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European and Mediterranean Plant Protection Organization
Organisation Européenne et Méditerranéenne pour la Protection des Plantes PM 9/1 (6)

Systèmes de lutte nationaux réglementaires
National regulatory control systems

PM 9/1 (6) *Bursaphelenchus xylophilus* and its vectors: procedures for official control

Specific scope

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

Specific approval and amendment

First approved in 2002-09.

First revision approved in 2009-09.

Second revision approved in 2010-09.

Third revision approved in 2011-09.

Fourth revision (editorial changes only) approved in 2012-09.

Fifth revision approved in 2018-09

1. Introduction

This Standard presents the basis of a national regulatory control system for the eradication of *Bursaphelenchus xylophilus* and associated surveillance to enable early detection. It also provides guidance on containment measures to prevent further spread, whether from an outbreak during eradication or from an area of established infestation where eradication is no longer achievable.

Eradication and containment are defined in ISPM 5 as, respectively, 'The application of phytosanitary measures to eliminate a pest from an area' and 'The application of phytosanitary measures in and around an infested area to prevent spread of a pest'. Unless it can be achieved quickly, a strategy of eradication has to be accompanied by implementation of measures for containment to avoid spread while the outbreak is eliminated.

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). During the feeding of the adult insects (maturation feeding by either sex) the nematode can be transmitted to the shoots of living trees, and during oviposition by the females it can be transmitted to the trunks or larger branches, cutting residues or debris, or weakened trees or trees that have recently died or those situated in storm- or fire-affected areas, depending on the *Monochamus* species involved (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 growth conditions.

Transmission of *B. xylophilus* to other tree genera by maturation feeding may also occur, but does not result in the development of wilt. 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* species or to be hosts of the nematode. Thus, *B. xylophilus* can be found in the wood of trees of any coniferous species (except *Thuja* and *Taxus*) that has been weakened enough to allow *Monochamus* species to oviposit and transmit the nematode and not only in wood of *Pinus* species expressing wilt disease. The nematode is very easily carried by the movement of infested, untreated wood. There are known to be differences in susceptibility between different genera of conifers and between species of *Pinus* (Evans *et al.*, 1996; EFSA, 2013), but given the associated uncertainties these have not been considered further in this revision of the Standard.

This Standard addresses the situation when infestation of host trees with *B. xylophilus* leads to expression of pine wilt disease as well as the situation when infestation does not lead to expression of pine wilt disease. Data from North America, Asia and Europe (Gruffudd *et al.*, 2016) indicates that tree mortality arising from expression of pine wilt disease is determined largely by whether or not mean summer isotherms are above 20°C. Other factors, including soil moisture, host species, tree condition and the level of the nematode population within the tree, also have an impact on symptom expression. The lack of wilt symptoms in cooler conditions or during the latent period before symptom expression may make it difficult to detect *B. xylophilus* infestation(s) in time for successful eradication.

This revision of the Standard takes into account new information on the flight capacity of the vector, the availability of an effective pheromone/kairomone attractant for trapping it, the possibility of remote sensing for early detection of symptoms and the efficacy of emamectin benzoate as a treatment in certain situations. Some demarcated areas were increased on account of evidence presented about the flight potential of the vector. Conversely, the size of the clear-cut area was reduced on account of evidence presented about the lack of infestation found, in practice, in asymptomatic trees felled in clear-cut areas. The overall intention of the revision was to rebalance efforts to increase the intensity of surveillance over a wider area while reducing resources going into felling of probably uninfested trees.

Key uncertainties remain: firstly, the probability distribution of actual flight distances of the vector under different field and physiological conditions (e.g. whether through continuous or fragmented pine stands and whether or not it is bearing eggs and carrying nematodes) and, secondly, the significance of asymptomatic presence of the nematode. Asymptomatic presence can occur where temperatures are high enough for symptom expression, in more tolerant host species and during early, latent, stages of infestation. There is some evidence that infested trees can be attractive to vectors even in the absence of symptoms (Futai & Takeuchi, 2008) The mechanism for this attraction is thought to be a change in the volatiles emitted from an asymptomatic tree (Futai & Takeuchi, 2008), However, since these changes in the spectra of volatiles are not known, early detection of asymptomatic trees remains a challenge.

EPPO Standard PM 8/2 (1) on commodity-specific phytosanitary measures for Coniferae (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 (such as sawn wood, round wood, wood packaging material, wood chips, hogwood and wood residues) of coniferous species. It recognizes that the risk associated with 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. Official application of this system should enable a country to qualify for less stringent measures required by other countries to which it exports.

