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European and Mediterranean Plant Protection Organization
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PM 9/1(5)

**National regulatory control systems**
**Systèmes de lutte nationaux réglementaires**

# *Bursaphelenchus xylophilus* and its vectors: procedures for official control

## Specific scope

This standard describes the procedures for official control with the aim of containing and eradicating *Bursaphelenchus xylophilus*.

## Specific approval and amendment

First approved in 2002–2009.
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## Introduction

*Bursaphelenchus xylophilus*, the pine wood nematode, is an EPPO A2 pest. Details about its biology, distribution and economic importance can be found in EPPO/CABI (1997). Its natural means of transmission from tree to tree is by transfer through activity of the adult stages of wood-inhabiting longhorn beetles of the genus *Monochamus* (Coleoptera: Cerambycidae). These can transmit the nematode either to the shoots of living trees during the feeding of the adult insects (maturation feeding by either sex), or to the trunks or larger branches, including cutting residues or debris, or weakened trees or trees that had recently died, during oviposition by the females, depending on *Monochamus* species (Schröder *et al.*, 2009). Transmission of *B. xylophilus* to live trees during feeding by adult beetles can result in the development of wilt disease in the tree, but only in susceptible species of *Pinus* under suitable climatic and edaphic conditions.

Nevertheless, transmission of *B. xylophilus* by maturation feeding may also occur to other tree genera, but without resulting in wilt development. Transmission during oviposition can occur on most if not all coniferous species, provided the trees are weakened, dying from any cause or have recently died, thus making them suitable for *Monochamus* oviposition. Transmission at oviposition can also occur on timber and cutting residues. Known exceptions are *Thuja* and *Taxus*, which are not known to be used for maturation feeding and egg-laying of *Monochamus* spp. or to be hosts of the nematode. Thus, *B. xylophilus* can be found in wood of *Pinus* spp. expressing wilt disease after *B. xylophilus* infestation, or in wood of trees of any coniferous species (except *Thuja* and *Taxus*) that has been weakened enough to allow *Monochamus* spp. to oviposit and transmit the nematode. The nematode is very easily carried by the movement of infested, untreated wood.

This standard addresses the situation that infestation of host trees with *B. xylophilus* leads to expression of pine wilt disease; and the situation that infestation does not lead to expression of pine wilt disease. Data from North America and Japan indicate that tree mortality arising from expression of pine wilt disease is determined largely by summer-temperature isotherms and factors such as soil type or moisture content. Other factors, including host species and tree condition, also have an impact on symptom expression, but further research is needed to assess that impact. The lack of wilt symptoms in cooler conditions implies a problem in detecting *B. xylophilus* infestation/s in time for successful eradication.

The EPPO Commodity-specific Phytosanitary Measures Standard on Coniferae PM 8/2(1) (EPPO, 2009a) specifies requirements for commodities with respect to *B. xylophilus*. It covers plants for planting, cut branches, isolated bark and various types of wood (squared wood, round wood, wood packaging material, particle wood and wood residues) of coniferous species generally. It recognizes that the risk from commodities from countries where *B. xylophilus* is widespread is significantly greater than that from countries where the pest is of limited distribution and under official control with the prospect of eradication.

 

This standard presents the basis of a national regulatory control system for the containment and eradication of *B. xylophilus*. Official application of this system should enable a country to qualify for less stringent measures required by other countries to which it exports.

## Outline of requirements

A national regulatory control system is recommended to all EPPO countries for the detection, containment and eradication of *B. xylophilus* if present, and provides sufficient guarantees to allow export of host commodities within and outside the region. This system is described in the present standard.

Detection surveys should be carried out annually and be pathway-based. The survey should concentrate on potential points of introduction of *B. xylophilus*, pine wilt disease symptoms, and a time of year when they are likely to develop. In situations where wilt symptoms do not occur, detection surveys should be focused on trees and wood debris with signs of *Monochamus* activity. A delimiting survey in a situation where wilt symptoms occur should focus on sampling from dead or dying trees, as recommended by this standard. If *B. xylophilus* is found in a situation where wilt symptoms do not occur, surveys should focus on sites with *Monochamus* activity.

