EPPO Datasheet: *Bursaphelenchus xylophilus*

Last updated: 2020-04-22

IDENTITY

Preferred name: *Bursaphelenchus xylophilus*
Authority: (Steiner & Bührer) Nickle
Taxonomic position: Animalia: Nematoda: Chromadorea: Rhabditida: Aphelenchoididae
Other scientific names: *Aphelenchoides xylophilus* Steiner & Bührer, *Bursaphelenchus lignicolus* Mamiya & Kiyohara
Common names: pine wilt disease, pine wood nematode, pinewood nematode
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EPPO Categorization: A2 list
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EU Categorization: Emergency measures, Quarantine pest ((EU) 2019/2072 Annex II B)
EPPO Code: BURSXY

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Notes on taxonomy and nomenclature

Bursaphelenchus xylophilus was first described in the USA as *Aphelenchoides xylophilus* (Steiner & Bührer, 1934). Later it was described, as *B. lignicolus*, when determined 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 of a mucro (finger-like projection) in the female tail terminus in the former species, which was absent in the latter. However, an intraspecific morphological variability of this character has been detected in *B. xylophilus* populations from North America, Asia and Portugal (Wingfield *et al*., 1983; Penas *et al*., 2004; Gu *et al*., 2011; Fonseca *et al*., 2008). There has thus been much discussion about the taxonomic relationships between these two species, and also with *B. fraudulentus* Rühm, 1956, a nematode from deciduous trees in central Europe. Molecular studies of several populations of these nematodes have clearly confirmed the distinctness of the three species (Webster *et al*., 1990; Abad *et al*., 1991; Filipiak *et al*., 2019). *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 has been shown to be an introduction from North America. In Europe, this nematode species was first identified in continental Portugal (Mota *et al*., 1999) and later in Spain (Abelleira *et al*., 2011) and Madeira island (Fonseca *et al*., 2012). Another closely related species, *B. kolymensis* Korenchenko, 1980 was described from *Larix* from the far east of the former USSR. However, in 2011, Braasch *et al*. found that this species corresponds to the European type of *B. mucronatus* and designated it as a subspecies, *B. mucronatus kolymensis*, and the East Asian type as the subspecies *B. mucronatus mucronatus*.

HOSTS

Bursaphelenchus xylophilus

B. xylophilus is found mainly on *Pinus* spp. that have a wide distribution in the northern hemisphere. Nevertheless, only a limited number of pine species are considered susceptible to the nematode infection; the Far Eastern species *P. bungeana, P. densiflora, P. luchuensis, P. massoniana* and *P. thunbergii* (in their native habitats), and the European species *P. mugo*, *P. nigra*, *P. sylvestris* and *P. pinaster* are the species known to be killed by the nematode as mature trees in the field (Evans *et al*., 1996). In Spain, *P. radiata* was also considered as a host species under European conditions (Zamora *et al*., 2015). Inversely, stone pine (*P. pinea*) has never been found infected by *B. xylophilus* even in locations with high incidence of the pathogen, which has led to the assumption that it is tolerant/resistant under natural conditions (Naves *et al*., 2018). Many other species have been found to be damaged or killed by the nematode but only under experimental conditions (mainly as seedlings in glasshouses). Other conifers can also act as hosts (*Abies, Cedrus, Larix, Picea* and *Pseudotsuga*) but reports of damage are rare. Within the EPPO region, *P. sylvestris* would be the species of *Pinus* most at risk in northern and central areas, whereas *P. nigra* and *P. pinaster* would be threatened in central and southern areas.

Monochamus spp.

The *Monochamus* spp., which are of concern as known or possible vectors of *B. xylophilus,* mainly develop in *Pinus* species, but other coniferous genera can also act as hosts (see Table 1). In addition, *Monochamus* spp. will oviposit in most coniferous species, even those in which they may not be able to complete development to adult stages. The most important hosts of *M. galloprovincialis*, the European vector of *B. xylophilus,* are pines, mainly *P. sylvestris*, *P. nigra*, *P. halepensis* and *P. pinaster,* but also *P. brutia*, *P. peuce* and *P. mugo*, and spruce (*Picea*). The remaining European members of the *Monochamus* genus prefer other conifers, such as fir (*Abies*), larch (*Larix*) and spruce (Naves *et al*., 2016). Some conifers appear to be unsuitable hosts and *Thuja plicata* has insecticidal properties that prevent oviposition. Other species of *Monochamus* attack other plant hosts, such as broadleaf, coffee and cocoa trees, but are not relevant in the present context.

