Hylastes* spp. and probably also *Hyllobius* spp. (Lindelow, 1992). It is possible that chips could act in a similar way, leading to contamination of any beetles trapped in nematode-infested chip piles. The fact that these beetles also breed in freshly dead trees points to possibilities of nematode transmission during

oviposition. Linit *et al.* (1983) compared the numbers of nematodes carried by the known vector *M. carolinensis* with those carried by a number of other cerambycid and weevil species emerging from nematode-infested *P. sylvestris* and showed that the former carried on average more than 19 000, while the non-specific vectors carried a maximum of 300. The possibilities of vector transport from chips after they have arrived in Europe cannot therefore be ruled out.

The increasing use of wood chips as soil-covering mulch and as a surface layer on paths increases the probability of infested chips coming into contact with native trees or native insects. The possibilities of movement of nematodes from chips buried in soil into tree roots were discussed in section 2.2. In summary, there is some experimental evidence for transfer of nematodes from wood chips to susceptible trees when chips are buried among wounded or unwounded tree roots (Kiyohara & Tokushige, 1971; Halik & Bergdahl, 1992). There is also evidence that nematodes from chips can move to freshly cut tree stumps (Braasch, pers. comm.). Movement to adjacent trees may be possible by root grafting but permanent establishment of *B. xylophilus* depends on interaction with *Monochamus* spp. for transfer between trees without root contact within and between forest blocks.

This route of transmission therefore presents a low to moderate risk. Trade is very low at present.

Sawdust

No *Monochamus* spp. would survive the sawing process and remain in shipments of sawdust.

B. xylophilus can reproduce in sawdust provided that sufficient fungal mycelium is present to support nematode growth (Braasch, pers. comm.). The question of whether the nematodes could survive the heat generated by the friction of the saw blade has not been investigated. Even if nematodes were present in sawdust, the most common end uses (burning and manufacture of particle boards) would not permit establishment in the PRA area. If used as mulch or animal bedding, the possibility of reaching the ecosystem approaches that of wood chips.

The overall innate risk from this pathway is probably very low.

Isolated bark

Although larvae of *Monochamus* spp. require the inner bark to feed they are unable to complete development without boring into wood. There is therefore no risk of vector transfer via isolated bark.

B. xylophilus will reproduce in the inner bark layer in live cambial cells (Evans *et al.*, 1993). It will also reproduce on any suitable fungal contaminants present on the bark. This pathway therefore has risks very similar to those for wood chips, particularly as the only significant international trade in bark is for use as a mulch in agriculture, horticulture and gardening. Bark that has not been composted represents the highest risk within this category because it will not have been subjected to the high temperatures associated with the composting process (OEPP/EPPO, 1994a). The same constraints for further transmission of the nematode to mature trees suitable for vector transfer apply (see section on *Wood chips*).

This route of transmission represents a similar risk to that for wood chips.

There is no trade in this commodity from countries where *B. xylophilus* occurs and therefore the risk is zero under current EU regulations.

Green-wood products

This category applies to items that are made from green wood but which are not covered under the categories above, for example, rustic garden furniture and buildings, shelving, etc. sold as a

finished commodity. If *Monochamus* spp. are present, the risks for transfer of nematode are the same as for any other green-wood category and can be regarded as high.

Presence of *B. xylophilus* in the absence of *Monochamus* spp. falls into a very low-risk category because the wood is sold as a finished product that is extremely unlikely to come into contact with living susceptible trees in the field.

It is difficult to assess the overall risk from this category because it is possible that any form of wood from roundwood with bark to sawn wood could be present. However, the high value of the products and the specialised end use will tend to lower the risk in all cases.

Accidental contamination by Monochamus spp.

It is possible that adults of *Monochamus* spp. could be present in a timber shipment accidentally. *B. xylophilus* present within the beetles could survive and the beetles could still be viable after the journey. For nematode transmission to take place, the beetles would have to undertake maturation feeding on the crowns of living trees or else oviposit. Since it is extremely unlikely that sufficient numbers would be present for mating to be probable, any eggs laid would be unlikely to be viable.