For containment and eradication of *B. xylophilus*, a minimum regulated area of at least 6 km radius around the infested tree(s) should be established, and measures should be taken in the sequence recommended by this standard. No findings of *B. xylophilus* over at least the duration of two vector life cycles, with a minimum of 3 years of annual monitoring and sampling in the regulated area, can be considered as evidence of successful eradication of *B. xylophilus*.

## Surveillance

Surveillance for the presence of *B. xylophilus* in a country or area not known to have pine wood nematode is usually based on a detection survey (the method used for a detection survey is described in Appendix 1). If *B. xylophilus* is found and confirmed, a delimiting survey has to be carried out in order to delimit the infested area (the method used for a delimiting survey is described in Appendix 2). Surveillance should continue in the infested area until *B. xylophilus* is eradicated.

The collection and processing of samples is described in Appendix 3. Identification of nematodes extracted from samples is described in EPPO standard PM 7/4 *Diagnostic Protocol for B. xylophilus* (EPPO, 2009b).

## Containment and eradication

### I. Situations where pine wilt occurs

Upon detection of *B. xylophilus* in situations where pine wilt symptoms occur, in the initial tree(s) cutting debris, wood residues or dead trees in a country or region, official containment and eradication measures should be taken in the following sequence:

1) A minimum initial regulated area of at least 6 km radius around the infested tree(s)[1] should be established;
2) The infested tree(s) should be felled immediately and destroyed completely *in situ* including the cutting residues, and an intensive delimiting survey of 5 km radius around the positive tree/s should commence immediately. New findings of infested trees should extend the delimiting survey further, with a 5 km radius around the last finding;
3) Depending on the results of the delimiting survey, the regulated area for application of containment and eradication measures should be re-defined to extend outward from the boundary of the area occupied by infested trees;
4) On the basis that *Monochamus* adults tend to only fly short distances within dense conifer forests and further distances in more open environments with fewer trees, the measures below differentiate between these situations.
   (a) For a localized and small infestation, a clear-cut area with a minimum of 500 m radius (in a pure stand of host trees, managed as forest) up to 3 km around the infested tree(s) should be established. Expert judgement should determine the precise necessary radius of the clear-cut area, based on maturation feeding (in relation to density of trees) and the presence of sites suitable for oviposition (in relation to tree conditions) in the regulated area. Sites suitable for oviposition include weakened trees, trees that recently died or felled trees as well as logging residues such as top branches and any trunk wood with bark remaining after felling. The felling of host species should be carried out from the outside of the area towards the centre and should be carried out outside the flying period of the vector. Samples taken from the trees felled in the clear-cut area (Appendix 3) should be analysed for presence of *B. xylophilus*. If any infestation is found, a further delimiting survey (as described in step 2) should be carried out and a new clear-cut area established;
   (b) For a wider and more dispersed infestation, the edge of the known infested area should be delimited and a clear-cut area with a minimum of 500 m width (in a pure stand of host trees, managed as forest) up to 3 km around the edge should be established. Expert judgement should determine the precise necessary width of the clear-cut, based on tree density and condition, maturation feeding and the presence of sites suitable for oviposition (see above) in the regulated area. The felling of host species should be carried out from the outside of the area towards the

[1]The 6 km is based on the minimum radius of the regulated area, consisting of a 500 m to 3 km clear-cut area immediately around the infested tree(s) and an adjacent area of at least 3 km which should be monitored intensively for the presence of *B. xylophilus*.

centre, and should be carried out outside the flying period of the vector. Samples of trees felled in the clear-cut area (Appendix 3) should be analysed for the presence of *B. xylophilus*. If any sample tests positive, a further delimiting survey (as described in step 2) should be carried out and a new clear-cut area established.

5) In both cases, a minimum of a 3 km wide intensive survey area around the edge of the clear-cut area should be set up.

### II. Situations where pine wilt symptom do not occur

Upon detection of *B. xylophilus* in cutting debris, wood residues or dead trees in a country or area in situations where pine wilt symptoms do not occur, two basic situations can be distinguished.

A. Sites suitable for oviposition (see above) for *Monochamus* spp. are scattered throughout the area (e.g. area partly or completely covered by host tree species, often not in use as production forest).