Host list: *Abies balsamea*, *Abies firma*, *Abies*, *Cedrus atlantica*, *Cedrus deodara*, *Cedrus*, *Larix decidua*, *Larix gmelinii var. olgensis*, *Larix gmelinii var. principis-ruprechtii*, *Larix kaempferi*, *Larix laricina*, *Larix*, *Picea abies*, *Picea pungens*, *Pinus armandii*, *Pinus banksiana*, *Pinus bungeana*, *Pinus cembra*, *Pinus clausa*, *Pinus contorta var. murrayana*, *Pinus densiflora*, *Pinus echinata*, *Pinus elliottii*, *Pinus koraiensis*, *Pinus lambertiana*, *Pinus luchuensis*, *Pinus massoniana*, *Pinus mugo*, *Pinus nigra subsp. pallasiana*, *Pinus nigra*, *Pinus palustris*, *Pinus pinaster subsp. escarena*, *Pinus pinaster*, *Pinus ponderosa*, *Pinus radiata*, *Pinus resinosa*, *Pinus strobus*, *Pinus sylvestris var. mongholica*, *Pinus sylvestris*, *Pinus tabuliformis*, *Pinus taeda*, *Pinus thunbergii*, *Pinus virginiana*, *Pinus yunnanensis* , *Pinus*, *Pseudotsuga menziesii*, *Pseudotsuga*, *Tsuga canadensis*, *Tsuga*

