Volume 55(2):213-217, 2011
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

REVIEW ARTICLE

Bursaphelenchus xylophilus, the pinewood nematode: its significance and a historical review

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ABSTRACT This paper reports on the biology, introduction, spread, damage and the control of *Bursaphelenchus xylophilus* (STEINER and BUHRER, 1934) NICKLE, 1970. Presenting a significant economic threat, the pinewood nematode is a unique quarantine status species of the genus *Bursaphelenchus*. The risk of its spread in European coniferous forests is especially high where the insect vectors are present. In Hungary, the pests *B. mucronatus* and *B. vallesianus* are present as well as its vector species *Monochamus*. Considering the health status of domestic planted pines, the severity of storm-damages, global warming, the increased volume of imported wooden packages and insect migration, the likelihood of *B. xylophilus* invading Hungary and finding favourable conditions keeps increasing. **Acta Biol Szeged 55(2):213-217 (2011)**

KEY WORDS

Bursaphelenchus xylophilus
Hungary
Monochamus sp.
pathway
spread
survey

The North American pinewood nematode, *Bursaphelenchus xylophilus* was identified as the main causal agent of the Pine Wilt Disease (Kiyohara and Tokushige 1971). A serious invasive and destructive species, it is listed as a quarantine pest in the legislation of more than 40 countries; is on the EPPO (European and Mediterranean Plant Protection Organization) A2 list (no.158.) and on the EU II/A2 list meaning that it is a regulated pest of quarantine significance present in at least one EPPO Memer State (PPO 211).

Taxonomic position, Bursaphelenchus species, hosts and symptoms

Its taxonomic position is the following: Nemathelminthes, Nematoda, Secernentia, Tylenchida, Aphelenchina, Aphelenchoidoidea, Parasitaphelenchidae, Bursaphelenchinae. The *Bursaphelenchus* genus contains about one hundred species (Hunt 2008), which are split into six groups, namely *xylophilus, hunti, aberrans, eidmanni, borealis* and *piniperdae* (Ryss et al. 2005). Braasch (2008) expanded the *xylophilus* group with species described after 2000 so this group currently contains *B. xylophilus, B. fraudulentus*, *B. mucronatus*, *B. kolymensis, B. conicaudatus*, *B. baujardi*, *B. luxuriosae*, *B. doui* and *B. singaporensis*. Presenting a significant economic threat to conifers, *B. xylophilus* is a unique quarantine species of *Bursaphelenchus* genus. With the help of its vector, *Monochamus* spp., this nematode is responsible for the Pine Wilt Disease, yet it does not cause significant mortality to native conifers in North America (Linit 1988). Pine Wilt Disease first appeared on the EPPO quarantine list in 1986. Effective early detection is a prerequisite for surveillance and eradication programs, as well as for the establishment of standards for pest risk analysis and the determination of pest-free areas (Schrader and Unger 2003). Most *Bursaphelenchus* species live on woody plants – mainly Coniferales: *Pinus, Abies, Chamaecyparis, Cedrus, Larix, Picea, Pseudotsuga* – and are mycophagus, while some species are phytophagus. A known exception among coniferous plants is *Thuja plicata*, which is considered to be immune to *Monochamus* spp. and thus, to the nematode. The most important feature of the damage is a sudden wilting. On infection, trees display the following symptoms: at first the production of oleoresin in the wounds significantly drops, secondly the transpiration of leaves decreases and finally stops, and later yet, the needles show yellow and red discoloration. All infection leads to the death of infected trees. Mortality rate is expected to peak from late August, not earlier than 30-50 days after the first symptoms. A tree may contain 10 million nematodes within its trunk, branches and roots (Braasch 1983). When scarred, healthy trees cover the surface of the scar with resin within a short time while infected trees produce less, if any, resin at all. However, these symptoms atypical and do not necessarily indicate the presence of the nematode: they might be caused by physical factors or by other pathogens. At the moment, there are no known symptoms to aid visual distinction between trees that are dying from Pine Wilt Disease and those dying for other reasons. It is important to note that the infection may or may not result in the quick appearance of symptoms: coniferous trees may remain symptomless for a considerable period of time (in experimental studies, up to 14 years). Latent infection is a key feature of the biology of nematode infestations. The pine nematode has gained importance throughout the European Union, especially in