B. Sites suitable for oviposition (see above) for *Monochamus* spp. are concentrated in logging areas of 1–2 years old or stands where trees are weakened due to snowfall, storms, forest fires, or biotic agents such as bark beetles. This covers areas completely covered by forest and in use as production forest.

In practice, there might be variable mixtures of situations.

In situation A, upon detection, official containment and eradication measures should be taken in the following sequence.

- A.1 An initial regulated area of at least 6 km radius around the infested site should be established.
- A.2 The infested oviposition site(s) should be removed immediately and an intensive delimiting survey of 5 km radius around the positive site should commence immediately (see Appendix 2, 2nd survey strategy for situations where wilt symptoms do not occur). New positive findings should extend the delimiting survey further, with a 5 km radius around the last finding;
- A.3 Depending on the results of the delimiting survey, a regulated area for application of containment and eradication measures should be established;
- A.4 For a localized and small infestation a clear-cut area with a minimum of 100 m radius (the exact radius to be determined by expert judgement based on testing for *B. xylophilus* presence) around the infested site should be established in order to remove the possible presence of *B. xylophilus* resulting from maturation feeding of *Monochamus*. The felling of host species should be carried out from the outside of the area towards the centre and should be carried out outside the flying period of the vector;
- A.5 For a wider and more dispersed infestation, it has to be decided whether eradication is still feasible. In the case that eradication measures will be applied, the edge of the known infested area should be delimited. Around every infested site within the infested area, a clear-cut area with a minimum of 100 m should be established in order to remove the possible presence of *B. xylophilus* resulting from maturation feeding. All material that can be used for oviposition should be removed continually from the infested area during the pest eradication. In the case that eradication measures are not taken, the regulated area should be demarcated and put under official control to limit further spread and suppress the presence of the pest;
- A.6 In both cases (A.4 and A.5), a minimum of a 3 km wide intensive survey area around the edge of the infested area should be set up and maintained for at least the duration of 2 vector life cycles, with a minimum of 3 years.

In situation B, upon detection, official containment and eradication measures should be taken in the following sequence:

- B.1 An initial regulated area of at least 6 km radius around the infested site should be established;
- B.2 All conifer wood suitable for vector oviposition from the *B. xylophilus* infested site should be removed immediately and an intensive delimiting survey of at least 5 km radius around the positive site should commence immediately, focusing on priority areas with material attacked by *Monochamus* spp. (see Appendix 2, 2nd survey strategy for situations where wilt symptoms do not occur). New positive findings should extend the delimiting survey further, with a 5 km radius around the last finding;
- B.3 Depending on the results of the delimiting survey, a regulated area for application of containment and eradication measures should be established;
- B.4 For a localized and small infestation (e.g. one infested logging site) all conifer wood suitable for vector oviposition should be removed immediately from the logging site and at least before the flight period of *Monochamus* in the infested areas. A clear-cut area with a minimum of 100 m radius (the exact radius to be determined by expert judgement based on testing for *B. xylophilus* presence) around the infested site should be established in order to remove the possible presence of *B. xylophilus* resulting from maturation feeding by *Monochamus* spp. Expert judgement should determine whether sites suitable for oviposition (see above) in the immediate vicinity of the clear-cut area should be removed. The felling of host species should be carried out from the outside of the area towards the centre and should be carried out outside the flying period of the vector;
- B.5 For a wider and more dispersed infestation (e.g. several infested logging sites spread over a larger area and infestation apparently present for several years), it has to be decided whether eradication is still feasible. If it is decided to eradicate the infestation, the measures recommended for a localized and small infestation (B.4) should be applied to every infested site. The edge of the known infested area should be delimited. All material that can

be used for oviposition should be removed continually from the infested area during the pest eradication. Samples should be taken to confirm the absence of *B. xylophilus*. In the case that eradication measures are not taken, the regulated area should be demarcated and put under official control to limit further spread and suppress the presence of the pest;

- B.6 In both cases (B.4 and B.5), a minimum of a 3 km wide intensive survey area around the edges of the clear-cut areas should be set up and maintained for the duration of two vector life cycles, with a minimum of 3 years.