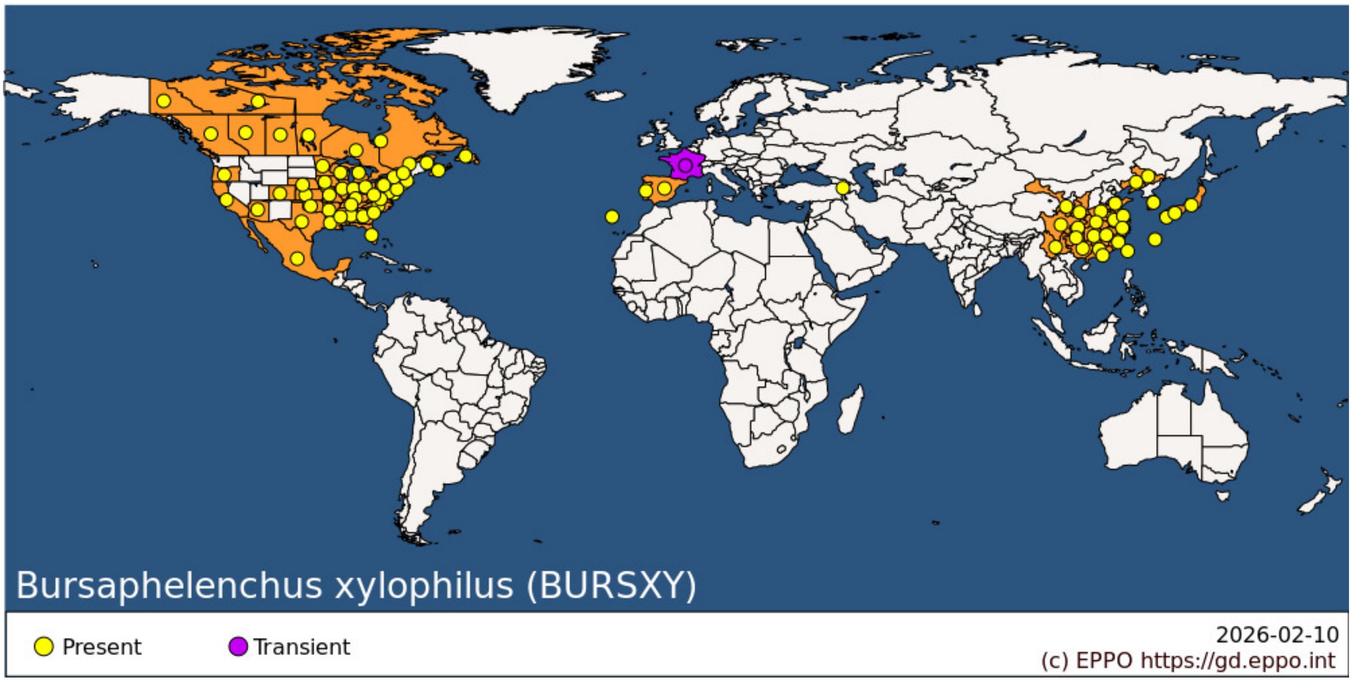
GEOGRAPHICAL DISTRIBUTION

Bursaphelenchus xylophilus

It is presumed that *B. xylophilus* originated in North America and was transported from there to the southern Japanese island of Kyushu in infected timber in the beginning of the 20th century (Nickle *et al*., 1981; Mamiya, 1983; Malek & Appleby, 1984). The fact that native American conifers are mostly resistant, whereas Japanese species are susceptible, tends to support this view. From Japan, *B. xylophilus* has spread to other Asian countries (Li *et al.*, 1983) and then, at the end of the 20th century, it was introduced in Europe, in Portugal (mainland and Madeira island) and Spain (isolated outbreaks) (Mota *et al*., 1999; Abelleira *et al*., 2011; Fonseca *et al*., 2012).

Monochamus spp.

The species of *Monochamus* which are of concern as known or possible vectors of *B. xylophilus* occur only in the northern hemisphere. A list, showing their geographical distribution, whether their range currently overlaps with that of *B. xylophilus* and whether they are known to be vectors of *B. xylophilus* is provided (Table 1). It may be noted that many species are very widely distributed, and the genus is represented in most areas of the northern hemisphere. Exceptions are the islands of Great Britain and Ireland. The genus *Monochamus* is represented elsewhere in the world, but the species concerned do not attack conifers.



EPPO Region: Armenia, France (mainland), Portugal (mainland, Madeira), Spain (mainland)
Asia: China (Anhui, Chongqing, Fujian, Gansu, Guangdong, Guangxi, Guizhou, Henan, Hubei, Hunan, Jiangsu, Jiangxi, Jilin, Liaoning, Shaanxi, Shandong, Sichuan, Xianggang (Hong Kong), Yunnan, Zhejiang), Japan (Honshu, Kyushu, Ryukyu Archipelago, Shikoku), Korea, Republic of, Taiwan
North America: Canada (Alberta, British Columbia, Manitoba, New Brunswick, Newfoundland, Northwest Territories, Nova Scotia, Nunavut, Ontario, Québec, Saskatchewan, Yukon Territory), Mexico, United States of America (Alabama, Arizona, Arkansas, California, Colorado, Connecticut, Delaware, Florida, Georgia, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Maryland, Massachusetts, Michigan, Minnesota, Mississippi, Missouri, Nebraska, New Jersey, New York, North Carolina, Ohio, Oklahoma, Oregon, Pennsylvania, South Carolina, Tennessee, Texas, Vermont, Virginia, West Virginia, Wisconsin)

BIOLOGY

Bursaphelenchus xylophilus

B. xylophilus is transmitted from one host to a new host by species of *Monochamus*, the insect vectors (see section on *Monochamus* spp. below). The transmission can be accomplished during the insect maturation feeding period on healthy trees, designated as primary transmission, or during the oviposition activity of the female vector on trees which recently died or weakened trees and known as secondary transmission (Naves *et al*., 2016).

The primary transmission is the most important pathway in regions where *B. xylophilus* was introduced (Asia and Europe), due to the occurrence of susceptible plant hosts, while in North America the secondary transmission is the most frequent pathway for nematode dissemination.

The life cycle of these nematodes, that comprises eggs, four juvenile stages (J1, inside the egg, J2, J3, J4) and adults with sexual dimorphism (the propagative forms) and four moults, can occur in the phytophagous phase (nematodes feed on cells of healthy trees) and in the mycophagous phase (nematodes feed on fungi).

When the environmental conditions are adverse, such as periods of desiccation or food shortage, morphological and physiological changes occur in the second-stage juveniles (J2) to give rise to the third and fourth dispersal stages (JIII and JIV) also designated as ‘pre-dauer’ and ‘dauer’ juveniles respectively. The JIII are attracted to the pupal chambers of the insects and moult into non-feeding JIV that are adapted to be carried out mainly in the tracheal system of immature adult insect vectors, entering through the spiracles, before the insect’s emergence from the wood. Nematodes JIV can also be found in other body segments, head, antennae and wings, using fungal hyphae, present in the interior of the pupal chambers, to achieve proximity to the immature adult beetle.