Entry by this route is therefore unlikely.

Factors affecting entry to pathways and transfer to EU forests

Entry to pathway

The likelihood of any tree having *Monochamus* spp. and *B. xylophilus* is greatest in pine-wilt areas, reflecting the densities of attacked trees and the higher numbers of nematodes in trees killed by the disease. In all areas, if trees are damaged by various biotic and abiotic factors or are felled during timber extraction, they will be susceptible to oviposition by *Monochamus* spp. with consequent transmission of *B. xylophilus*.

Throughout North America, *Monochamus* spp. have a long history as secondary pests causing degradation of timber through the boring activities of the larvae and the introduction of blue-staining fungi (Morley, 1939; Raske, 1973; Gardiner, 1975). Any coniferous forest has a high probability of a resident population of *Monochamus* spp. and, by association, of *B. xylophilus*. The widespread presence of *B. xylophilus* noted in surveys in North America indicates that there is probably no region of coniferous forest that can be regarded as free from the organisms (Dropkin *et al.*, 1981; Kondo & Taylor, 1986; Rutherford & Webster, 1987).

Within a forest, the numbers of trees attacked by *Monochamus* spp. will be related to the availability of breeding material arising from biotic and abiotic damage to the trees. In some cases, such as major wind storms, fires, snow damage, etc. there will be a large number of trees available and the population of *Monochamus* spp. will increase accordingly. This relationship between availability of breeding resources and population density of *Monochamus* spp. is recognized in the recommendations for management of these pests by early removal of weakened or recently felled trees (Raske, 1973). However, the severity of attack depends on local conditions and is generally not predictable.

Even if a low global average incidence, as quoted in North American surveys (Marshall & Favinger, 1981; Riedel, 1981; Esser *et al.*, 1983; Kondo & Taylor, 1986; Van Sickle, 1991; Bowers *et al.*, 1992), is taken as a measure of the size of *Monochamus* spp. populations, the volume of wood exported still results in large numbers of infested pieces of wood arriving in Europe. In addition, the incidence of infested wood may be much higher on a local basis because of large fluctuations in severe damage conditions that would favour oviposition by *Monochamus* spp.

Transfer from pathway to EU forests

A number of factors will influence the likelihood of transfer and successful establishment of *B. xylophilus* in EU forests. In some cases, these will act in concert to increase further the probability of establishment. However, in the long term, the risk to Europe depends on whether establishment can occur once over time and therefore it is not the risk from a pathway on a single occasion that is important but the accumulated risk of that, and other pathways, over the entire period over which trade takes place that influences the ultimate risk of establishment.

Degree of afforestation and patterns of trade

The combination of increasing coniferous afforestation and changing patterns of trade in wood in recent years (greater volume, more rapid movement, more commodity types, more pathways) has considerably increased the likelihood of introduction. The fact that North American wood has been imported to Europe for more than 200 years without introduction of *B. xylophilus* should not be taken to indicate that introduction in the future is unlikely.

Proximity to EU forests

The proportions of land covered by forests of conifers, especially *Pinus*, clearly influence the probability that the nematode could find a suitable breeding site in Europe. The primary risk arises from the distance over which *Monochamus* spp. have to fly in order to find trees for maturation feeding or oviposition. Initial risk will arise at the point of entry to the country when cargoes are being offloaded at the ports. In some cases these will be close enough to forests to pose a direct risk of transfer. Clearly, the Nordic countries with an extremely high forest cover would be at highest risk from transfer direct from the ports.

A greater risk is posed by transfer after the wood, in whatever form, has reached an inland destination, which may be a storage area or the point of end use. It is not possible to control the fate of wood at these final points and, thus, any piece of wood containing the vectors and *B. xylophilus* could end up near suitable forest breeding sites. The likelihood of this happening is increased if the forest is large but, as discussed below, there is also a further risk factor in relation to regional temperature and tree conditions.

