

Report of a Pest Risk Analysis for *Bursaphelenchus xylophilus*

This summary presents the main features of a pest risk analysis which has been conducted on the pest, according to EPPO Decision support scheme for quarantine pests.

Pest: *Bursaphelenchus xylophilus*
PRA area: EPPO region
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STAGE 1: INITIATION

Reason for doing PRA: The PRA for *Bursaphelenchus xylophilus* (Pine Wood Nematode - PWN) has been performed by EPPO for the territory of EU (of that time) in 1996 and published in the EPPO Bulletin (v.26, pp. 199-249). The pest was included in the EPPO A1 list absent from the territory of the organization. Revision of this PRA has been done for the following reasons:

- 1) the pest has established in Portugal and its phytosanitary status for the EPPO territory has changed
- 2) new data have been gathered on natural and man-assisted capacities of spread of the pest
- 3) new data have been gathered on the pest impact
- 4) under the new EPPO procedure on conducting PRA, it is necessary to perform it for the whole of the EPPO region
- 5) the pest is being considered for inclusion in the EPPO A2 list

Taxonomic position of pest: Nematoda: Aphelenchoididae, Parasitaphelenchinae

STAGE 2: PEST RISK ASSESSMENT

Probability of introduction

Entry

Geographical distribution: *B. xylophilus* is believed to be native in North America and has been spread to Asia (Japan, China, Taiwan, Korea) and Portugal (Europe) (OEPP/EPPO, 1986; EPPO/CABI, 1996; Evans *et al.*, 1996). *B.*

xylophilus is widespread in Canada and USA (Ryss *et al.*, 2005; Sutherland 2008) and there is a single report of its presence in Mexico (Dwinell, 1993). It occurs in practically all states/provinces of Canada and USA where pine and other conifer forests exist. The northernmost limit to its distribution in North America is uncertain. In Japan, *B. xylophilus* is now widespread in three of the four main islands, Kyushu, Shikoku and Honshu, but has not yet been reported from the prefectures Hokkaido and Aomori (Futai, 2008). It has spread into China (Zhao *et al.*, 2008), Korea (Shin 2008) and Taiwan during the past 35 years and is thought to have reached these locations from Japan. In all these new Asian areas, *B. xylophilus* has become associated with *M. alternatus* as principal vector (Nakamura-Matori 2008). In China, it is restricted to the provinces of Jiangsu, Anhui, Fujian, Jiangxi, Guangxi, Guizhou, Hubei, Hunan, Guangdong, Shandong, Sichuan, Yunnan, Zhejiang and Chonqing City (Zhao *et al.*, 2008; Robinet *et al.*, 2009). In the Republic of Korea it is present in 54 districts, counties, and cities in 11 provinces (Shin 2008; Shin *et al.* 2009). Pine wilt symptoms are evident in all these locations. There is a record of the presence of *B. xylophilus* in dying pines in Nigeria but this has not been confirmed by specialist taxonomists (Khan & Gbadegesin 1991). In the EPPO region, PWN has established in continental Portugal where his main vector is *Monochamus galloprovincialis* (Mota *et al.* 1999, Sousa *et al.* 2001), and there is a single recorded incursion in Spain.

Geographic distribution of *B. xylophilus*

EPPO region: Portugal (OEPP/EPPO, 1986; EPPO/CABI, 1996; Evans *et al.*, 1996, Mota *et al.*, 1999, Sousa *et al.*, 2001).

Asia: China (Jiangsu, Anhui, Fujian, Jiangxi, Guangxi, Guizhou, Hubei, Hunan, Guangdong, Shandong, Sichuan, Yunnan, Zhejiang and Chonqing City) (Zhao 2008; Robinet *et al.*, 2009), Japan (main islands, Kyushu, Shikoku and Honshu) (Futai, 2008), Republic of Korea (54 districts, counties, and cities in 11 provinces) (Shin 2008; Shin *et al.*, 2009), Taiwan.

Africa: Nigeria – not confirmed (Khan & Gbadegesin, 1991).

North America: Canada and USA – widespread (Ryss *et al.*, 2005; Sutherland, 2008), Mexico (Dwinell, 1993).

Major host plants or habitats:

PWN prefers *Pinus* species, but is also able to attack other Coniferae: *Abies*, *Picea*, *Larix*, *Cedrus* and *Pseudotsuga*. These genus are considered as PWN host plants.

Its vectors in the genus *Monochamus* can also attack trees of above mentioned species and some other Coniferae: *Juniperus*, *Chamaecyparis*, *Cryptomeria* and sometimes *Tsuga* (OEPP/EPPO, 1986; EPPO/CABI, 1996; Evans *et al.*, 1996), but it is uncertain whether these genera are hosts for PWN. They may become infested. Neither *Thuja* nor *Taxus* are regarded as hosts of PWN and its vectors.

The EU Directive 2006/133 EC includes plants of *Abies*, *Cedrus*, *Larix*, *Picea*, *Pinus*, *Pseudotsuga* and *Tsuga* and all coniferous wood and bark except *Thuja* as hosts of PWN.