After the emergence, the infested insects feed on young branches of a new host and transmit the JIV (primary transmission) that penetrate through the feeding wounds and moult into adults within 48 hours. Afterwards, the nematodes reproduce rapidly and migrate through the vascular system, entering the conductive vessels, responsible for the circulation of water and minerals (crude sap), and feed on parenchyma and epithelial cells of the resin canals (phytophagous phase) causing cavitation and embolism of tracheids that stop the sap circulation. A decrease in foliar transpiration, needle discoloration, wilting, and death of the tree can be seen within two or three months of the initial infection (Vicente *et al*., 2012; Futai, 2013).

In the laboratory, *B. xylophilus* can be maintained on fungal (*Botrytis cinerea*) cultures. The nematodes reproduce in 12 days at 15°C, 6 days at 20°C and 3 days at 30°C and the females lays on average 79 eggs in 28 days. The second-stage-juveniles (J2) hatch in 26-32 h at 25°C. The temperature threshold for development is 9.5°C (Futai, 1980, 2013).

***Monochamus* spp.**

Species of *Monochamus* inhabiting conifers are the principal vectors of *B. xylophilus* worldwide, *M. alternatus* being the major vector in Asia, whereas *M. carolinensis* and *M. scutellatus* are the major vectors in North America. In Europe (Portugal and Spain), *M. galloprovincialis* is the main vector. Other less efficient *Monochamus* vectors have been recorded in Japan and North America (Table 1). Many of the *Monochamus* spp. from conifers have been recorded as having non-pathogenic *Bursaphelenchus* spp. as associates; it is supposed, therefore, that most, if not all, species would also be capable of transmission of *B. xylophilus* to a greater or lesser extent.

Other genera of the Cerambycidae (e.g. *Acalolepta, Acanthocinus, Amniscus, Arhopalus, Aromia, Asemum, Corymbya, Neacanthocinus, Rhagium, Spondylis, Uraecha, Xylotrechus*) and other Coleoptera (e.g. *Chrysobothris, Hylobius, Odontotermes, Pissode*s, *Tomicus*) have been found to carry *B. xylophilus* in or on their bodies, but there is no evidence that they have any role as vectors in nature. In Europe, *B. xylophilus* has never been found associated with insects of any of these genera (Naves *et al*., 2016).

The life cycle of *Monochamus* species comprises eggs, a variable number of larval instars, pupae and adults. *Monochamus alternatus*, *M. galloprovincialis* and *M. scutellatus* have four larval instars, whereas *M. sutor* has a fifth instar and three to eight larval instars have been recorded for *M. carolinensis*.

The selection of the egg-laying site is made by the female, based on characteristics of the host such as bark thickness and moisture content of the sapwood. Adult females excavate small niches, known as ‘egg-pits’, in the bark, with their mandibles, and use the ovipositor to insert the egg under the bark, on the cambium/sapwood interface, plugging the oviposition scar with a gelatinous substance to protect it from fungi and adverse environmental conditions. Due to the relatively small size of the ovipositor, bark which is more than a few millimetres thick is a limiting factor. Usually, the egg-pits have a single egg, although two or three can also be found, while many do not contain eggs. Females can live for up to 113 days laying 40 to more than 300 eggs during a lifetime. In general, bigger females live longer and lay more eggs.

After the laying of the eggs, the larvae hatch within six to 21 days, depending on the environmental temperature. The first two larval instars (L1 and L2) feed on the phloem and cambium tissues under the bark, constructing asymmetrical galleries which can have lengths of 15 cm or more, becoming obstructed by frass and excrements over time. The third instar larva (L3) starts to bore into the xylem through an oval hole, making an internal gallery, which is usually U-shaped or S-shaped, or has an intermediate form. Later, larval instars complete the gallery by digging an enlarged pupal chamber (usually within less than 20 mm) close to the wood surface. Then, the larvae plug the opening with coarse fibrous debris, and some species enter a period of dormancy (diapause) for several months, experiencing visible physiological and morphological changes in body coloration (from milky white with faecal material to vivid yellowing without faecal material), while ceasing feeding. After the cold season, the mature larvae undergo morphological and physiological changes and moult into pupae.

The pupal stage lasts for two or three weeks and when the adult insect is fully sclerotized, it emerges from the pupal chamber through a round hole (about 4-12 mm in diameter) at the wood surface.

The newly emerged sexually immature adults fly to healthy trees to feed on the bark and phloem tissues of small branches (maturation feeding). This feeding period is essential for sexual maturation, and extends for the first 10-20 days after emergence, although adult *Monochamus* will continue feeding on conifer tissues throughout their lives. Mature adults are attracted to weakened host trees or recently felled logs where they mate, and females start laying eggs.