The aim of the measures applied within the regulated area is to locally eradicate the nematode and to prevent its spread to other areas of the country and to other countries, to limit spread within the regulated area and to eradicate the nematode by continually removing foci of infestation for a wider and more dispersed infestation. Methods for preventing spread to other areas and for reducing infestation levels are described in Appendix 4.

## Verification of pest eradication

*Bursaphelenchus xylophilus* can be considered eradicated when the following condition is fulfilled: no findings of *B. xylophilus* over at least the duration of 2 vector life cycles, with a minimum of 3 years of annual monitoring and sampling in the regulated area.

## Enquiries

Enquiries may be addressed to the EPPO Secretariat, 21 Boulevard Richard Lenoir, Paris 75011, France or at hq@eppo.inthq@eppo.int.

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This standard was originally drafted by H. Evans, Forest Research in Wales (GB) and C. Magnusson, Institute for Agricultural and Environmental Research in Norway.

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## Appendix 1 - Survey in an area not known to have *Bursaphelenchus xylophilus*

### Purpose

In order to verify that a country is free from *B. xylophilus*, regular surveys should be carried out.

### Strategy

Survey criteria to determine both presence and absence of *B. xylophilus* are based on biological characteristics of both the vector *Monochamus* and *B. xylophilus*; they target those trees and wood (such as cut stems, cutting residues material and naturally occurring debris) most likely to be exploited by *Monochamus* spp. (either for maturation feeding or oviposition) and, consequently, to have potential *B. xylophilus* transmission. In situations where pine wilt symptoms do not occur, only sites suitable for oviposition[2] are considered. Therefore, the following characteristics should be used in designing a survey strategy: known host range and distribution of *Monochamus* spp., recent logging history and areas of commercial forest, wood processing facilities, state of health (fire, windblow, snow damage, etc.), which affect the presence and distribution of *Monochamus* spp.,

[2]Sites suitable for oviposition include weakened or freshly dead or felled trees as well as logging residues such as top branches and any trunk wood with bark remaining after felling

points of wood import (including wood packaging), handling or storage. Other *Bursaphelenchus* species in the *xylophilus* group (particularly *B. mucronatus*) may also be recorded as a valuable component in designing the overall survey strategy (in particular, *B. mucronatus* has a similar relationship to *Monochamus* spp. as vectors for its dispersal). Records of *B. mucronatus* in the survey may confirm the correct choice of sample substrates and help to design the intensity of sampling programmes.

It is important to take into account that a relatively low-intensity detection survey may result in late detection of an infestation, which has already spread over a larger area.

Surveys should concentrate on the following.

1. Weakened trees (e.g. damaged by biotic agents, windblown, snow-damaged, drought-stressed, affected by forest fire);
2. 1–2-year-old logging sites (targeting e.g. cutting residues, broken or cut stumps, snags, standing dead trees left for biodiversity purposes);
3. Trees in non-forest locations (e.g. parks, gardens, street trees) close to potential points of introduction of *B. xylophilus*; wood-processing yards, either stored logs or chips, shavings or sawdust. In this case, for reasons of traceability, it is important to be sure of the exact origin of the wood, as processing yards may contain wood of diverse origins, including imported material and trees in the immediate vicinity;
4. Collection stations of fuel wood and trees in the immediate vicinity. For reasons of traceability, it is important to be sure of the exact origin of the wood, as collection stations may contain wood of diverse origins, including imported material;

Surveys should be carried out annually. In situations where wilt symptoms occur, the survey should concentrate on a time of year when both potential pine wilt disease symptoms and vector activity are likely to be detected. Direct tree surveys can also be augmented by the use of traps (pheromone/kairomone traps, trap trees, etc.) (Pajares *et al.*, 2010) to capture adult *Monochamus* that can themselves be sampled for the presence of *B. xylophilus*. If it is not possible to survey during the main activity period, sampling of trees can be carried out over the winter months. Sampling should be focused on trees/wood debris with signs of *Monochamus* activity. In the case of cutting residues, this would require snow-free conditions. Examples of some useful survey techniques can be found in Magnusson *et al.* (2007).