Pest:

Which pathway(s) is the pest likely to be introduced on:

Bursaphelenchus xylophilus

The EWG considered the following possible pathways:

- 1) Plants for planting (except seeds) of host species (including bonsai plants)
- 2) Cut branches (including Christmas trees) of host species
- 3) Wood (except particle wood and waste wood) of host species (including any wood products made from raw untreated coniferous wood)
- 4) Particle wood and waste wood of host species
- 5) Coniferous wood packaging material
- 6) Isolated bark of host species

Other pathways discussed

- 1) Seeds and cones of host species

There is no report up to now that *B. xylophilus* has been isolated from cones or seeds. Fresh green cones may be a possible commodity to harbour nematodes as *Monochamus* species use them for maturation feeding (Hellrigl, 1971). Mature cones are dry. Size and morphology of the cones and seeds 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 some nematodes can be associated with coniferous seeds. 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 transfer 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 negligible.

- 2) Hitchhiking *Monochamus* beetles

Beetles of *Monochamus* emerging from PWN-infested trees/wood are able to carry PWN and transmit it to non-infested trees during maturation feeding. Theoretically, hitchhiking beetles could present a risk of introducing PWN to new areas/countries but the lack of information on hitchhiking *Monochamus* risk would require expert judgement to answer most of questions in the corresponding section of PRA. The risks from this pathway are unknown but are likely to be negligible.

Establishment

Plants or habitats at risk in the PRA area:

Most of *Pinus*, *Abies*, *Picea* and *Larix* species are known hosts of PWN. Some other conifers could also be infested. Large numbers of these tree species are present in the PRA area.

The host plants of PWN are very widely spread in the PRA area (Schütt et al. 2004; Kindel 1995).

Monochamus species are required for the pest spread and are widely distributed in the PRA area on different coniferous trees (Hellrigl 1971, Bense 1995).

Climatic similarity of present distribution with PRA area (or parts thereof):

Most of the EPPO region has climatic conditions similar to the current area of PWN distribution including its native range in North America where environmental conditions are generally unsuitable

for wilt expression and the nematode remains in its saprophytic phase (Evans et al. 1996).

Characteristics (other than climatic) of the PRA area that would favour establishment:

Large part of the EPPO region has abiotic factors (including soil types, range of slopes, etc.) similar to the current area of PWN distribution but they do not much affect PWN establishment.

Local wood nematode species (e.g. *Bursaphelenchus mucronatus*) are unlikely to be an obstacle for the PWN establishment.

It is extremely unlikely that natural enemies existing in the PRA area (if any) could be an obstacle for the PWN establishment.

Which part of the PRA area is the endangered area:

Coniferous plants are present in all EPPO countries. PWN is likely to establish throughout the distribution range of suitable hosts in the PRA area. Tree damage will be different in different parts of the EPPO region, but even in areas where direct damage will be negligible, the presence of the pest will have important impacts on international trade. So, the whole of the PRA area where host plants are present is considered as an endangered area. Climate change is likely to increase the zones within the PRA area where PWN can result in wilt expression in susceptible host trees. Previous indications were that the 20°C July or August isotherms would delimit the area of wilt expression (De Guiran 1990) and new process modelling methods are now being used to refine this gross assumption (Evans, Evans & Ikegami, 2008). The impact of climate change on productivity of existing and future forests must be taken into account and include possible effects of PWN and other biotic damaging agents.

POTENTIAL ECONOMIC CONSEQUENCES

How much economic impact does the pest have in its present distribution:

The current area of PWN includes the area of the pest origin (North America) and the areas where it has been introduced (Japan, China, Korea, Portugal). While the negative effect in the area of origin is low, the negative effects in other parts of the current area are massive. Very few host trees in the native area of origin succumb to the disease but the impact on export markets is substantial.

It is clear that PWN is able to cause significant damage to plants in the PRA area (Kulinich & Kolossova, 1993, 1995; Evans *et al.*, 1996; Mota et al 1999). This damage would be expressed in tree mortality in the southern part of the EPPO region (as demonstrated in Portugal) and in restrictions to trade in its northern part. In Portugal, almost 24 mln euros during 2001 – 2009 were spent to control/eradicate PWN (CIRCA information). In Spain, almost 344 thousand euros were spent in 2009 and almost 3 mln euros will be spent in 2010.

Describe damage to potential hosts in PRA area:

In areas where wilt expression is predicted there will be significant tree mortality. In the young *Pinus* shoots, *B. xylophilus* multiplies in the resin canals, attacking their epithelial cells. The tree shows first symptoms of 'drying out', in the form of reduced oleoresin exudation. The nematodes can now move freely throughout the dying tree. As a consequence of the reduction of its defence

mechanisms (e.g. reduced oleoresin), the tree becomes attractive to adult insects which gather on the trunks to mate. At this stage, intensified wilting and yellowing of the needles is seen. The tree dies 30-40 days after infection, and may then contain millions of nematodes throughout the trunk, branches and roots.

How much economic impact would the pest have in the PRA area:

In areas where wilt expression is predicted there will be significant tree mortality. Simulation presented in the final report of the EU PHRAME project (QLK5-CT-2002-00672) suggests that up to 90% of susceptible pine trees could die in the Setubal region of Portugal.

An increase in production costs is likely to be major due to costs of phytosanitary and control measures, commodity treatments, early replanting compared with expected rotation age, possible changes of tree species to be grown and other costs. In Japan, tens of millions of dollars have been spent for PWN control annually (Kulinich, Kolossova, 1993). In Portugal, almost 24 mln euros during 2001 – 2009 were spent to control/eradicate PWN (information SANCO). In Spain, almost 344 thousand euros were spent in 2009 and almost 3 mln euros will be spent in 2010.

In areas where wilt expression is not predicted and where the direct damage will be negligible, the presence of the pest will have important impacts on the international trade.