European *Monochamus* species share similar phenology and are typically univoltine, completing their life cycle in one or two-years. Latitude and altitude have influence on the length of the lifecycle, which is related to variations in mean annual temperatures (Naves *et al*., 2016). *Monochamus alternatus* and *M. galloprovincialis* usually have one generation per year while the North American *M. carolinensis* can have up to two annual generations.

DETECTION AND IDENTIFICATION

Symptoms

Bursaphelenchus xylophilus

The symptoms in the trees infected by *B. xylophilus* are not specific. However, the first indication of the presence of nematodes in the tree is a reduction of resin exudation, due to the disruption of the resin canals and to the diffusion of oleoresins into tracheids. Another symptom is the yellowing and wilting of the needles, partial or total dryness of the crown leading to eventual death of the tree, which is variable according to the host plant, temperature and time of the year. In some trees the infection with *B. xylophilus* does not lead to expression of wilt symptoms (asymptomatic trees). Details are given in PM 9/1 (6) *Bursaphelenchus xylophilus* and its vectors: procedures for official control EPPO, 2018).

Monochamus spp.

These insects only oviposit on recently felled trees and logs or trees already under stress. The larval instars feeding produces large galleries on the sapwood under the bark and bore holes into the wood. The emergence of immature adults give rise to a round hole, but other insects, such as *Sirex* spp. wood-wasps and *Xilocopa* spp. carpenter bees, can cause similar exit holes.

Morphology

Bursaphelenchus xylophilus

B. xylophilus has the general characters of the genus *Bursaphelenchus* (Nickle, 1970; Hunt, 1993): small to long and slender nematodes; cephalic region high and offset by a constriction, with six lips; stylet well developed (length less than 30 µm), usually with weak basal knobs; median bulb or metacorpus, oval to quadrangular, well developed; oesophageal glands overlapping the intestine.

Female

Female with post-uterine sac, usually three to six body widths in length; vulva with a conspicuous overlapping anterior lip (vulval flap); usually at 70–80% of the body length; tail sub?cylindrical and, in most populations, with a broadly rounded terminus.

Male

Male tail with a strong dorsal curvature; small terminal bursa at the tail tip; robust spicules, strongly arcuate with large rounded apex and a prominent rostrum; spicules tip with a disc?like projection (cucullus); gubernaculum absent; seven caudal papillae (one adanal pair, just pre-anal, a single papilla just pre-anal centered and two post?anal pairs).

B. xylophilus can be distinguished by three main characters. In males, spicules with a cucullus and in females, vulva with vulval flap plus a rounded tail terminus. This last character has been considered as the main morphological character to differentiate *B. xylophilus* from the non-pathogenic species *B. mucronatus* that has a mucro at the posterior end (mucronate tail). However, an intraspecific morphological variability of this character has been detected.

Measurements of morphological characters of *B. xylophilus* are given in the Diagnostic Protocol PM 7/4 (3) *Bursaphelenchus xylophilus* (EPPO, 2023) along with information on identification by molecular methods.

***Monochamus* spp.**

Eggs

The eggs, in general 4.0 mm long by 1.2 mm wide, are elongate, slightly rounded at the poles, and the chorion has a fine, faint reticulate sculpture. Initially, eggs are white and matt, acquiring a light-brownish colouration over time.

Larvae

The larvae are robust, legless, and have a white or yellowish white and opaque coloration, with small black and red hairs all over the body, and an amber-black head capsule with well-developed chewing mouthparts. Body is elongate, soft, with ten abdominal segments, including the anal mamilla, and all abdominal tergites are simple, without spines or sclerified plates.

Pupae

The pupae initially have a white colour and a few days before adult's emergence become darker with a harder outer cuticle. This development stage morphologically resembles the adults, with visible mouthparts, legs, antennae and wings.

Adults

Adults are 15 to 32.8 mm long (without antennae). 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.