*Transfer of *Bursaphelenchus xylophilus* by maturation feeding of *Monochamus* spp.*

Adult *Monochamus* spp. emerging from wood imported into Europe will attempt to carry out their maturation feeding as a means of sustenance and, for the females, egg maturation. Adults carry out this form of feeding throughout their lives. *B. xylophilus* will be transferred to trees during this process but further development of the nematodes will depend on the susceptibility of the tree into which they have been introduced. Tree species that are susceptible intrinsically may still not die from wilt symptoms unless the temperature is high enough and tree resistance is low enough to sustain reproduction of *B. xylophilus*. If so, these trees may not exhibit wilt symptoms for years and will constitute a source of nematodes that would be very difficult to detect and manage. Thus, the likelihood of nematode development and expression of wilt symptoms arising from primary transfer of *B. xylophilus* to European conifers during maturation feeding of *Monochamus* spp. is greatest in the southern Member States (see sections 5 and 8). Taking the EU as a whole therefore, this route of transfer and establishment of *B. xylophilus* is only of significance in the southern Member States.

Transfer of Bursaphelenchus xylophilus by oviposition of Monochamus spp.

Female *Monochamus* spp. will attempt to lay eggs even though they may not have been mated. In these cases, the eggs laid will be infertile and no further development of the exotic *Monochamus* spp. will take place. However, the process of cutting an oviposition scar may result in transfer of *B. xylophilus* into the tree (Edwards & Linit, 1992). Thus, each female emerging in Europe and carrying *B. xylophilus* poses a risk of transmission of the nematode by the oviposition pathway. *B. xylophilus* will reproduce readily in dead or damaged trees of most species of conifer and therefore would become established in the wood. Further transfer between trees will depend, to a very great extent, on the presence of native *Monochamus* spp. that may oviposit in the same tree. The presence of *Monochamus* spp. throughout mainland Europe indicates that such an event is possible in any forest in these locations. Forests in UK and Ireland do not have resident *Monochamus* spp. and therefore it would be necessary for both the nematode and its vector species to establish for further transfer of *B. xylophilus* to take place.

Although forest hygiene measures to avoid timber degradation tend to keep vector populations low, there is also an increasing trend in Europe for retention of deadwood as a valuable resource for increasing biodiversity, especially for invertebrates. For example, German and Norwegian work indicates that the number of species of saproxylic insects increases in relation to the amount of deadwood left in a forest. German research, in particular, distinguishes between the low conservation value of managed versus natural forests, attributing this to only 1–5 m³ ha⁻¹ deadwood in the former and 50–200 m³ ha⁻¹ in the latter (Albrecht, 1991). Increased availability of deadwood over much larger areas was the principal recommendation. Other German work recommended an increase from the current 1–3 m³ ha⁻¹ to a level of 5–10 m³ ha⁻¹ of deadwood, with half of this as standing trees of > 20 cm dbh (Ammer, 1991). In all cases, although there is ultimate conservation value from leaving deadwood, there is an initial period of 1–3 years when bark beetles and cerambycid beetles colonize the trees and therefore increase in numbers locally.

Transfer by oviposition has a high probability of success and is therefore the likeliest route by which *B. xylophilus* could become established in Europe.

Non-vector routes of transfer

B. xylophilus has a number of characteristics that indicate that its introduction to Europe by means of any type of imported untreated wood would, over time, be a highly probable event, even in the absence of specific insect vectors. It can survive in wood for long periods of time after entry; it has been shown to be capable of moving (by its own means) out of pieces of wood into healthy susceptible trees via their roots or into fresh cut stumps of trees; it can move onto the bodies of adjacent insects, including non-specific vectors. The unrestricted import of infested wood could therefore lead to a potentially dangerous situation in which pieces of wood of many types (sawn wood, off-cuts, wood chips, sawdust) carrying nematodes would be transported throughout the region and would offer multiple occasions for transfer to native trees.

PART C. RISK MANAGEMENT

The PRA concludes with an evaluation of the risk management options to reduce the likelihood of successful transfer of *Bursaphelenchus xylophilus* to the EU.