CONCLUSIONS OF PEST RISK ASSESSMENT

Summarize the major factors that influence the acceptability of the risk from this pest:

Estimate the probability of entry:

The overall probability of entry of PWN is high.

In order of priority, the probability of entry of PWN and its vectors is:

- 1) untreated coniferous wood packaging materials (but the implementation of ISPM No 15 reduces this risk to an acceptable level),
- 2) wood (except particle and waste wood) of host species,
- 3) plants for planting (except seeds) of host species (including bonsai plants),
- 4) particle and waste wood of host species,
- 5) cut branches (including Christmas trees) of host species,
- 6) isolated bark (including Christmas trees) of host species.

Nevertheless the risk of PWN entry, but not necessarily establishment, with those commodities is substantial.

Estimate the probability of establishment:

The establishment of PWN in new areas of the EPPO region is highly likely, which is shown by experience in Portugal and non-EPPO countries and by climate comparison with the area of current distribution as well as widespread availability of *Monochamus* spp. in the EPPO region.

Spread of PWN in the PRA area is likely without eradication/containment measures, which should be based on biological characteristics of the pest.

Spread of PWN in the PRA area is likely without eradication/containment measures, which should be based on

biological characteristics of the pest and its vectors, particularly on the capacity of PWN to be present in host trees that are not exhibiting symptoms of wilt expression.

Elements favouring establishment

Host plants, including wild hosts, are widely available.

Climatic conditions are suitable both outdoors and under protected conditions.

Biological control agents are not known.

Key biological characteristics of this pest are its ability to establish in trees without symptoms expression, and its capacity to live saprophytically.

Few (10s to low hundreds) specimens transmitted to a host tree are sufficient to enable a PWN population to establish in that tree.

Elements inhibiting establishment (in protected crops)

No elements.

The whole of the PRA area where host plants are present is considered as an endangered area. Climate change is likely to increase the zones within the PRA area where PWN can result in wilt expression in susceptible host trees.

Estimate the potential economic impact:

Tree damage will be different in different parts of the EPPO region, but even in areas where the direct damage will be negligible, the presence of the pest will have important impacts on international trade. So, the whole of the PRA area where host plants are present is considered as an endangered area.

Degree of uncertainty

The main uncertainties arise from the lack of information available on the risk of infestation of "secondary" hosts (such as *Juniperus*, *Tsuga*, etc.) and "secondary" commodities (such as bark or cut branches) by PWN and especially by its *Monochamus* vectors. *Juniperus*, *Chamaecyparis* and *Cryptomeria* could be hosts of *Monochamus* but not of PWN: the lack of evidence creates some uncertainty. Considerable uncertainties remain concerning transmission of PWN from certain consignments to suitable hosts and its possible transmission to trees in the absence of vectors. These involve consignments that do not carry *Monochamus* and cannot be attacked by *Monochamus* because non-vector transmission has only been demonstrated in experiments, but has never been reported in field conditions. Although there is substantial information on flight capacities of *M. alternatus* and *M. carolinensis*, there is only fragmentary information on flight distances for *Monochamus* spp. in the EPPO region. Other uncertainties (e.g. on the degree of social damage) are not important for overall conclusions on the phytosanitary risks involved.

OVERALL CONCLUSIONS

The EWG considered that the pest was an appropriate candidate for pest risk management given the high potential for establishment and the potential for economic impact in the whole of the PRA area.

STAGE 3: PEST RISK MANAGEMENT

IDENTIFICATION OF THE PATHWAYS

Pathways studied in the pest risk management	Pathway 1: 1) Plants for planting (except seeds) of host species (including bonsai plants) Pathway 2: Cut branches of host species (including Christmas trees) Pathway 3: Wood (except particle wood and waste wood) of host species Pathway 4: Particle wood and waste wood of host species Pathway 5: Coniferous wood packaging material Pathway 6: Isolated bark of host species
Other pathways identified but not studied	Pathway 7: Seeds and cones of host species. Pathway 8: Hitchhiking <i>Monochamus</i> beetles.

IDENTIFICATION OF POSSIBLE MEASURES

Possible measures for pathways

Pathway 1: Host plants for planting (except seeds) of host species (including bonsai plants).

Measures related to consignments:

Specified testing

This testing requirement is not sufficient when used alone. The plants should have been tested and found free from *Bursaphelenchus xylophilus* and its vectors and should be produced under vector-proof conditions according to EPPO National Regulatory Control System No 9/1.

Measures related to the crop or to places of production:

Pest Free Area for *Bursaphelenchus xylophilus*

Growing in specified vector-proof conditions could ensure place of production freedom of plants for planting, but this measure could not be regarded as efficient alone and should be combined with testing.

Any of identified measures taken alone could not reduce the risk to an acceptable level and should be combined.

Combination of measures

The pest-free area requirement in combination with measures preventing the infestation of the commodity in transit (transportation outside of *Monochamus* flight period, or through areas not infested with *Bursaphelenchus xylophilus*, or in sealed containers or packaging to prevent infestation) could reduce the risk to an acceptable level. The pest-free area requirement could be replaced by testing plants for PWN freedom in combination with production under vector-proof conditions, and always in combination with measures preventing the infestation of the commodity in transit mentioned above.

Measures in the importing country

Portugal experience shows that even a very intensive survey and phytosanitary measures do not make successful eradication of PWN easily achievable. In principle, PWN eradication is possible but very complicated and expensive. It could not be considered as an effective measure alternative to phytosanitary measures taken in the exporting country and in transit.

Pathway 2: Cut branches of host species (including Christmas trees).