Detection and inspection methods

Bursaphelenchus xylophilus

Before any symptoms appear in trees suspected of being infected, the reduction of oleoresin production can be detected by making a hole 10-15 mm in diameter through the bark and cambium, due to the rupture of the resin canals and to the dissemination of oleoresins into adjacent tracheids. Nematodes can be extracted from trees, by collecting wood samples from different zones, including the upper part of the trunk and the crown. Guidance on sampling is given in EPPO Standard PM 9/1 (6) (EPPO, 2018a). Afterwards, wood samples should reach an incubation temperature of approximately 25°C for at least 14 days to allow any nematodes present to breed and maximise the likelihood of detection. Then, nematodes can be extracted from the samples (wood ships, shavings, sawdust, branches) using the standard nematode extraction method (Baermann funnel method) or modifications (Whitehead and Hemming tray method) for 48 h. These methods are based on the motility of the nematodes that migrate, at room temperature, from the wood into the water. Guidance on nematode extraction is given in EPPO Standard PM 7/119 (1) (EPPO, 2013).

The fourth dispersal juvenile stage (IV) can be extracted from the vectors by cutting the insects into pieces and using the Baermann funnel extraction method over 24 h (EPPO, 2013).

***Monochamus* spp.**

The conical oviposition scars (egg-pits) on the bark can indicate where the eggs have been laid. Egg-pits are flat and slit-like when the bark is thin and assume a pit-like structure on wood sections with thicker bark. When the bark is removed, sinuous galleries made by young larvae (L1 and L2), during their feeding activity, can be seen on the sapwood. These galleries remain detectable for several years after the larvae´s presence. The third larval instar (L3) begin tunneling in the heartwood (xylem) by boring an oval hole and the majority of the fourth instar larval (L4) are found in the heartwood galleries. The pupal chamber, constructed by the last-instar larvae, is usually located near the wood surface to enable the subsequent emergence of the adults. The more distinct circular holes of the adults, visible at the wood surface, are the exit holes of the adults, indicating that the insects have completed their development in the wood and have emerged. Traps and lures are highly effective for monitoring *Monochamus* flying adults and are efficient tools to sample populations in the field (Torres-Vila *et al*., 2015).

PATHWAYS FOR MOVEMENT

The nematodes, within the wood tissues, can move actively and can leave one part of wood to move into an adjoining part., However, they need an insect vector to move from one host tree to another. Adults of the vector beetles can fly actively, and a peak of flight activity is reached about 5 days after emergence. In dense woodland, the flights of the beetles are short but, in more open areas and/or in the absence of suitable hosts, long dispersal flights (>2 km up to 40 km or longer) have been recorded (Kobayashi *et al.*, 1984).

Infected wood is the most probable mean of international transport of *B. xylophilus*, and the species has been intercepted on a few occasions on sawn wood, round wood and wood chips imported into the EPPO region from the USA, Canada and Asia.

The most likely pathway of introduction of *B. xylophilus* is when imported together with insect vectors which may carry and transmit the nematodes to coniferous trees. Mature *Monochamus* larvae can survive in processed timber for several months and can be transported over long distances with international trade of goods and merchandise, being occasionally found alive in assorted and untreated wood packaging material (Sousa *et al*. 2011a). Survival of the larvae is higher on larger pieces of timber and, therefore, round wood and sawn wood present a greater risk than smaller pieces of wood. Wood chips can have a high moisture content, allowing nematode survival, but the shredding of the wood to small sections reduces the possibility of insect vectors surviving.

Whether introduced with or without a vector insect, it is still necessary for long-term establishment for the nematode to find a mean of coming into contact with an insect vector, and this can probably only be achieved if the nematode first invades wood which contains larvae or pupae of a potential vector. Nematodes can survive for long periods in wood and they can move from infected wood to timber or damage trees (Sousa *et al*., 2011b; Hopz-Biziks *et al*., 2017).

PEST SIGNIFICANCE

Economic impact

Bursaphelenchus xylophilus

Pine wilt disease was first reported in Japan in 1905 in Nagasaki, Kyushu, but the causal agent was only identified as *Bursaphelenchus* sp. in 1971 (Kiyohara & Tokushige, 1971). In 1972, the species was described as *B. lignicolus* in Japan (Mamiya & Kiyohara, 1972) and then synonymized as *B. xylophilus* (Nickle *et al*., 1981). The symptoms were first attributed to wood-boring insects, which are found abundantly on infected trees, but it was then found that first symptoms precede attack by the insects. The disease then began to spread northwards causing very severe losses throughout the country, reaching 2.4 million m^3 of annual timber loss in the late 1940s (Mamiya, 1988), and now being on average of 1 million m^3/year (Suzuki, 2002).