In situations where wilt symptoms do not occur, the timing of the survey is less critical. Surveys should be pathway-based, which will allow resources to be targeted to those pathways with the highest likelihood of *Monochamus* spp. and/or *B. xylophilus* being present and causing introduction in new areas. In order of importance, the main pathways or types of wood capable of supporting vectors and *B. xylophilus* are as follows.

- Round wood with bark (as logs or as standing trees): trees that have been weakened or have recently died can be utilized for breeding by *Monochamus* spp. The larval stages of the vector may initially be present in the cambial zone, but later they enter the wood where they complete their life cycle through the pupal stage and eventually emerge as new adults;
- Wood packaging/sawn wood with or without bark: if trees have been infested by *Monochamus* spp. and/or *B. xylophilus*, they may still be present in sawn wood, even if the outer layers with bark have been removed. Survey effort should concentrate on wood processing facilities that have such trees in their inventories, whether the wood is used for packaging or as a product in its own right;
- Conifer plants for planting: larger trees that are moved internationally may have been used by *Monochamus* spp. for maturation feeding or, if the trees were already weak, for breeding by the vector. The likelihood of finding either or both *Monochamus* spp. and *B. xylophilus* in such plants will depend on the state of health of the plants for planting. Survey effort should concentrate on larger specimens, with emphasis on detection of maturation feeding in the crowns and signs of breeding on the stems.

As the purpose of the surveys is to optimize the prospects of detecting *B. xylophilus* as soon as possible after arrival in a previously *B. xylophilus*-free area, emphasis should be on the pathways as they enter the area, or on sampling in woodlands close to the end points of the pathways. Emphasis should, therefore, be on points of import of wood products and/or processing facilities and points of import of wood packaging material or large conifer plants for planting and the distribution of these products into the area not known to have *B. xylophilus*.

## Appendix 2 - Delimiting surveys in an area where *B. xylophilus* has been found

### Purpose

During any form of survey for *B. xylophilus*, if nematodes are found and confirmed to be *B. xylophilus*, then an intensive delimiting survey to establish the full extent of the infestation should be carried out immediately. The purpose of this will be to determine geographical limits of the infested area (or areas) and then to demarcate the regulated area.

### Strategy

If *B. xylophilus* is found in a situation where wilt symptoms occur, a delimiting survey covering an area of 5 km radius surrounding the infested tree/s should be carried out. If further positive results are obtained, the edges of the delimiting survey should be moved outwards (by 5 km from the additional infested trees) until no further positive results are recorded.

Samples should be taken from dead or dying trees, including cut stems, cutting residues and naturally occurring

debris, especially those showing signs of activity of insect vectors of the genus *Monochamus*. Nematodes may be detectable for 3 years after tree death (Malek & Appleby, 1984). In principle, apparently healthy trees are unlikely to contain *B. xylophilus*, but in some cases *B. xylophilus* does not cause symptoms, and trees that appear to be healthy may contain *B. xylophilus*, usually in the crown, reflecting entry of the nematodes to that part of the tree during maturation feeding by adult *Monochamus* spp. Sampling from apparently healthy trees would, therefore, be valuable, especially in areas where *Monochamus* activity is higher than normal.

If *B. xylophilus* is found in a situation where wilt symptoms do not occur, the following strategy can be followed for the delimiting survey.

A delimiting survey covering an area of at least 5 km radius surrounding the infested tree(s) or wood material should be carried out. This is particularly important for areas in which sites suitable for oviposition[3] are evenly spread around. In those areas where sites suitable for oviposition are concentrated in clear-cut areas (managed as production forests), providing cutting residues, and the delimiting survey will focus particularly on those sites. If further positive results are obtained, the edges of the delimiting survey should be moved outwards (by 5 km from the additional infested trees) until no further positive results are recorded.

It is important to take into account that a relatively low-intensity delimiting survey may indicate a localized and small infestation, while in reality the infestation is wider and more dispersed.

If possible, the pathway of introduction and the length of time the pest has been present should be taken into account to set the size of the delimiting survey.