Measures related to consignments:

Specified testing

This testing requirement is not sufficient when used alone. The cut branches should have been tested and found free from *Bursaphelenchus xylophilus* and its vectors, and must come from a pest-free place of production whose immediate vicinity was free from *Bursaphelenchus xylophilus*.

Measures related to the crop or to places of production:

Pest Free Area for *Bursaphelenchus xylophilus*

Growing in specified vector-proof conditions could ensure place of production freedom of cut branches, but this measure could not be regarded as efficient alone and should be combined with testing for PWN freedom. But these measures are not considered practical for cut branches.

Any of identified measures taken alone could not reduce the risk to an acceptable level and should be combined.

Combination of measures

The pest-free area requirement in combination with measures preventing the infestation of the commodity in transit (transportation outside of PWN vectors flight period, or through areas not infested with PWN, or in sealed containers or packaging to prevent infestation) could reduce the risk to an acceptable level. The pest-free area requirement could be replaced by testing the consignments in combination with place of production and immediate vicinity freedom, always in combination with measures preventing the infestation of the commodity in transit mentioned above.

Measures in the importing country

Portugal experience shows that even a very intensive survey and phytosanitary measures do not make successful eradication of PWN easily achievable. In principle, PWN eradication is possible but very complicated and expensive. It could not be considered as an effective measure alternative to phytosanitary measures taken in the exporting country and in transit.

Pathway 3: Wood (except particle wood and waste wood) of host species.

Measures related to consignments:

Specified testing. Reliable testing method is available but testing all volume is not practical.

Three treatments are effective against PWN: (1) heat treatment (by using a reliable method/process to achieve 56°C in the core of wood for at least 30 min), (2) methyl-bromide fumigation (according to the EPPO Phytosanitary Procedure No 10/7) and (3) irradiation according to PM 10/8. Debarking could prevent infestation if PWN-free consignment is transported through or stored on territories infested both by PWN and its vectors.

Measures related to the crop or to places of production:

Pest Free Area for *Bursaphelenchus xylophilus*

Any of identified measures taken alone could not reduce the risk to an acceptable level and should be combined.

Combination of measures

The pest-free area requirement in combination with debarking or other measures preventing the infestation of the commodity in transit or during storage (transportation outside of PWN vectors flight period, or through areas not infested with PWN, or in sealed containers or packaging to prevent infestation) could reduce the risk to an acceptable level. Heat treatment or methyl-bromide fumigation¹ or irradiation could reduce the risk to an acceptable level in combination with debarking or other measures preventing the infestation of the commodity in transit or during storage.

¹ Prior removal of bark must be carried out for the efficacy of methyl bromide treatment

Measures in the importing country

Portugal experience shows that even a very intensive survey and phytosanitary measures do not make successful eradication of PWN easily achievable. In principle, PWN eradication is possible but very complicated and expensive. It could not be considered as an effective measure alternative to phytosanitary measures taken in the exporting country and in transit.

Pathway 4: Particle wood and waste wood of host species.

Measures related to consignments:

Specified testing. This testing is possible in theory but difficult in practice. Wood chips tend to be imported in large quantities and could encourage development and breeding of PWN. Testing of particle wood and waste wood is more feasible than testing other wood because only large peaces of wood (suitable for development of PWN vectors) require testing.

One treatment is effective against PWN: heat treatment (by using a reliable method/process). If PWN-free particle and waste wood originate from debarked wood, it could prevent further infestation if consignments are transported through or stored in territories infested both by PWN and its vectors.

If particle/waste wood is imported outside of *Monochamus* flight period (e.g. during winter) and processed before *Monochamus* flight period starts, there is no risk of PWN introduction, but it may not be feasible for NPPOs to ensure that all imported particle/waste wood is completely processed before *Monochamus* flight period.

Measures related to the crop or to places of production:

Pest Free Area for *Bursaphelenchus xylophilus*

Any of identified measures taken alone could not reduce the risk to an acceptable level and should be combined.

Combination of measures

The pest-free area requirement in combination with production from debarked wood or other measures preventing the infestation of the commodity in transit (transportation outside of PWN vectors flight period, or through areas not infested with PWN, or in sealed containers or packaging to prevent infestation) could reduce the risk to an acceptable level. Heat treatment could reduce the risk to an acceptable level in combination with production from debarked wood or measures preventing the infestation of the commodity in transit.

Measures in the importing country

Portugal experience shows that even a very intensive survey and phytosanitary measures do not make successful eradication of PWN easily achievable. In principle, PWN eradication is possible but very complicated and expensive. It could not be considered as an effective measure alternative to phytosanitary measures taken in the exporting country and in transit.

Pathway 5: Coniferous wood packaging material.

The EWG believed that the implementation of ISPM No 15 reduces the risk to an acceptable level.

Pathway 6: Isolated bark of host species.

Measures related to consignments:

Specified testing. This testing is possible in theory but not practical used alone. Testing of bark is however more feasible than testing of wood (see above). Sampling procedures may limit the confidence level of detection if PWN is present

One treatment of bark is effective against PWN: heat treatment (by using a reliable method/process).

Measures related to the crop or to places of production:

Pest Free Area for *Bursaphelenchus xylophilus*

Each of two identified measures taken alone could not reduce the risk to an acceptable level and should be combined. The pest-free area requirement could reduce the risk to an acceptable level. Heat treatment could reduce the risk to an acceptable level.