2. Outline of the system

A national regulatory control system is recommended to all EPPO countries for the early detection, containment and eradication of *B. xylophilus*. Elements of this Standard are also relevant to a long-term containment strategy when eradication is no longer a feasible objective. The system described provides sufficient guarantees to allow export of host commodities within and out of infested and regulated areas.

Annual surveys should be carried out for early detection of new outbreaks and for monitoring spread using appropriate methods depending on whether or not wilt symptoms are likely to be seen.

Countries are encouraged to develop and test a contingency plan for outbreaks of the pest, drawing on guidance in this Standard and in EPPO Standards PM 9/10 (1) *Generic elements for contingency plans* (EPPO, 2009b) and PM 9/18 (1) *Decision-support scheme for prioritizing action during outbreaks* (EPPO, 2014a). The contingency plan should take account of climate, host plant distribution (likelihood of symptoms or of latency), vectors present in the country and the silvicultural practices and socio-economic structure of the forestry sector. In larger countries, different contingency plans may be needed for different regions and circumstances.

Publicity should be prepared and disseminated to explain the risks posed by *B. xylophilus* to landowners, forestry operators and members of the public who may be affected by measures taken. In particular operators of transport and wood handling facilities near the boundary of infested and uninfested areas should be encouraged to take simple hygiene precautions to avoid inadvertently moving vectors, as well as complying with any measures in place against moving the nematode on specified commodities.

For containment and eradication of *B. xylophilus*, in the case of new outbreaks, a minimum regulated area around the infested tree(s) with a radius of at least 20 km should be established, and measures should be taken in the sequence recommended by this Standard. This radius is justified by recent data on the flight capacities of *Monochamus galloprovincialis* (Etxebeste *et al.*, 2016).

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 its successful eradication.

3. Surveillance

Early detection of new outbreaks is a very important factor in determining the likelihood of eradication.

Detection surveys should be carried out annually on susceptible plants, susceptible wood and bark and on the vector to determine the presence of *B. xylophilus.* Surveys should also be partly pathway-based, concentrating on potential points of introduction of *B. xylophilus*. Surveys should include tree sampling, focusing on dying trees, and the use of traps containing a mixed pheromone/kairomone attractant, followed by testing of any trapped *Monochamus* species for the presence of *B. xylophilus*. Appendix 1 should be consulted for more details.

Bursaphelenchus xylophilus may infest trees without expression of wilt. This occurs on coniferous species which are not susceptible to *B. xylophilus* and in areas that are not warm enough for wilt expression. 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 *et al.*, 2008). The impact of climate change on productivity of existing and future forests must be taken into account and include possible effects on pine wood nematode and other damaging biotic agents.

In situations where wilt disease is likely to occur, surveys should focus on the pine species which are most likely to show symptoms (*Pinus nigra*, *Pinus pinaster*, *Pinus radiata*, *Pinus sylvestris*) and the time of year when symptoms are likely to develop. Remote sensing from aircraft or drones, with or without automated image analysis, may be used for early detection of new suspect cases and to help target ground-based inspection and trapping of vectors for laboratory analysis to check for the presence or absence of the nematode. Following detection, a delimiting survey should focus on sampling from dead or dying trees, but should also include some vector trapping. Even in situations where wilt disease is likely to occur, survey design should take account of latency, particularly in more tolerant species.

In situations where wilt symptoms do not occur, detection surveys should be based on trapping with a pheromone and kairomone attractant, supplemented by inspection of trees and wood debris showing signs of *Monochamus* activity. Trees felled during the flight period of *Monochamus* may be used as trap trees to detect the presence of adult *Monochamus* and associated *B. xylophilus.* If *B. xylophilus* is found in a situation where wilt symptoms do not occur, delimiting surveys should be based on trapping and inspection of sites with *Monochamus* activity. Trapping should not be used within a zone of 200 m outside the infested area, to avoid drawing infected vectors into areas which were previously free from the nematode (the attraction radius of pheromone traps, e.g. a cross vane model baited with a Galloprotect pack, being around 100 m; Jactel *et al.*, 2018).

Where a confirmed interception occurs on susceptible material, such as wooden packaging material, subsequent action should be taken if there is a likelihood that the vector has escaped into surrounding host trees. The probability of this should be assessed according to factors including the presence of insect vector exit holes, the time of year and the proximity of host species. If the probability is considered high then an area should be demarcated and measures taken in the same way as for other findings of *B. xylophilus,* including the use of vector trapping and laboratory analysis.'