The forest resource within the EU is large, with a total area of conifers exceeding 45%. It is not possible to give such a resource a precise monetary value but, for example, the 1994 exports from Sweden alone amounted to 1,062 million m³, which was 71% of the total North American

export to Europe, reflecting the major importance of the EU forest estate in terms of international trade. Regional differences in risk would, of course, affect the likely loss from wilt expression, especially for the more southerly Member States of the EU. However, the important point for risk management is the recognition that establishment of *B. xylophilus* anywhere within the Community places the most vulnerable forests at risk. For example, establishment of *B. xylophilus* in northern Europe would result in restrictions on trade between regions of the Community and would carry a monetary cost. In this respect, it is not considered that regional risk management options are appropriate.

There are a number of risk management options that would provide benefits regardless of which pathway in Fig. 10 was being considered. We therefore distinguish between management in the forest, management during processing, management during transportation and, finally, management after arrival in Europe.

Risk management options

Option 1. Management in the forest to avoid selection of infested wood for felling

Preventive measures in the forest could be established to select healthy trees on the basis of scientific criteria, followed by felling, removal and debarking of selected trees before the flight period of *Monochamus* spp. Similar methods for reduction in the damage caused by *Monochamus* spp. are already established in forest management but the stringency applied is low and does not seek to avoid attack but to harvest material before beetles develop to the stage of larval entry into the wood (Raske, 1973; Webb, 1909).

The efficacy of such methods (forest selection) for avoidance of *B. xylophilus* colonization will depend on extremely high reliability in selection of healthy trees and strict inventory control to ensure rapid processing of wood before the flight period of *Monochamus* spp. to prevent attacks both in the forest and in the sawmill. Selection of healthy, unattacked, trees during the winter period when trees are not growing rapidly is unlikely to prove reliable, even if the trees show no external symptoms of attack. Selection, by appropriate methods, would therefore need to be carried out during the growing season when a number of criteria for tree health could be assessed and suitable trees marked for later felling (visual inspection is not sufficient). For example, oleoresin pressure measurement has been used in Japan as a reliable indication of tree health; other techniques such as infra-red thermography, foliage analysis, etc., could also be evaluated and employed if shown to be reliable. Assessment of background populations of *Monochamus* spp., using various trapping techniques, would be a further check to determine the risk posed at the particular forest block. Marking of suitable trees would have to be as late in the flight period of *Monochamus* spp. as possible to avoid the risk of later damage to selected trees that would make them vulnerable to attack after the selection procedure. Marked trees would then be felled during the winter period, ensuring that this was done when there was no flight of *Monochamus* spp. Provided that the trees are debarked immediately on reaching the sawmill, there would be no further risk of attack by *Monochamus* spp. and the trees could be guaranteed to be either free from the vector or, because they will not be subject to risk of transmission by oviposition, to have an extremely low likelihood of *B. xylophilus* being present (any branches that may have been inoculated with *B. xylophilus* during maturation feeding would be removed on felling).

A further consideration in forest selection is whether all conifer genera or species are equally susceptible to attack by *Monochamus* spp. and/or *B. xylophilus*. There is a considerable body of data on the susceptibility to *B. xylophilus* of living trees of a number of species in the genus *Pinus*, and in a number of other coniferous genera (see section 4.1, Table 2). This information concentrates on potential effects on living specimens of a given tree species and does not differentiate between growth of the nematode in dead

trees. Section 2 deals with the biological characteristics of the nematode and concludes that it can grow in a wide range of media, particularly when suitable fungal species are present. The question of risks from different tree species therefore revolves around whether the tree can support nematode growth and reproduction and also on whether *Monochamus* spp. can also be supported. The evidence presented in section 2 indicates that most conifer genera can support reproduction of *B. xylophilus* on dead trees. Indeed, there is no clear evidence that any conifer species have a complete lack of susceptibility and hence present an effectively zero risk. *Thuja plicata* has been exempted from heat-treatment requirements because of the circumstantial evidence that neither *B. xylophilus* nor *Monochamus* spp. has been recorded breeding successfully in the tree. In addition, the wood is so distinctive that it can be easily separated for the purposes of phytosanitary inspection. It has been suggested that other species of conifers, particularly *Tsuga heterophylla*, should also be regarded as resistant to the organisms. However, the evidence in this case is less clear from both field and laboratory experiments carried out by Canadian scientists (Ring *et al.*, 1992). Results indicated that *Monochamus* spp. can oviposit in *T. heterophylla* but that survival of larvae is poor. Further experiments and field observations would be necessary to ensure that these results would be applicable across the full geographic range of both *T. heterophylla* and *Monochamus* spp. The question of exemption of conifer species from the full phytosanitary requirements for conifers needs to be addressed on a case-by-case basis. Unequivocal evidence is required that neither *B. xylophilus* nor, particularly, *Monochamus* spp. can breed successfully in the wood.