Measures in the importing country

Portugal experience shows that even a very intensive survey and phytosanitary measures do not make successful eradication of PWN easily achievable. In principle, PWN eradication is possible but very complicated and expensive. It could not be considered as an effective measure alternative to phytosanitary measures taken in the exporting country and in transit.

EVALUATION OF THE MEASURES IDENTIFIED IN RELATION TO THE RISKS PRESENTED BY THE PATHWAYS

CONCLUSION:

Recommendation for possible measures:

<p>Plants for planting (except seeds) of Coniferae host species</p>	<p>PC² and, if appropriate, RC³</p>
<p>Plants for planting (except seeds) of host species originating in countries where <i>Bursaphelenchus xylophilus</i> occurs</p>	<p>Pest-free area for <i>Bursaphelenchus xylophilus</i> or The plants should have been tested and found free from <i>Bursaphelenchus xylophilus</i> and its vectors <u>and</u> produced under vector-proof conditions according to EPPO National Regulatory Control System No 9/1</p> <p style="text-align: center;">AND</p> <p>Transported outside of <i>Monochamus</i> flight period or Not transported through areas infested with <i>Bursaphelenchus xylophilus</i> or Transported in sealed containers or packaging to prevent infestation</p>
<p>Cut branches of Coniferae host species</p>	<p>PC and, if appropriate, RC</p>
<p>Cut branches (including Christmas trees) of host species originating in countries where <i>Bursaphelenchus xylophilus</i> occurs</p>	<p>Pest-free area for <i>Bursaphelenchus xylophilus</i> or Tested and found free from <i>Bursaphelenchus xylophilus</i> and its vectors <u>and</u> must come from a pest-free place of production whose immediate vicinity was free from <i>Bursaphelenchus xylophilus</i></p> <p style="text-align: center;">AND</p> <p>Transported outside of <i>Monochamus</i> flight period or Not transported through areas infested with <i>Bursaphelenchus xylophilus</i> or Transported in sealed containers or packaging to prevent infestation</p>
<p>Wood of Coniferae host species</p>	<p>PC and, if appropriate, RC</p>
<p>Wood (including squared wood, but excepting packaging wood, particle wood and waste wood) of host species originating in countries where <i>Bursaphelenchus xylophilus</i> occurs</p>	<p>Fumigated according to EPPO Phytosanitary Procedure PM 10/7⁴</p> <p style="text-align: center;">OR</p> <p>Pest-free area for <i>Bursaphelenchus xylophilus</i> or</p>

² PC – Phytosanitary Certificate

³ RC – Re-export Phytosanitary Certificate

⁴ Prior removal of bark must be carried out for the efficacy of methyl bromide treatment

	<p>Heat treated (commodity is heated until the core temperature reached at least 56°C for at least 30 min according to an officially recognized technical specification)</p> <p>or</p> <p>Irradiation treatment according to EPPO Phytosanitary Procedure PM 10/8</p> <p style="text-align: center;">AND</p> <p>Debarking</p> <p>or</p> <p>Transported outside of <i>Monochamus</i> flight period</p> <p>or</p> <p>Not transported through areas infested with <i>Bursaphelenchus xylophilus</i></p> <p>or</p> <p>Transported in sealed containers or packaging to prevent infestation</p>
<p>Particle wood (sawdust, chips, particles) and waste wood (shavings, scrap) of host species originating in countries where <i>Bursaphelenchus xylophilus</i> occurs</p>	<p>Pest-free area for <i>Bursaphelenchus xylophilus</i></p> <p>or</p> <p>Heat treatment (commodity is heated until the core temperature reached at least 56°C for at least 30 min according to an officially recognized technical specification)</p> <p style="text-align: center;">AND</p> <p>Originating from debarked wood</p> <p>or</p> <p>Transported outside of <i>Monochamus</i> flight period</p> <p>or</p> <p>Not transported through areas infested with <i>Bursaphelenchus xylophilus</i></p> <p>or</p> <p>Transported in sealed containers or packaging to prevent infestation</p>
<p>Wood packaging material of Coniferae</p>	<p>Requirements of ISPM No 15</p>

<p>Isolated bark of Coniferae</p>	<p>PC and, if appropriate, RC</p>
<p>Isolated bark of Coniferae originating in countries where <i>Bursaphelenchus xylophilus</i> or its vectors occur</p>	<p>Pest-free area for <i>Bursaphelenchus xylophilus</i></p> <p>or</p> <p>Heat treatment (commodity is heated until the core temperature reached at least 56°C for at least 30 min according to an officially recognized technical specification)</p>

References

Akbulut, S. & Linit, M.J. (1999) Flight performance of *Monochamus carolinensis* (Coleoptera : Cerambycidae) with respect to nematode phoresis and beetle characteristics. *Environmental Entomology*, **28**, 1014-1020.

Anonymous (2008): RESOLUCIÓ N de 12 de noviembre de 2008, de la Direcció n General de Explotaciones y Calidad Alimentaria, por la que se declara contaminada por el nematodo de la madera del

pino *Bursaphelenchus xylophilus* (Steiner et Buhner) Nickle et al. Determinada a área forestal del término municipal de Villanueva de la Sierra y se establece una zona demarcada de 20 kilómetros de radio, adoptándose en ella diversas medidas fitosanitarias tendentes al control y erradicación del agente patógeno. Diario Oficial de Extremadura Numero 233, Martes, 2 de diciembre de 2008, 32028–32036

Bense U (1995) Longhorn Beetles. Illustrated Key to the Cerambycidae and Vesperidae of Europe. Markgraf Verlag Weikersheim: 512 p.