The collection and processing of samples are described in Appendix 2. Identification of nematodes extracted from samples is described in EPPO Diagnostic Standard PM 7/4 Bursaphelenchus xylophilus (EPPO, 2013b).

4. Containment and eradication

When there is suspicion that *B. xylophilus* is present in an area previously believed to be free from the pest, immediate action should be taken, pending laboratory confirmation, to reduce the risk of spread, including prohibiting movement of host material from the vicinity of the suspicious finding and an appropriate provisional buffer zone based on rapid analysis of the potential distribution of the pest. Laboratory examination should be arranged urgently and the actions given below should be taken if presence of the pest is confirmed.

The infested area should be delimited. The delimitation should be adjusted in response to any new findings.

In this section 'infested area' means the area which includes all confirmed infested trees and is delimited based on all available information and expert judgement. In the early stages of an investigation, this may be limited to single trees or groups of trees from which samples have tested positive.

'Clear-cut area' means an area with a radius of at least 50 m from which all host plants (conifers other than *Thuja* and *Taxus*) are removed.

'Intensive survey area' means the area surrounding the infested area in which an intensive survey must be carried out.

4.1 Situations where pine wilt is likely to occur

Upon detection of *B. xylophilus* in situations where pine wilt symptoms are likely to 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 with a radius of at least 20 km (Fig. 1) around the infested area should be established in which measures are taken to prevent spread (David *et al.*, 2014; Sanchez-Husillos *et al.*, 2015). These measures include prohibition of any movement of susceptible plant species, wood and bark that can be infested with *B. xylophilus* or its vectors unless effectively treated against *B. xylophilus* and its vectors. The regulated area consists of the infested area and a surrounding buffer zone.
2) The infested tree(s) should be felled immediately, inspected for the presence of *Monochamus* or exit holes and then destroyed completely *in situ*, including the harvesting residues. An intensive delimiting survey should commence immediately, prioritizing areas with signs of

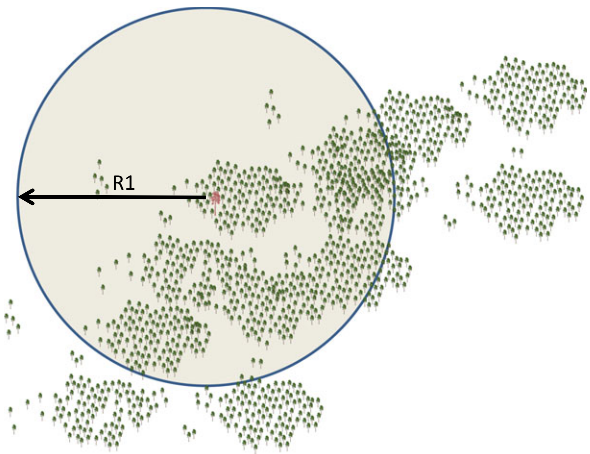

Fig. 1 Regulated area with a radius of at least 20 km (R1) around the infested area. In the initial stages of investigation the infested area may be limited to the first finding of *B. xylophilus* on one tree or group of trees. [Colour figure can be viewed at wileyonlinelibrary.com]

Monochamus activity (Fig. 2). The survey area should have a radius of at least 5–10 km (Hernández *et al.*, 2011; Jactel *et al.*, 2015) depending on whether exit holes were seen around the positive tree(s). Traps should also be placed between the outer limit of this intensive survey and 20 km of the finding. The design and density of the sampling and traps will depend on the structure and density of the susceptible trees around the detection site. New findings of infested trees or infested *Monochamus* from the traps should extend the delimiting survey further in the same way (Fig. 3).

3) Depending on the results of the delimiting survey, the regulated area for application of containment and eradication measures should be redefined to extend outward from the boundary of the infested area (Figs 3 and 4).
4) A clear-cut area (Fig. 5) with a minimum radius of 50 m should be established around each infested area.