Accepted Nov 7, 2011
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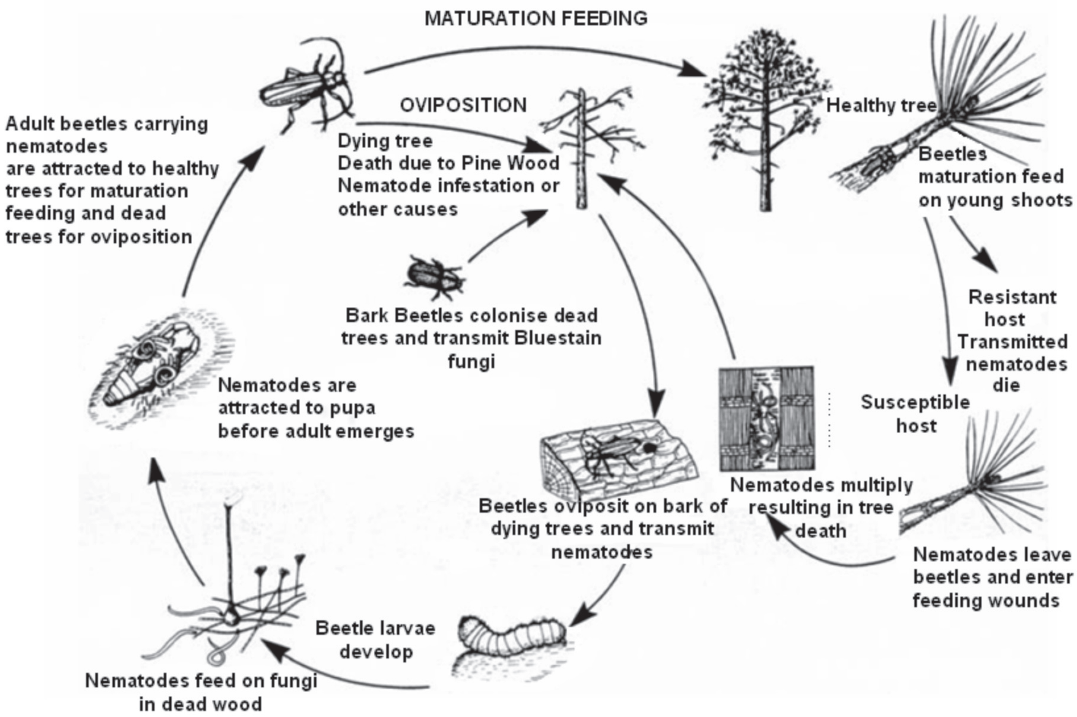

Figure 1. Transmission cycle of *B. xylophilus* by its *Monochamus* sp. insect vector (Wingfield 1987).

Northern countries (European Commission 2009).The only known solution to stop spreading the infection is to burn the infected plants after cutting. The infected plant dies within 1 to 3 months. Live *B. xylophilus* can also be found in roots, even when the upper parts of the tree are already dead, dried out or cut. Depending on climatic conditions and on the speed of desiccation of the wood, nematodes can be detected in trees up to 3 years after the trees died from Pine Wilt Disease (Malek and Appleby 1984). *B. xylophilus* can be found in coniferous plants for planting, cut branches, wood, isolated bark and wood shavings, but never in needles, cones or seeds. Under favourable conditions (25°C) this nematode can complete its life cycle from egg to adult in just four to five days (Ishibashi and Kondo 1977; Mamiya 1984).