Bergdahl DR (2008) Personal communication (Report to the International Seminar on Control Strategies for Pine Wood Nematode in Portugal, Lisbon, 7-9 October 2008).

Bergdahl DR, Halik S (2003) Persistence of the pine wood nematode in asymptomatic Scots pines. In Mota MM, Vieira P (eds) In Mota M, Vieira P (ed) (2003 the pinewood nematode, *Bursaphelenchus xylophilus*, proceedings of an international workshop 20-22.08.2001, Nematology monographs and perspectives Vol I: 177-185.

Braasch H (2001) Studies on the transmissibility of the pine wood nematode (*Bursaphelenchus xylophilus*) from wood chips to *Pinus* seedlings and stumps. Nachrichtenbl. Deut. Pflanzenschutzd. 48 (8/9): 173-175 (in German).

Braasch H, Enzian S (2003) The pine wood nematode problem in Europe – present situation and outlook, In Mota M, Vieira P (ed) (2003 the pinewood nematode, *Bursaphelenchus xylophilus*, proceedings of an international workshop 20-22.08.2001, Nematology monographs and perspectives Vol I: 77-91.

Braasch H, Burgermeister W, Gu J (2009) revised intra-generic grouping of *Bursaphelenchus* Fuchs, 1937 (Nematoda: Aphelenchoididae). J. Nematode Morphol. Syst. 12 (1): 65-88.

de Guiran, G. (1990) Pine wood nematodes (*Bursaphelenchus* spp.). Biological, taxonomical and epidemiological aspects. *Comptes Rendus de l'Academie d'Agriculture de France*, 76, 13-20.

Dwinell, L.D. (1993): First report of pinewood nematode (*Bursaphelenchus xylophilus*) in Mexico. Plant Disease 77: 846.

Eriko K, Hiroki O, Koich S (1998) Migration of pine wood nematodes (*Bursaphelenchus xylophilus* (Steiner et Buhner) Nickle between two connected Japanese black pine (*Pinus thunbergii* Parl.) seedlings. Research Bulletin of the Kagoshima University Forests 26: 33-36.

EPPO/CABI (1996) *Bursaphelenchus xylophilus*. In *Quarantine Pests for Europe* (2nd edition). CAB International, Wallingford (GB).

EPPO (2000) Diagnostic protocols for regulated pests: *Bursaphelenchus xylophilus*. EPPO standard PM 7/4 (1). EPPO website: www.eppo.org.

EPPO (2009) Diagnostic protocols for regulated pests: *Bursaphelenchus xylophilus*. EPPO standard PM 7/4 (2). EPPO website: www.eppo.org.

EPPO/EPPT (2009) EPPO Plant Protection Thesaurus system, EPPO website: www.eppo.org.

EUROSTAT (2009) http://epp.eurostat.ec.europa.eu/portal/page/portal/external_trade/data/database

Evans S., Evans HF & Ikegami M. (2008) Modeling PWN-Induced Wilt Expression: A Mechanistic Approach. *Pine Wilt Disease: A Worldwide Threat to Forest Ecosystems* (eds M. Mota & P. Vieira), pp. 259-278. Springer.

- Evans H, McNamara DG, Braash H, Chadoeuf J, Magnusson C (1996). Pest Risk Analysis (PRA) for the territories of the European Union (as PRA area) on *Bursaphelenchus xylophilus* and its vectors in the genus *Monochamus*. *Bulletin OEPP/EPPO Bulletin* **26**, 199-249.
- Fujioka H (1993) A report on the habitat of *Monochamus alternatus* Hope in Akita prefecture. *Bull Akita For Tech Cent* 2: 40–56.
- Futai K (2003) Role of asymptomatic carrier trees in epidemic spread of pine wilt disease. *Journal of Forestry Research* **8**, 253–260.
- Futai K (2008) Pine Wilt in Japan: From First Incidence to the Present. In: Zhao BG, Futai K, Sutehrland JR, Takeuchi Y (ed) 2008: Pine Wilt Disease. Springer :5-12
- Halik, S. & Bergdahl, D.R. (1987) Infestation of wounded roots of *Pinus strobus* by *Bursaphelenchus xylophilus* from contaminated wood chips in soil. *Phytopathology*, **77**, 1615.
- Halik S & Bergdahl DR (1994) Long-term survival of *Bursaphelenchus xylophilus* in living *Pinus sylvestris* in an established plantation. *European Journal of Forest Pathology* **24**, 357–363.
- Halik S, Bergdahl DR (1992) Survival and infectivity of *Bursaphelenchus xylophilus* in wood chip - soil mixtures. *Journal of Nematology* **24**, 495-503.
- Hellrigl KG (1971) Die Bionomie der Europäischen *Monochamus*-Arten (Coleopt., Cerambycid.) und ihre Bedeutung für die Forst- und Holzwirtschaft. *Redia* **52**: 367-509.
- Humphry SJ, Linit MJ (1989) Tethered flight of *Monochamus carolinensis* (Coleoptera : Cerambycidae) with respect to beetle age and sex. *Environmental Entomology*, **18** (1), 124-126.
- Humphry SJ, Linit MJ (1989) Effect of pinewood nematode density on tethered flight of *Monochamus carolinensis* (Coleoptera : Cerambycidae). *Environmental Entomology*, **18** (4), 670-673.
- Hunt DJ (1993) Genus *Bursaphelenchus* Fuchs, (1973). In: *Aphelenchida, Longidoridae and Trichodoridae, their Systematics and Bionomics*, pp.129-142. CAB International, Wallingford (GB)
- Hunt DJ (2008) A checklist of the Aphelenchoidea (Nematoda: Tylenchina) J. Nematode Morphol. Syst. **10** (2): 99-135.
- Ido N, Kobayashi K (1977) Dispersal of *Monochamus alternatus*. In . Studies on the control of pine wilt disease. Secretariat of Agriculture, Forestry and Fisheries Research Council, Ministry of Agriculture, Forestry and Fisheries, Tokyo: 87-88. (In Japanese, cited in Togashi and Shigesada 2006)
- Kasuya, S., Sakura, T., and Kishi, Y. (1990) Selective breeding of resistant pines against pine wood nematode--Resistance of the seedlings and the graftings from resistant mother trees--.*Bull. Tokyo Univ. For.* **83**: 19-30. (in Japanese with English summary cited in: Kishi Y (1999) Survival of *Pinus densiflora* and *P. thunbergii* Forests in Ibaraki Prefecture Severely Damaged by *Bursaphelenchus xylophilus*. *J. For. Res.* **4**::287-290)
- Kawabata K (1979) Migration of the pine sawyer among small islands. *Trnas. 32nd Ann Meet. Kyushu Branch Jpn For. Soc.*: 281-282. (In Japanese, cited in Togashi 1990a)
- Khan FA, Gbadegesin RA (1991) On the occurrence of nematode induced pine wilt disease in Nigeria. *Pak. J. Nematol.* **9** (1): 57-58.
- Kindel K-H (1995) *Kiefern in Europa*. Gustav Fischer Stuttgart: 204 S.