In 1979, *B. xylophilus* was associated with the death of *Pinus* trees in Missouri (USA), primarily of *P. sylvestris* growing in amenity plantings (Malek & Appleby, 1984), and, in North America in general, losses arise almost exclusively among exotic species and in artificial forest ecosystems like ornamental conifer plantings, wind-breaks and Christmas tree plantations. *B. xylophilus* is widespread in natural coniferous forests but significant losses are not recorded.

In China, in a year, tree mortality reached 2.3 million just in a single county (Robinet *et al*., 2009) with over one million trees killed/year from 1995 to 2006 (Zhao, 2008) while in South Korea over one million trees were affected by pine wilt disease in 2005–2006 (Shin, 2008).

Predicted losses in wood production in the European Union from the spread and damage from *B. xylophilus* were estimated at 22 billion euros over 22 years, with losses in standing volume in Portugal and Spain greater than 80% (Soliman *et al*., 2012).

***Monochamus* spp.**

Monochamus larvae can cause economic losses to felled logs by boring holes in the wood, which is normally only of significance if the logs are left for a long time in the forest after felling. Most European species appear to be attracted to scorched and slightly burned trees after forest fires, where they can cause significant damage to the wood (Naves *et al*., 2016). Under well-managed forest conditions, *Monochamus* spp. are not generally considered to be serious pests, although they can occasionally demonstrate aggressive behaviour as a result of outbreaks with very high population levels (Hellrigl, 1971). During outbreaks in the boreal forests of Russia, the maturation feeding by millions of insects caused severe crown damage, leading to tree weakening which predisposed the trees to subsequent attacks by other pests (Gavrikov & Vetrova 1991). Nevertheless, in general, the only economic impact arises in countries where *B. xylophilus* is present and local *Monochamus* species transmit the nematode to healthy hosts.

Control

The tactics and strategies developed to manage pine wilt disease rely on the control of the insect vector and the nematode, with complementary approaches designed to reduce the impact of both organisms (Naves *et al*., 2016). So far, it has proved impossible to control *B. xylophilus* once introduced into a tree. Therefore, control of pine wilt disease in Japan has concentrated on a combination of cultural practices, in removing dead or dying trees from the forest to prevent their use as a source of further infection, and the control of the vector beetles by insecticidal treatment. The Japanese government has spent large amounts of money on extensive control programmes involving aerial spraying and removal of diseased trees (Ikeda, 1984). In the case of individual trees with a particular (e.g. religious) significance, infection can be prevented by a prophylactic chemical treatment. Research is continuing to search for effective management strategies for the insect vector while inside the host tree [i.e. mechanical (felling the trees, elimination of plant material, soaking in water), chemical (treatment of wood, protection with insecticide nets), biological (parasitoids, entomopathogenic fungi and microsporidia, entomopathogenic nematodes, predators)], as well as for the insect vector while outside the host tree [i.e. biotechnical (kairomones, pheromones, traps), chemical (insecticides spraying and tree or soil injection), biological (entomopathogenic fungi, entomopathogenic nematodes and bacteria, predators), others (moulting inhibitors and adult sterilization)]. For the insect-host tree interaction, the strategies can be based on chemical (feeding repellents and inhibitors, oviposition inductors) methods. Concerning the nematodes, management strategies can be based on chemical (fumigation), biological (nematophagous fungi), heat and irradiation treatments while for the nematode-host tree interaction there are chemical possibilities (nematicides and biocides). A long-term strategy is the breeding for resistance/tolerance of pine species/varieties, which has been initiated in eastern Asia and in Europe.

Phytosanitary risk

B. xylophilus is included in the EPPO A2 list of pests recommended for regulation as quarantine pest. It is a quarantine pest for the European Union and many other EPPO member countries. The risk of introduction into other countries of the EPPO region is high and Evans *et al*. (2009) prepared a detailed report of a Pest Risk Analysis for *B. xylophilus*. European *Pinus* species have proved very susceptible in North America and the introduction of *B. xylophilus* into other areas of Europe is considered a very serious risk. Several species of *Monochamus* are present in Europe (Table 1). In view of the non-specific nature of the phoretic relationship between *Monochamus* and *Bursaphelenchus* spp., it is probable that these European species would transmit *B. xylophilus*. Since the disease is favoured by hot summers, areas in the Southern EPPO region are most at risk. Direct introduction of the nematode into the Northern EPPO region certainly also presents a risk, since symptoms could be expressed when trees suffer from any other form of stress (e.g. drought or occasional periods of high temperatures).