Phytosanitary risk, pathway: vector and human

Pinewood nematode is a major threat to European pine forests today with an estimated mortality risk of >50% of pine trees in southern Europe. Their introduction and spreading may have a highly destructive effect on European forests (~82 million ha in the EU). The risk of its spreading in European coniferous forests is especially high where the insect vectors are present. *B. xylophilus* is transmitted from infected trees into healthy ones mainly by *Monochamus* beetles (Coleoptera: Cerambycidae). Alive individuals of the nematode species can be identified to species level directly from the bodies of their vectors. In healthy trees, nematodes favour the place of maturation feeding of *Monochamus* beetles, whereas on infected, or on dead trees they assemble where *Monochamus* lay their eggs (Fig .1). The pinewood nematode is transported in its fourth stage of development to new host trees by longhorn beetles (Cerambicydae). *Monochamus* vectors damage the trees during maturation feeding by chewing on young shoots and during finishing development by boring at least 3-mm wide holes under the bark. One usually finds the typical flat-headed *Monochamus* larvae /apodous/ under the bark or within oval larval galleries (grub holes). While the natural spread of nematode by insect vectors is limited (Braasch 2000), coniferous wood consignments and wood packaging material may both contain the nematode and the vector(s), making these the primary methods of spreading the nematode. To obtain adults and larvae for identification, one has to search for them in wood and in the insect vector. The number of nematodes per each vector may total from 15,000 to 230,000. Once the host tree is diseased, the food source for the pinewood nematode weakens and the pest starts feeding on various wood-inhabiting fungi such as blue-stain fungi, although these fungi, including *Trichoderma* spp. living on dead pines are unsuitable for *B. xylophilus* for propagation (Kobayashi et al. 1975). For a short period after having been introduced by vectors during oviposition or maturation feeding, nematodes are found in the vicinity of these locations.

They spread and multiply rapidly in all parts of the infected tree except in needles, cones and seeds. Nematodes have been observed to invade the root system as well, where they are able to survive for a certain period once the tree has died, dried or cut out. Under unfavourable climatic or host conditions however, the nematode infection does not achieve its usual systemic form (that is, the infection established in the crown does not spread) (European Commission 2009). *Monochamus* species prefer physiologically weakened trees. *B. xylophilus* were found on or within the bodies of cerambycid beetles (of the following genera: *Acalolepta*, *Acanthocinus*, *Amniscus*, *Arhopalus*, *Asemum*, *Corymbia*, *Neacanthocinus*, *Rhagium*, *Spondylis*, *Uraecha* and *Xylotrechus)* and on and within other coleopterans (eg *Chrysobothris*, *Hylobius*, *Pissodes*) (EPPO/CABI 1996). According to a 2001 publication on the European appearance and spreading of *Bursaphelenchus* species by Braasch, not all insect vectors are completely known, but the most important transmitters belong to families *Scolitydae, Cerambycidae* and *Curculionidae.*

Dispersal and economic impact

B. xylophilus was introduced to Eastern Asia and Western Europe by human activities and international trade of untreated and contaminated wood. Outside its native range, the nematode is one of the most important pests of pine trees and other conifers worldwide. It is the casual agent of Pine Wilt Disease, which, in certain conditions leads to the death of infected trees (Kobayashi et al. l984; Kishi 1995; Evans et al. 1996). With timber consignments imported from the American continent, *B. xylophilus* was introduced to Asia in the late 1970's. The pest rapidly spread and has became one of the most important forest pests in Japan, China, Taiwan, Korea, and also in European countries, namely in Portugal and Spain (OEPP/EPPO, 1986; EPPO/CABI, 1996; Evans et al. 1996; Anonymous 2008). *B. xylophilus* is widespread in Canada and USA (Ryss et al. 2005; Sutherland 2008) and it appeared in Mexico as well (Dwinell 1993). In Japan tens of millions of dollars are spent annually to control *B. xylophilus* (Kulinich and Kolossova 1993). In 2000, approximately 580,000 hectares of pine forest that is 28% of the total pine forest area in Japan were estimated to have been infected by the species (Mamiya 2004). The nematode spreads easily by wood moving in trade, either as a commodity (live plants, log, sawn timber etc.), or as wood packaging material (being transported with other commodities), not meeting the requirements of ISPM No. 15 'Regulation of Wood Packaging Material in International Trade' (FAO 2009). *B. mucronatus* which is morphologically and biologically very similar to *B. xylophilus* does not infect trees of European native pine forests but its presence indicates the potential penetration of the pine wood nematode. According to speculations, *B. mucronatus* may threat Northern American coniferous stands when introduced with imported European (or Russian) pinewood into the USA. *B. mucronatus* is wide-spread in European pine forests and in Japan (Mamiya and Enda 1972). Damage done by *B. xylophilus* is the most significant where the average temperature exceeds 20°C in July and August (Braasch and Enzian 2004). Both *Bursaphelenchus* species contain a number of different pathogenic strains with various effect on coniferous species. It is very difficult to tell a harmful strain from a non-harmful one. North Europe has had import restrictions on coniferous timber and wood chips since the 1980's, when *B. xylophilus* was discovered in pine chips imported from Canada and the USA. Later, those restrictions were adopted by the EU and applied to most countries of Europe (Dwinell 1997). To prevent the introduction of *B. xylophilus* and its vectors from infected countries into the EU, a number of phytosanitary regulations (Directive 77/93 updated as 2000/29/EC) were implemented. Nevertheless in 1999, the first establishment of European pinewood nematode was found in Portugal (Økland et al. 2010). Findings of *B. xylophilus* in wood pallets exported from Portugal to other European countries triggered measures, and the European Commission banned Portuguese imports of all coniferous wood that were not proved of having undergone the required heat treatment (European Union 2008). *B. xylophilus* has been present in Portugal since 1999 and the infection was not stopped ever since despite of the important amounts of European Union subvention of nearly 24 Million Euros between 2001-2009 (EPPO 2009). The most important insect vector in continental Portugal is *Monochamus galloprovincialis*. The main transmitters of pinewood nematodes are *Monochamus* beetles and each geographical region has its own *Monochamus* species: for example *M. alternatus* (Japan and China), *M. saltuarius* (Japan and Korea), *M. carolinensis* and *M. titillator* in North America and *M. galloprovincialis* in Portugal (Schröder et al. 2009). In Portugal, *Pinus pinaster* is the coniferous species most affected by Pine Wilt, corresponding to an area of about 1 million ha (34% of the total forest) (Mota et al. 1999; Sousa et al. 2001). In Spain 344,000 Euros were spent in 2009 and 3 Million Euros in 2010 for control measures (EPPO 2009). In 2009 several countries identified live *B. xylophilus* individuals and their vectors in consignments of timber and in wood packing material arriving from Portugal.