- Kiyohara T & Tokushige Y (1971) Inoculation experiments of a nematode, *Bursaphelenchus* sp., onto pine trees. *Journal of the Japanese Forestry Society*. 1971; 53(7): 210-218
- Kobayashi F, Yamane A, Ikeda T (1984) The Japanese pine sawyer beetle as the vector of pine wilt disease. *Annu Rev Entomol* 29: 115–135. DOI: 10.1146/annurev.en.29.010184.000555.
- Kulinich O & Kolossova V (1993) [Pinewood nematode - a potential danger for the coniferous forests of Russia.] *Zashchita Rastenii* **8**, 22-24 (in Russian).
- Kulinich O & Kolossova V (1995) The potential of the pinewood nematode *Bursaphelenchus xylophilus* to become established in countries of the former USSR. *Russian Journal of Nematology* **3**, 35-48.
- Linit MJ (1988) Nematode-vector relationships in the pine wilt disease system. *Journal of Nematology* **20**, 227-235.
- Linit M, Akbulut S (2003) Pine wood nematode phoresis: the impact on *Monochamus carolinensis* life functions. *Nematology Monographs & Perspectives*, **1**, 227-237.
- Luzzi MA, Wilkinson RC & Tarjan AC (1984) Transmission of the pinewood nematode *Bursaphelenchus xylophilus*, to slash pine trees and log bolts by a Cerambycid beetle, *Monochamus tilliator*, in Florida. *Journal of Nematology* **16**, 37–40.
- Magnusson C (1986) Potential for establishment of *Bursaphelenchus xylophilus* and the pine wilt disease under Nordic conditions. *Bulletin OEPP/EPPO Bulletin* **16**,465-471.
- Malek R.B. & Appleby J.E. (1984). Epidemiology of pine wilt in Illinois. *Plant Disease* **68**, 180-186.
- Mamiya Y, Shoji T (1989) Capability of *Bursaphelenchus xylophilus* to inhabit soil to cause wilt in pine seedlings. *Jpn. J. Nematol.* **18**: 1-5
- Mamiya Y (2003) Pine Wilt Disease in Japan. *Nematology Monographs and Perspectives*. **1**, 9–20.
- Ministério da Agricultura (2008) Portuguese Ministério da Agricultura, do Desenvolvimento Rural e das Pescas, Portaria n.º 358/2008 de 12 de Maio, Diário da República, 1.ª série N.º 91 12 de Maio de 2008, 2527–2528.
- Mota, M.M.; Braasch, H., Bravo; M.A.; Penas, A.C.; Burgermeister, W., Metge, K.; Sousa, E. (1999): First record of *Bursaphelenchus xylophilus* in Portugal and in Europe. *Nematology* **1**, 727-734.
- Mota, M.M., Futai, K. & Vieira, P. (2009) Pine Wilt Disease And The Pinewood Nematode, *Bursaphelenchus Xylophilus*. *Integrated Management and Biocontrol of Vegetable and Grain Crops Nematodes* (eds A. Ciancio & K. G. Mukerji), pp. 253-274. Springer.
- Myers RF (1986) Cambium destruction in conifers caused by pinewood nematodes. *Journal of Nematology* **18**, 398–402.
- Nakamura-Matori K (2008) Vector-Host Tree Relationships and the Abiotic Environment. In: Zhao BG, Futai K, Sutehrland JR, Takeuchi Y (ed) 2008: Pine Wilt Disease. Springer :144-161.
- Nickle WR, Golden AM, Mamiya Y, Wergin WP (1981) On the taxonomy and morphology of the pinewood nematode, *Bursaphelenchus xylophilus* (Steiner & Buhrer) Nickle 1970. *Journal of nematology* **13**, 385-392.