## Appendix 3 - Sampling procedures

It must be borne in mind that the likelihood of detecting *B. xylophilus* in a tree is determined by the distribution of nematodes through the tree. If a tree is already weak or has died recently from causes other than wilt expression caused by pine wood nematode, then the nematodes will be localized in oviposition sites, which may not be in the main trunk area; for example, *M. galloprovincialis* lays its eggs only in the upper trunk and thicker branches of host trees. If a tree has actually been killed by *B. xylophilus* infestation, there is increased likelihood of nematodes being present through the whole trunk area and so detection at breast height is possible, but not certain. If a tree has been infested during maturation feeding but is not yet showing strong wilt symptoms, the nematodes are likely to be more localized in the upper part of the tree closest to the maturation feeding sites and, therefore, will not be detected in the main trunk. Consequently, the strategy that gives the highest likelihood of detecting *B. xylophilus* in all circumstances is to take samples from several positions along the trunk, but always including the upper trunk and canopy area and preferably at places where *Monochamus* activity (e.g. signs of maturation feeding, grub holes, galleries) is found.

Symptoms which can be used as indicators for sampling include: discoloration (e.g. yellowing or reddening) of needles, wilting of foliage, partial die-back of branches, evidence of insect attack (e.g. the typical Lamiinae larvae of *Monochamus* beneath the bark or the oval larval galleries ('grub holes') or round exit holes of adults), blue-stain fungal growth in the wood, and lack of oleoresin flow from wounds. The rate of oleoresin flow may be checked while trees are still green by removing part of the bark from the cambial layer; this method is used for early detection of pine wilt disease in some countries. However, these symptoms are non-specific and may be caused by physical factors, such as wind or fire damage, or by other insect pests or pathogens. There is currently no method to distinguish visually between trees that are dying from pine wilt disease and those dying for other reasons. It should also be borne in mind that presence of *B. xylophilus* in trees is not always associated with wilt symptoms, and nematode presence in a tree may be localized to *Monochamus* oviposition sites, although this will always be in a tree that is weakened or has recently died.

In situations where pine wilt symptoms do not occur, sampling should target material used by *Monochamus* for oviposition, such as cutting residues and weakened trees.

For extended surveys and intensive sampling of *B. xylophilus* in trees and cutting residues, a powerful, water-tolerant, cordless drilling machine may be the best solution for collecting wood samples. It is important to operate it at a slow speed, using a bit-size of at least 17 mm diameter, to produce shavings for the samples. The diameter of the drill is not critical, but smaller drills may generate more heat than larger ones. For dead trees, an alternative way of sampling for detecting *B. xylophilus* is to cut wood discs from three positions along the length of the felled tree, with particular emphasis on taking samples from the upper trunk/canopy of the tree. Bark should be removed before cutting to enable the presence of staining fungi or insect gallery systems or grub holes to be observed. Bark removal will reduce contamination of the sample with saprophytic nematodes. Nevertheless *B. xylophilus* occurs also in the bark. The discs should be taken from such contaminated wood, and should be cut into small pieces using a method that does not generate heat.

A chainsaw may also be used to produce sawdust from several parts of the tree. In all cases, at least 60 g of wood should be taken from each tree. In all cases, it is important to avoid cross-contamination between samples from different geographical locations; use of e.g. a mini-burner to sterilize instruments or alcohol (>70%) to clean instruments,

[3]Sites suitable for oviposition include weakened or freshly dead or felled trees as well as logging residues such as top branches and any trunk wood with bark remaining after felling

and avoidance of previously used containers, will reduce the risk. The samples should be collected in new plastic bags, labelled (location, including GPS coordinates where possible), sealed and kept out of direct sunshine while being taken to the laboratory.

In areas with a known population of *Monochamus* beetles, logs felled during the flight period of the beetles may be used as trap logs. Beetles are attracted to them for oviposition and it has been proven that nematode transmission can take place in such cases (Luzzi *et al.*, 1984; Dwinell, 1997). Sampling the wood or the emerged beetles can be used to monitor for the presence of *B. xylophilus* in a limited area. It is also possible to accelerate beetle development by taking the trap log material into the laboratory in the autumn and maintaining it at higher than ambient winter temperatures: beetles will emerge several weeks before they would have emerged under natural conditions (Schönfeld *et al.*, 2008). Wood samples can be taken from the trap logs by the methods described above for trees.