Phytosanitary measures and survey

In compliance with EU regulations, measures against the pest include monitoring coniferous forests and coniferous wood, wood packing, nursery plants and coniferous plant material in international trade (OEPP/EPPO 2009a). Import restrictions vary among Member States. One of the recommended treatments is the correct use of heat treatment (HT) that kills both the vector and the nematodes as the wood reaches a core temperature of 56°C for a minimum of 30 minutes (Dwinell 1990 and 1997). EU Member States shall annually conduct official national surveys for pinewood nematode on susceptible plants and for vector species occurring in their territory and on susceptible wood and bark originating in their territory, to determine whether there is any evidence of the presence of *B. xylophilus* in their territory or parts of their territory where pinewood nematodes were not yet detected. In Hungary the Central Laboratory for Pest Diagnosis, Central Agriculture Office, Directorate of Plant Protection and Soil Conservation has been conducting nation-wide surveys since 2003 for B. *xylophilus* in coniferous forests, within 25 km radius around pine forests and at all potential sources of danger (wood depot, airport, border post, international transport lines). Morphological and molecular diagnostic tests were also conducted according to international protocols (OEPP/EPPO 2009b, c) (Tóth 2010). As the chance to detect *B. xylophilus* on trees that seem healthy is very low, survey for the pest should be linked to trapping and testing for *Monochamus* beetles (European Commission 2009). Surveillance may include trapping and testing of *Monochamus* beetles. Official survey detected no individuals of *B. xylophilus* and other European *Bursaphelenchus* species between 2003 and 2010. While Hungary is free from the quarantine pest pinewood nematode (Tóth and Elekes 2011), *B. mucronatus* and *B. vallesianus* as well as the vector species *Monochamus* are present.

Conclusions

Despite the worldwide importance of *B. xylophilus* as a quarantine pest and its economic importance in forestry and associated industries, including wood packaging industry, the biology, ecology, long-term survival and development of *B. xylophilus* in wood packaging materials is still largely unknown (Sousa et al. 2011). In Hungary, the pests *B. mucronatus* and *B. vallesianus* are present as well as its vector species *Monochamus*. Considering the health status of domestic planted pines, the severity of storm-damages, global warming, the increased volume of imported wooden packages and insect migration, the likelihood of *B. xylophilus* invading Hungary and finding favourable conditions keeps increasing. The only protection against *B. xylophilus* is prevention, trapping and early detection. Monitoring may include trapping and testing of *Monochamus* beetles. Where the presence of *B. xylophilus* is confirmed, the strictest quarantine measures are to be taken: all infected trees should be totally destructed in a several km radius.

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