- Mota MM, Futai K, Vieira P (2009) Pine Wilt Disease and the pinewood nematode *Bursaphelenchus xylophilus*. In: A. Ciancio & K. G. Mukerji (eds.) Integrated Management of Fruit Crops and Forest Nematodes Springer Science+Business Media B.V. : 253-274
- OEPP/EPPO (1986) Data sheets on quarantine organisms No. 158, *Bursaphelenchus xylophilus*. *Bulletin OEPP/EPPO Bulletin* **16**, 55-60.
- Robinet C, Roques A, Pan H, Fang G, Ye J, Zhang Y, Sun J (2009). Role of Human-Mediated Dispersal in the Spread of the Pinewood Nematode in China. *PLoS ONE* **4** (2): e4646. doi:10.1371/journal.pone.0004646, www.plosone.org
- Ryss A, Vieira P, Mota M, Kulinich O (2005). A synopsis of the genus *Bursaphelenchus* Fuchs, 1937 (Aphelenchida: Parasitaphelenchidae) with keys to species. *Nematology*, **7** (3), 393-458.
- Schröder T, Mcnamara D, Gaar V (2009) Guidance on sampling to detect pine wood nematode *Bursaphelenchus xylophilus* in trees, wood and insects. *EPPO Bulletin* **39**, 179–188.
- Schütt P, Weisgerber H, Schuck HJ, Lang UM, Stimm B, Roloff A (ed) (2004) Lexikon der Nadelbäume. Nikol Verlagsgesellschaft Hamburg: 640 S.
- Shibata E (1986) Dispersal movement of the adult Japanese pine sawyer, *Monochamus alternatus* Hope (Coleoptera : Cerambycidae) in a young pine forest. *Appl. Ent. Zool.* **21** (1), 184-186.
- Shin, S-C (2008) Pine Wilt Disease in Korea. In:Zhao BG, Futai K, Sutehrland JR, Takeuchi Y (ed) 2008: Pine Wilt Disease. Springer :26-32.
- Shin S-C, Moon I-S, Han H (2009) Current research and management of pine wilt disease in Korea. Proceedings of the International Symposium on Pine Wilt Disease, Official meeting of the IUFRO working party 7.02.10, 20-23 July 2009 Nanjing China: 22.
- Sousa, E.; Bravo, M. A.; Pires, J.; Naves, P.; Penas, A.C.; Bonifácio, L.; Mota, M.M. (2001): *Bursaphelenchus xylophilus* (Nematoda: Aphelenchoididae) associated with *Monochamus galloprovincialis* (Coleoptera; Cerambycidae) in Portugal. *Nematology* **3** (1): 89-91.
- Sutherland JR (2008) A Brief Overview of the Pine Wood Nematode and Pine Wilt Disease in Canada and the United States. In:Zhao BG, Futai K, Sutehrland JR, Takeuchi Y (ed) 2008: Pine Wilt Disease. Springer :13-25.
- Suzuki, K. (2002): Pine wilt disease – a threat to pine forest in Europe. *Dendrobiology* **48**: 71-74.
- Takasu F, Yamamoto N, Kawasaki K, Togashi K, Kishi Y, Shigesada N (2000) Modeling the expansion of an introduced tree disease. *Biological Invasions* **2**, 141–150.
- Takeuchi Y & Futai K (2007) Asymptomatic carrier trees in pine stands naturally infected with *Bursaphelenchus xylophilus*. *Nematology* **9**, 243–250.
- Togashi K (1990) Change in the activity of adult *Monochamus alternatus* Hope (Coleoptera : Cerambycidae) in relation to age. *Appl. Ent. Zool.* **25** (2), 153-159.
- Togashi K (1990a) A field experiment on dispersal of newly emerged adults of *Monochamus alternatus* (Coleoptera : Cerambycidae). *Res. Popul. Ecol.* **32**, 1-13
- Togashi K, Shigesada N (2006) Spread of the pinewood nematode vectored by the Japanese pine sawyer: modeling and analytical approaches. *Popul Ecol* **48**, 271–283.

- Toda T, Kurinobu S (1999) Realized genetic gains observed in progeny tolerance of selected red pine (*Pinus densiflora*) and Black Pine (*P. thunbergii*) to Pine Wilt Disease. *Silvae Genetica* 51 (1): 42-44.
- Tomminen J, Halik S, Bergdahl DR (1991) Incubation temperature and time effect on life stages of *Bursaphelenchus xylophilus* in wood chips. *Journal of Nematology* 23(4): 477-484.
- Vincent B, Altemayer V, Roux-Morabito G, Naves P, Sousa E, Lieutier F (2007) Competitive interaction between *Bursaphelenchus xylophilus* and the closely related species *Bursaphelenchus mucronatus*. *Nematology* 10: 219:230.
- Wingfield MJ (1983) Transmission of pine wood nematode to cut timber and girdled trees. *Plant Disease* 67, 35–37.
- Wingfield MJ (1987), A comparison of the mycophagous and the phytophagous phases of the pine wood nematode. In: *Pathogenicity of the Pine Wood Nematode* (ed. Wingfield MJ), pp. 81–90. Symposium Series, APS Press, The American Phytopathological Society, St Paul (US).
- Yang BJ, Wang LF, Xu FY & Zhang P (2003) latent infection of *Bursaphelenchus xylophilus* and a new means of transmission by *Monochamus alternatus*. *Nematology Monographs & Perspectives* 1, 261–266.
- Zhao BG, Futai K, Sutehrlund JR, Takeuchi Y (ed) (2008) *Pine Wilt Disease*. Springer, 144-161.