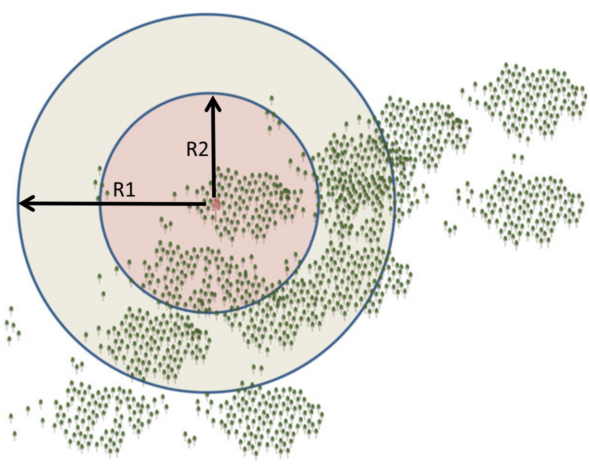

Fig. 2 Delimiting survey of radius at least 5 km (R2) depending on the situation around each new finding of *B. xylophilus* in order to provide a preliminary indication of the infested area. [Colour figure can be viewed at wileyonlinelibrary.com]

More than one clear-cut area may be created within a single regulated area (Fig. 6). Justification for the size of the clear-cut area is based partly on the need for a sufficient number of trees to be cut, sampled and tested for *B. xylophilus*. For example, in a dense forest a clear-cut of radius 50 m will produce a sample of about 1200 trees which are cut, sampled and tested for *B. xylophilus*. If all of the tests are negative this leads to a high level of confidence of freedom. Expert judgement should determine the precise radius of clear-cut area that is necessary, based on host tree distribution, the risk of spread before detection, the likelihood of symptomless infestation, evidence of 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 have died recently or felled trees, as well as logging residues such as tops, 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 or after appropriate phytosanitary treatments have been carried out against vector dispersal.
5) Samples should be taken from the canopy and trunk of each tree felled. If any sample tests positive, a further delimiting survey should be carried out and a new clear-cut area established. 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 (Fig. 6).
6) Any finding of *B. xylophilus* in a tree which was not showing symptoms should lead to a reconsideration of the assumptions made in deciding to apply this section of the Standard and therefore the survey methods and the clear-cut radius.
7) Where a number of infested trees are found, the infested areas around them can be merged into one for ease of management, and a single clear-cut area of radius at least 50 m implemented around them (Fig. 4).
8) Intensive surveys should continue around the clear-cut area as described in step 2.
9) Before the next flight season, vector pheromone/kairomone traps or trap logs should be set up in the centre of the clear-cut area to minimize the risk of dispersal of any remaining infested vectors by attracting vectors to the centre.

4.2 Situations where pine wilt symptoms are not likely to occur

Upon detection of *B. xylophilus*, for example in vectors from traps, cutting debris, wood residues or dead trees, in an area or situation where pine wilt symptoms are *not* likely to occur, two basic situations can be distinguished:

(A) Sites suitable for oviposition (see above) for *Monochamus* species are scattered throughout the area

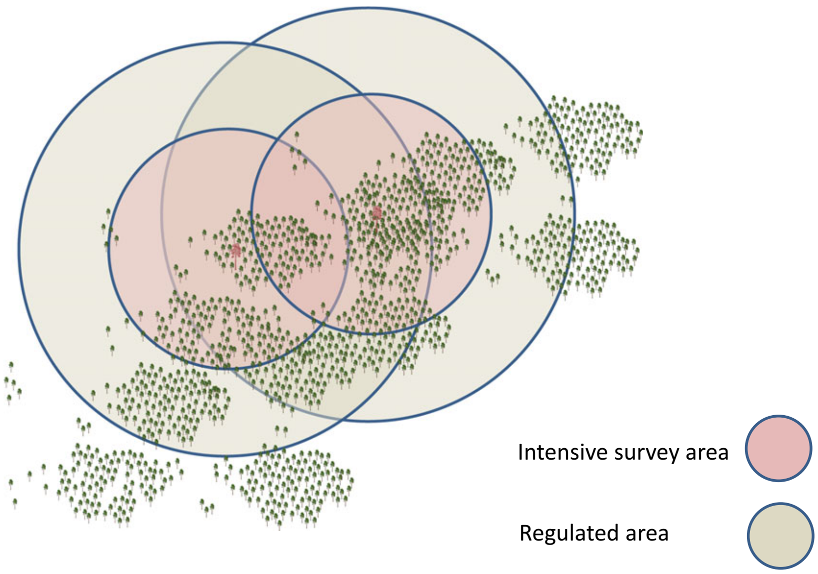

Fig. 3 The regulated area and the area of the intensive survey should be adjusted in response to any further findings. [Colour figure can be viewed at wileyonlinelibrary.com]