Direct control of *Monochamus* spp. to prevent maturation feeding and oviposition has been practised in Japan for a number of years (Morikawa, 1976; Yamane, 1981; Ishikura, 1983; Ikeda, 1984). Aerial application of insecticides, particularly of fenitrothion, has given some success but appears to be of value only in reducing the incidence of pine wilt rather than completely preventing attack. Such a method does not therefore provide a reliable or environmentally sustainable method of risk management for international trade in coniferous wood.

Option 2. Management during processing

If the stringency of selection in the forest is high, there should be little risk of infested material entering a sawmill. However, it is recognized that development of forest selection methods with sufficiently high reliability may prove impractical, so other measures will have to be considered that acknowledge the potential presence of *B. xylophilus* and *Monochamus* spp. in the wood.

Option 2.1 Inspection for grub holes to ensure freedom from Monochamus spp.

Measures to inspect wood during the cutting process in the mill are possible as part of normal quality control checks. For phytosanitary purposes, additional stringency would be needed, by increasing the level of inspection, allowing for possible occlusion of grub holes with sawdust, etc. This system has already been used in Canada under the previous Mill Certificate of Debarking and Grub Hole Control scheme and was refined under the Integrated *Monochamus* Eradication Programme proposed by Canada during 1994. The essence of the method is to avoid the presence of the vector and, thus, to eliminate the main pathway by which *B. xylophilus* could reach Europe.

It is clear from failures of the previous scheme that success depends as much on avoidance of infested material as on the quality of grub-hole elimination during processing. It seems unlikely that it can be improved sufficiently to act as a stand-alone scheme without increasing the level of inspection to such a degree that it would be impractical to administer. The scheme could be considered as an useful cross-check to assess the quality of forest selection but not as a primary system for risk management.

Option 2.2 Heat treatment

Heating wood to a core temperature of 56°C for 30 min has been shown to be an effective and guaranteed measure to eliminate both *B. xylophilus* and *Monochamus* spp. (Anon., 1991; Evertsen *et al.*, 1991). It is the only proven method for large volumes of wood and is the current measure required by the EU for conifer wood originating in *B. xylophilus*-infested countries. When carried out correctly, it gives the highest possible level of assurance of phytosanitary safety. Application of the minimum heat requirement does not change the moisture content of the wood and it is therefore necessary to have a means of certification to indicate compliance with the treatment. This places the onus on regulatory authorities to ensure that mills carrying out heat treatment do so according to the strict standards necessary. An infringement of the system in Canada during 1994 was a major worry because, despite frequent inspections by the regulatory authorities, failure of various control systems in the heating chambers were missed and only became apparent when living cerambycid beetles were found during port inspection in the UK. Temperature-sensitive indicators were developed to prototype stage during the EU/Canada heat treatment research programme but further development to a working system was not carried out (Evertsen *et al.*, 1991; Evans, 1992).

Such a system could be considered for future use within a heat treatment regime. Kiln drying, when wood is heated to the minimum temperature requirement for phytosanitary purposes during the process of drying, is an extremely effective procedure because it provides a measurable change in the wood that can be ascertained by phytosanitary inspectors in the receiving country.

Option 2.3 Chemical treatment

The use of fumigation or pressure impregnation under appropriately controlled conditions is known to kill both insect and fungal infestations in wood (French, 1970; Leesch *et al.*, 1989). There is also evidence that the chemicals employed, such as methyl bromide and phosphine, will kill *B. xylophilus*. It can therefore be assumed that established methods for wood treatment against insects and fungi would be effective against *B. xylophilus*.