#### Processing of samples

The samples should be incubated at 25°C for at least 14 days (Schröder *et al.*, 2009) to allow any nematodes present to breed and to maximize the likelihood of detection. Any nematodes present should be extracted from samples by a method that relies on the fact that live nematodes will emerge from wood when it is immersed in water and will settle to the bottom of the vessel in which the wood samples are placed (e.g. the Baermann funnel technique). Nematodes recovered in this way can be identified using EPPO Standard PM 7/4 *Diagnostic Protocol for B. xylophilus* (EPPO, 2009b).

## Appendix 4 - Measures in the regulated area

The measures to be applied to all types of host commodities, in order to prevent transfer of *B. xylophilus* and its vectors from the infested area to other parts of the country, should be at least as stringent as those applied for import, as recommended in the Commodity-specific Phytosanitary Measures Standard on Coniferae, PM 8/2(1).

The measures aimed at eradicating *B. xylophilus* are based on the principle of eliminating or preventing the spread of the vector, insects of the genus *Monochamus*, that carry this species of nematode. This is done by preventing the insects from emerging from wood infested with the nematodes and thus eliminating the possibility of transfer to other trees, where they could create new foci of infestation. These measures are applied in the regulated area to all coniferous species (except *Thuja* and *Taxus*).

#### Plants for planting

In the regulated area, plants for planting of coniferous species (except *Thuja* and *Taxus* species) should not be grown in a place of production unless that place of production is tested and no *B. xylophilus* is found and host plants for planting are grown under vector-proof conditions. In addition, in those situations where maturation feeding does not lead to wilt symptoms, an evaluation of whether the plants present at the place of production at the time of establishment of the pest free place of production are infested with *B. xylophilus* should be carried out.

#### Standing trees (living or dead)

*Clear-cut areas*

Clear-cut areas should be kept free from host plants until *B. xylophilus* is declared to be eradicated in the infested areas. To minimize the likelihood of breeding of *Monochamus* spp. in the stump, trees should be cut as close as possible to the soil surface.

*Larger infested area*

On the basis of visual inspection, it is generally not possible to distinguish living trees expressing wilt symptoms caused by *B. xylophilus* from those trees dying or dead from any other cause and, in an infested area, any dead or dying coniferous trees (except *Thuja* and *Taxus*) are therefore to be considered as being potentially infested with *B. xylophilus*. Throughout the infested area, all dead or dying coniferous trees should be felled immediately if detected during the *Monochamus* flight period. When felling dead or dying trees, some living coniferous trees without symptoms should be felled as possible oviposition sites to reduce further dispersal of the infested *Monochamus*, and these trees should be removed after the first flight season prior to the next flight season of *Monochamus* (trap trees). As long as the eradication measures take place in the infested area, some living coniferous trees without symptoms should be felled for every flight period, to function as trap trees. If detected outside the flight period, the trees should be felled before the next flight period starts. To minimize the likelihood of breeding in the stump, trees should be cut as close as possible to the soil surface. All felled trees should be assessed for the presence of *B. xylophilus*. If *B. xylophilus* is detected, all host trees within a radius of 100 m should be felled and destroyed (including all felling debris), since attacks by *Monochamus* tend to be grouped on neighbouring trees. Some of the felled trees should be sampled (see Appendix 3) and analysed for the presence of *B. xylophilus*. If any positive trees are found, a further 100 m felling area should be established. These measures should be applied if it is decided not to clear-fell the entire infested area, i.e. these are the minimum eradication measures to be applied in an infested area.

In the situation that no pine wilt symptoms occur, an annual survey should be carried out to sample possible oviposition material. All possible oviposition material should be destroyed in the area in order to exterminate the vector.

These surveys and removal of oviposition material should continue until the end of the verification period.