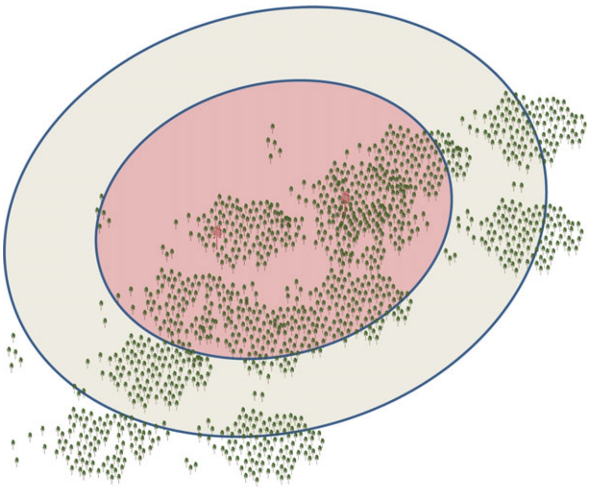

Fig. 4 Areas (infested areas, regulated areas, survey areas or clear-cut areas) may be consolidated for ease of management and communication. The figure shows an example of consolidated regulated and surveillance areas, which more than cover the areas defined by a radius shown in Fig. 3. [Colour figure can be viewed at wileyonlinelibrary.com]

(e.g. an 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 1–2-year-old logging sites or stands where trees are weakened due to snowfall, storms, forest fires or biotic agents such as bark beetles. This includes areas completely covered by forest and in use as production forest.

In practice, there might be a range 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 with a radius of at least 20 km around the infested area should be established (Fig. 1) in which measures are taken to prevent spread, including prohibition of movement of susceptible plant species, wood and bark that can be infested with *B. xylophilus* or its vectors (Appendix 3).

(A.2) Trees in the infested area which are in a weakened condition and recently felled material that is favourable for breeding of the vector should be removed and destroyed immediately and an intensive delimiting survey of an area of radius at least 5 km (Fig. 2) around the infested area should commence immediately (see Appendix 2 'Second survey strategy for situations where wilt symptoms do not occur'). Additional traps should be placed between 5 and 10 km from the finding. New positive findings should extend the delimiting survey further in the same way (Fig. 3).

(A.3) Depending on the results of the delimiting survey, the regulated area for application of containment and eradication measures should be extended outward at least 20 km from the boundary of the infested area.

(A.4) For a localized, small infestation an initial clear-cut area (Fig. 5) with a minimum radius of 50 m (the exact radius to be determined by expert judgement based on signs of dispersal of *Monochamus* species, trap findings and testing for presence of *B. xylophilus*) around the infested area should be established in order to remove any *B. xylophilus* that may be present as a result of 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.

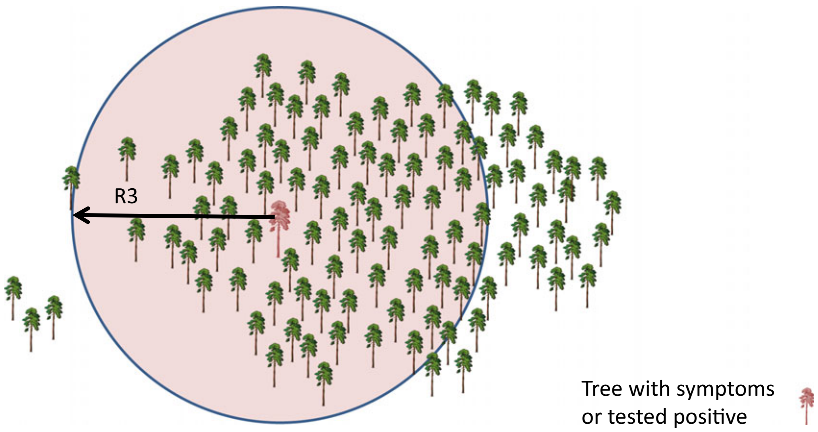

Fig. 5 All infested trees should be destroyed and all host trees within a radius of at least 50 m (R3) (depending on the situation) should be felled. Each felled tree should be checked meticulously for the presence of *B. xylophilus* and *Monochamus* species. [Colour figure can be viewed at wileyonlinelibrary.com]