There are well developed procedures for fumigation as a phytosanitary measure and it would be necessary to ensure that an international standard was followed (OEPP/EPPO, 1994b). Of particular concern is the need to maintain a sufficiently high temperature (usually > 10°C) to volatilize the gases fully. Pressure impregnation has been developed primarily as a wood preservative treatment and was not designed specifically for phytosanitary purposes. Nevertheless, the conditions of high temperature and pressure should be sufficient to kill both *B. xylophilus* and *Monochamus* spp. regardless of the toxic chemicals employed. There are a number of different protocols available depending on the end use of the wood and it would therefore be necessary to ensure that a minimum standard was designated if the treatment was to be carried out to kill *B. xylophilus* and *Monochamus* spp.

Option 2.4 Other treatments

Experimental trials of other direct intervention treatments have been carried out. These include radio-frequency drying of wood (Dwinell *et al.*, 1994), and microwave treatments (Kishi, 1975). Neither of these has been developed to full-scale operational use but they could be considered as viable methods on the basis of further evidence of efficacy.

Option 3. Management during transportation

If any categories of wood are likely to attract vector insects during transportation, then the risk of transfer to European forests is increased. This applies particularly to chips that may contain

sufficient fresh bark to attract *Monochamus* spp. Thus, transportation in closed containers could be considered as an additional option to control the movement of wood to its final destination. It is not viable as a 'stand-alone' option.

This would not be a viable option for any category of wood that could enable *Monochamus* spp. to complete development and to emerge as an adult in Europe.

Option 4. Management to control the end use of imported wood within Europe

Wood containing *Monochamus* spp. and *B. xylophilus* poses a threat by direct transfer of the nematode to trees during maturation feeding or oviposition. The only way to avoid this is to ensure that the wood is kept several kilometres from the nearest forest.

A further risk arises from wood containing *B. xylophilus* alone. Although such wood presents a low risk intrinsically, there are possible pathways by which the nematode could reach suitable breeding material. It would be theoretically possible to control the end use of imported wood to prevent round, sawn or chipped wood from being used in forest locations. However, the difficulty of ensuring compliance with control of end use of wood makes these measures impossible to consider in practice.

Applicability to particular pathways

The risk management options above apply to all categories of wood but the degree to which they could be used in practice varies with the categories of wood in the pathways shown in Fig. 10.

Although forest selection should provide assurance for all pathways, it is only likely to have any potential when the value of the final product justifies the costs involved in pre-selection of trees in the forest. It is therefore appropriate for round wood without bark, sawn wood and packing wood.

The low value of dunnage and, to a lesser extent, wood chips, and the tendency for these categories of wood to be produced from salvage and poor quality wood makes it unrealistic to require that they should derive from a forest selection scheme. In these cases options 2.2 or 2.3 are the only practical methods.

Conclusions on risk management

The level of phytosanitary assurance of any method for reducing the incidence of both *Monochamus* spp. and *B. xylophilus* is related to the consistency with which the method is applied over the long term. The degree of quality control required in all cases is extremely high and requires to be consistently carried out over time. It differs considerably between the procedures, reflecting the fact that failures may lead to higher probabilities of successful transfer for some methods than for others.

Forest selection is a scientifically sound and environmentally benign method based on common practice in forest pest management. However, in terms of quality control, it is the most difficult to carry out consistently over time. Failures in the previous Mill Certificate of Debarking and Grub Hole Control scheme, which included avoidance of degraded wood when felling trees, indicate some fundamental problems in carrying out selection procedures consistently. On the other hand, it should be possible to develop, through increased stringency in objective selection criteria, a method for pre-selection, marking and winter cutting that could be quality-checked at a number of stages during processing. Inclusion of the procedure in section 2.1 would be one of these quality checks. In addition, random samples for the presence of *B. xylophilus* should be included to assess the potential for transfer of the nematode to the trunks of trees as a result of maturation feeding by *Monochamus* spp. in the forest, a possibility even for healthy trees.