#### Wood

Wood from clear-cut areas:

- Can be transported freely out of the area provided that it is either heat-treated so that the wood-core temperature is maintained at 56°C for 30 min, or fumigated with a fumigant, according to EPPO Standards PM 10/6 *Heat treatment of wood to control insects and wood borne nematodes* (EPPO, 2009c) and PM 10/7 *Methyl bromide fumigation of wood to control insects* (OEPP/EPPO, 2009d);
- If not treated using an approved procedure, the wood should be destroyed completely by burning (avoiding fire damage to adjacent trees which could act as an attractant to vector insects of the genus *Monochamus*);
- Can be used for industrial purposes within the regulated area or chipped and transported, under official control, to an approved processing facility. If the chips are not to be used immediately for industrial purposes, they should be heat-treated (at 56°C for 30 min) or fumigated with a suitable fumigant, according to EPPO Standard PM 10/7;
- Outside the vector flight period, wood can be moved under official control outside the area to an approved processing facility, and processed or treated before the start of the next flight period.

Wood from trees in the infested area:

- Can be transported freely out of the area provided that it is either heat-treated so that the temperature is maintained at 56°C for 30 min throughout the entire profile of wood, or fumigated with a suitable fumigant, according to EPPO Standards PM 10/6 and PM 10/7;
- If not treated using an approved procedure, the wood should be destroyed completely by burning (avoiding fire damage to adjoining trees which could act as an attractant to vector insects of the genus *Monochamus*);
- Can be used as industrial fuel or chipped and kept within the infested area. Wood chips to be left on site must not exceed 3 cm in any dimension. If the chips are not to be used immediately for industrial purposes, they should be heat-treated (at 56°C for 30 min) or fumigated with a suitable fumigant, according to EPPO Standards PM 10/6 and PM 10/7;
- Can be processed into sawn wood for use within the infested area, provided that it is tested and found free from *B. xylophilus*. If the wood derives from trees felled in summer and is not processed immediately into sawn wood, it should be debarked directly after felling. Wood from trees felled in the winter period (November 1–March 31) should be treated, processed or destroyed before the end of this period.
- In addition, outside the vector flight period, wood tested and shown to be free from *B. xylophilus* can be moved under official control outside the infested area to an approved processing facility and processed or treated before the start of the next flight period.

#### Bark

Isolated bark removed from trees in the infested area should either be destroyed by burning, or used as industrial fuel within the regulated area, or heat-treated (minimum 56°C for at least 30 min throughout the bark), or fumigated with a suitable fumigant. If heat-treated or fumigated, the bark can be transported freely out of the regulated area. Bark can also be transported in closed containers and under official control to approved processing facilities at any time of the year.

#### Wood residues and debris

Wood residues and debris produced during felling in the infested area and the clear-cut areas should be destroyed completely by burning at or near the place where the tree was felled, or transformed into chips not exceeding 3 cm in any dimension. However chips should not be left on site in the clear-cut areas. Especially during the summer period, this should be done as soon as possible after felling. Wood residues produced during other processing procedures should be destroyed by burning, used as industrial fuel, or fumigated with a suitable fumigant. Residual and wood residues can also be transported in closed containers and under official control to approved processing facilities outside the vector flight period, and utilized before the start of the next flight period.

#### General measures

General measures taken in the infested area should aim to decrease the likelihood of build-up and dispersal of *Monochamus* spp., and hence reduce the likelihood of spread of *B. xylophilus* via its vectors that could lead to new foci of *B. xylophilus* infestation. This requires the maintenance of a high degree of forest hygiene. To eliminate breeding sites for *Monochamus* spp., wood residues should be removed as soon as possible, and certainly before the flight period of the beetles. In order to avoid damage from forest machinery that could impair tree vigour, forest operations should be limited to salvage activities of storm-damaged or killed trees and to the removal of dying and deteriorating trees. There should be efficient control of forest fires, which are important ecological factors in enhancing the build-up of populations of *Monochamus* spp. The presence of weakened, dying trees or trees that have recently died should be kept to a minimum to avoid build-up of populations of *Monochamus* spp. Both visual examination of trees for presence of beetle attack and the use of baited insect traps and trap logs will provide specimens of the beetle

that can, especially in the case of adults, be assessed for the presence of *B. xylophilus*. If trap logs are employed to attract and retain *Monochamus* spp., they should be destroyed before emergence of adult vectors that could have completed their development in the logs. Use of trap logs provides information on vector populations, and can also contribute to population reduction when the logs are destroyed.