PHYTOSANITARY MEASURES

EPPO's recommendations to prevent the introduction of *B. xylophilus* and its vectors from countries where the nematode occurs are detailed in PM 8/2 (3) Coniferae (EPPO, 2018). These recommendations cover plants for planting, cut branches, wood (round wood and sawn wood), particle wood (wood chips, hog wood) and isolated bark of Coniferae. For wood packaging material (including dunnage), the requirements of ISPM 15 (FAO, 2019) should be fulfilled to prevent the introduction of *B. xylophilus* (and other pests) via international trade into new areas.

Several quarantine treatments for round wood, sawn wood, wood chips, isolated bark and wood packaging materials have been proposed, such as steam/heat treatment or irradiation according to PM 8/2 (3) Coniferae (EPPO, 2018b). The main risk of infection of cut timber is in the period between felling and removal from the forest. Cut logs can be protected from oviposition (and thus from introduction of nematodes) by chemical treatment but such treatment is more effective in killing the insect larvae already present under the bark; in the latter case, the treatment is too late to prevent nematode infection. Other means of reducing the risk of attack are to cover logs after felling, to leave trap logs exposed nearby and to ensure that the felling is conducted outside the flight period of the beetles.

The only known effective treatment for wood already infected with *B. xylophilus* and its vectors appears to be heat treatment, in which all parts of the wood reach a temperature of 56°C for at least 30 min. However, *B. xylophilus* can colonize heat-treated timber if kept in direct contact with nematode-infected timber, although infection can be prevented by kiln-drying (KD) the wood to moisture content around 18-22%, thus hampering nematode transfer (Sousa *et al*., 2011b). After heat-treatment and kiln-dry, old and dry wood does not support re-infection with *B. xylophilus* even in favorable conditions (Naves *et al*., 2019). Visual inspection of timber does not always reveal the presence of insect larvae or pupae, which can be hidden within internal galleries, but they can be detected by X-ray analysis.

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

***Monochamus* species**	**Geographical distribution**	**Main hosts**	**Vector status**
North America			
M. carolinensis Olivier	Canada, Mexico, USA	*Pinus banksiana, P. clausa, P. nigra, P. ponderosa, P. resinosa, P. strobus, P. sylvestris,*	+
M. clamator LeConte	Canada, USA	*Pinus contorta*	-
M. marmorator Kirby	Canada, USA	*Abies balsamea*	+
M. mutator LeConte	Canada, USA	*Pinus nigra, P. resinosa*	+
M. notatus (Drury)	Canada, USA	*Pinus strobus*	-

M. obtusus Casey	Canada, USA	*Abies, Pinus, Pseudotsuga*	-
M. rubigeneus Bates	Guatemala, Honduras, Mexico, USA	*Pinus*	-
M. scutellatus Say subsp. *scutellatus*	Mexico, USA	*Abies, Larix, Picea, Pinus*	+
M. scutellatus subsp. *oregonensis* LeConte	Canada, USA	*Picea*	-
M. titillator (Fabricius)	Canada, USA	*Abies, Picea, Pinus strobus, P. sylvestris, P. thunbergii*	+
Palaearctic region (overlapping with *B. xylophilus* in some countries/regions)			
M. alternatus Hope	China, Korea, Japan, Taiwan	*Abies, Cedrus, Larix Picea, Pinus densiflora, P. luchuensis, P. thunbergii,*	+
M. galloprovincialis (Olivier)	Belgium, Finland, France, Germany, Greece, Iraq, Italy, Kazakhstan, Kyrgyzstan, Mongolia, North Africa, Poland, Portugal, Russia, Sweden, Turkey	*Pinus*	+
M. saltuarius Eschscholz	China, Europe (central & eastern and south Italy), Korea, Japan, Siberia, Taiwan	*Picea, Pinus densiflora, P. thunbergii*	+
M. tesserula White	China, Japan	*Pinus*	-
M. rosenmuelleri Cederhielm	China, Finland, Japan, Poland, Russia (Caucasus), Siberia	*Abies, Larix, Picea, Pinus*	-

Palaearctic region (not overlapping with *B. xylophilus*)			
M. sartor Fabricius	Europe (eastern France to western Ukraine)	*Picea, Pinus*	-
M. sutor (Linnaeus)	China, Europe (north, central & eastern), Georgia, Kazakhstan, Mongolia, Russia (European), Siberia	*Larix, Picea, Pinus*	-

+ Known vector; - Potential vector

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