Any failure of the system (presence of grub holes and/or *B. xylophilus*) would indicate that forest-selection procedures were not being carried out effectively and would require a fundamental re-appraisal of the methods. Consideration of this method of management assumes a naturally low background level of *B. xylophilus* and *Monochamus* spp. and is therefore not applicable to areas where epidemic wilt disease is present or is potentially present.

Conclusion on option 1. Although it may be difficult to develop objective tree-selection criteria, forest selection should be given serious consideration for further development, provided that criteria for selection of trees can be developed and are verified in advance of full-scale use in the field. It is not applicable to areas where pine wilt is present.

Management during processing (methods in sections 2.1 to 2.3) are essentially procedures that have been used previously or, with some further development, offer potential for wider use in the future.

Grub-hole elimination is theoretically an effective method for avoiding wood infested by *Monochamus* spp. but does not guarantee freedom from *B. xylophilus*. It does not offer sufficient quality assurance to be a 'stand-alone' method but, in conjunction with option 1, it would provide a quality control check.

Conclusion on option 2.1. Grub-hole elimination should only be considered as an additional quality control check for option 1 and not as a stand-alone method of risk management.

Heat treatment is an effective and proven method of killing both *Monochamus* spp. and *B. xylophilus*. Unlike the other methods considered here, heat-treatment regimes have been developed, through detailed joint EU-Canada research, specifically for the purpose of killing *B. xylophilus* and *Monochamus* spp. This has resulted in the establishment of the minimum criteria required for phytosanitary safety, thus reducing the amount of heat needed to give complete pest elimination. This emphasis on minimum heat has required quality control to be developed for each heating chamber and, thus, the method relies heavily on adequate and frequent checks that all criteria are being complied with. Failure of this system carries a high risk because presence of grub holes and/or fungal contamination are ignored by phytosanitary inspectors who assume that treatment has been carried out. Some improvements to quality assurance methods could therefore be carried out.

Conclusion on option 2.2. Heat treatment is the most effective direct-intervention method for eliminating all risks from B. xylophilus and Monochamus spp. It has been refined to concentrate on minimum heat requirements but, consequently, carries a higher risk of quality control failure. However, it is certainly the method of choice for risk management, but requires further appraisal of methods to ensure compliance with the set criteria.

Chemical treatments offer viable direct intervention procedures for eliminating both *B. xylophilus* and *Monochamus* spp. Fumigation with lethal chemicals, such as methyl bromide or phosphine, has been shown to be effective in killing both organisms. Precise protocols for these procedures are available but, in all cases, the dependence on suitably high temperatures for volatilization of the gases involved requires very careful control over procedures. These should be included in detailed protocols specifically for eliminating *B. xylophilus*. Fumigation is a particularly suitable method for treating wood chips. Pressure impregnation is a viable alternative to fumigation but international standards for phytosanitary purposes need to be determined and agreed.

Conclusion on option 2.3. Chemical treatments offer reliable alternatives to heat treatment and could be developed for full-scale use. Particular attention needs to be paid to the temperature of the wood during fumigation procedures. Pressure-impregnation protocols need to be tailored for phytosanitary purposes. Quality assurance relies on certification,

which should be provided only by specialized contractors overseen by national regulatory authorities.

Other treatments offer potential for eliminating *B. xylophilus* and *Monochamus* spp. but would need to be evaluated fully before they could be introduced as viable options. However, research on such methods should be encouraged in order to increase the number of options that are available for international plant quarantine.

Conclusion on option 2.4. Other treatments should be investigated further with a view to development as viable alternative phytosanitary methods.

Management during transportation does not provide a 'stand-alone' method for risk management. Its value lies in avoidance of further potential contamination *en route* and applies particularly to transportation of wood chips.

Conclusion on option 3. Management during transportation has value only in preventing re-infestation of wood that is already known to be free from B. xylophilus and applies only to wood chips.

The above risk management options offer a range of methods for avoidance or elimination of *B. xylophilus* and *Monochamus* spp. All methods require some refinement to increase the level of quality assurance but, provided this is carried out with high stringency, they should then offer a suite of procedures that would increase the choice available for exporters in countries where *B. xylophilus* is present.

Risks of failure

The pathway analysis has already addressed the intrinsic risks from various commodities of wood. Each method of ensuring freedom from *B. xylophilus* and *Monochamus* spp. may fail in practice and would lead to the full potential of the intrinsic risk being realized.

Option 1 carries a high risk of failure because of the difficulty of applying forest-selection methods consistently over both time and location. The consequences of this failure are greater than for intervention methods because, by definition, the wood will be infested when it leaves the exporting country.

Option 2.1 serves as a quality control check on option 1 and is not regarded as sufficiently reliable as a stand-alone method.

Options 2.2 to 2.4 have a lower risk of failure because they rely on normal engineering standards for quality assurance. Failure of the system, such as that noted for the Heat Treatment regime in Canada during 1994, could lead to serious consequences because there would be no further check on arrival in Europe. However, failure does not automatically result in infested wood leaving the exporting country because the wood may have been free from the pests in the first instance.

PART D. OVERALL CONCLUSIONS OF THE PRA

Key conclusions

The key conclusions from this PRA are as follows.

1. *Bursaphelenchus xylophilus* is a quarantine pest justifying the use of phytosanitary measures to exclude it from the PRA area. This conclusion is based on the fact that:

- *B. xylophilus* does not occur in the PRA area;
- the entire PRA area is suitable for establishment of *B. xylophilus*;

- susceptible host species occur universally in the PRA area;
 - suitable vector insects occur in the PRA area;
 - B. xylophilus* is of potential economic importance to the PRA area.
2. Pine wilt can be expected in the Mediterranean and continental dry regions.
 3. *Bursaphelenchus xylophilus* is known to damage tree species of economic importance in the PRA area.
 4. The presence of *Bursaphelenchus xylophilus* would be prejudicial to Community trade.
 5. The risk from *Bursaphelenchus xylophilus* to the PRA area varies with different elements of each of the identified pathways:
 - origin;
 - commodity (including tree species);
 - volume of trade;
 - end use.
 6. The major risk comes from commodities carrying the vector insect but, even in the absence of the vector, the risks can be significant when volumes of trade are large (accumulated over time).
 7. All commodities of coniferous wood from infested areas/sources warrant phytosanitary measures.

Management options

Several potentially effective management options were considered, among which three:

- forest selection,
- heat treatment (including kiln-drying),
- chemical treatment,

could give adequate phytosanitary protection. Their precise implementation needs to be developed further, particularly in the case of forest selection which is unproven and does not rely on engineering controls for quality assurance.

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Analyse du risque phytosanitaire (PRA) présenté pour le territoire de l'Union européenne (en tant que zone PRA) par *Bursaphelenchus xylophilus* et par ses vecteurs dans le genre *Monochamus*

L'analyse du risque présenté pour les Etats de l'Union européenne dans le cas d'une éventuelle introduction de *Bursaphelenchus xylophilus* et de ses vecteurs du genre *Monochamus* a été faite sur la base d'un recueil de données biologiques, climatiques et commerciales. Les risques présentés par diverses filières commerciales sont évalués et des mesures phytosanitaires susceptibles de les réduire sont proposées. L'analyse du risque phytosanitaire, réalisée en conformité de la Directive PRA no. 1 de l'OEPP, arrive à la conclusion que ces organismes nuisibles présentent un grave danger pour les forêts européennes de conifères.

Анализ Риска Вредителя (PRA) для территорий Европейского Союза (как область PRA) на базе *Bursaphelenchus xylophilus* и его переносчиков в род *Monochamus*

Анализ риска для стран Европейского Союза возможного попадания *Bursaphelenchus xylophilus* и переносчиков в род *Monochamus* был сделан на основе сбора необходимой биологической, климатической и коммерческой информации. Был оценен риск, представленный различными торговыми путями, и предложены фитосанитарные меры, позволяющие уменьшить такие риски. Заключение PRA, основанного на директиве ЕОЗР N°1, посвященной PRA, сводится к тому, что эти вредители представляют собой серьезный риск для европейских хвойных лесов.

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