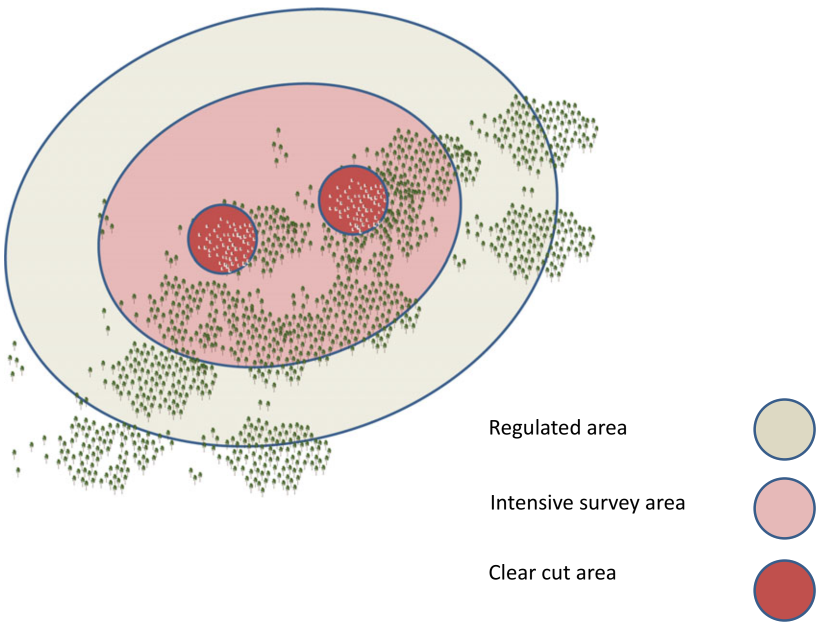

Fig. 6 More than one clear-cut area may be created within a single regulatory area. This figure, while not to scale, gives an indication of the relative size of the clear-cut, intensive survey and regulated areas. [Colour figure can be viewed at wileyonlinelibrary.com]

(A.5) For a wider and more dispersed infestation it has to be decided whether eradication is still feasible. Around the infested area, an initial clear-cut area with a minimum radius of 100 m should be established in order to remove any *B. xylophilus* that may be present as a result of maturation feeding. All material that can be used for oviposition should be removed continually from the infested area during pest eradication. If eradication measures are not taken, the regulated area should be demarcated and put under official control to limit further spread, suppress the presence of *B. xylophilus* and reduce the population of its vectors (see 'Long-term containment measures' below).

(A.6) In the case of both (A.4) and (A.5) an additional radius of at least 50 m around the infested area should also be clear-cut (Fig. 7) and an intensive survey area around the edge of the infested area with a minimum width of 5 km should be set up and maintained for at least the duration of two vector life cycles, for a minimum of 3 years.

(A.7) Before the next flight season for vector pheromone/kairomone traps or trap logs should be set up in the centre of the clear-cut to minimize the risk of dispersal of any remaining infested vectors by attracting vectors to the centre.

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

(B.1) An initial regulated area around the infested area with a radius of at least 20 km should be established in which measures are taken to prevent spread, including prohibition of the movement of susceptible plants, wood and bark that can be infested with *B. xylophilus* or its vectors (Appendix 3).

(B.2) All conifer wood suitable for vector oviposition from the infested area should be removed immediately and an intensive delimiting survey with a radius of at least 10 km around the infested area should commence immediately, focusing on priority areas with material attacked by *Monochamus* species (see Appendix 2, 'Second survey strategy for situations where wilt symptoms do not occur'). New findings should extend the delimiting survey further, with a radius of at least 10 km around the last finding;

(B.3) Depending on the results of the delimiting survey, the regulated area for application of containment and eradication measures should be extended outward at least 20 km from the boundary of the infested area.

(B.4) For a localized and small infested area (e.g. one infested logging site) all conifer wood suitable for vector breeding should be removed immediately from the logging site and at least before the flight period of *Monochamus* in the infested areas. An initial clear-cut area with a minimum radius of 50 m (the exact radius to be determined by expert judgement based on testing for the presence of *B. xylophilus*) around the infested area should be established in order to remove the possible presence of *B. xylophilus* resulting from maturation feeding by *Monochamus* species. Expert judgement should determine whether sites suitable for breeding (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 area. All material that can be used for oviposition should be removed continually from the infested area during pest eradication. If 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 (see 'Long-term containment measures' below).

(B.6) In the case of both (B.4) and (B.5) an additional area with a width of at least 50 m around the infested area should also be submitted to clear-cut (Fig. 7) and an intensive survey area with a minimum width of 5 km around the edges of the clear-cut areas should be set up and maintained for the duration of two vector life cycles, for a minimum of 3 years.

(B.7) Before the next flight season vector pheromone/kairomone traps or trap logs should be set up in the centre of the clear-cut to minimize the risk of dispersal of any remaining infested vectors by attracting vectors to the centre.

In either case, samples of trees felled in the clear-cut area should be analysed for the presence of *B. xylophilus* as

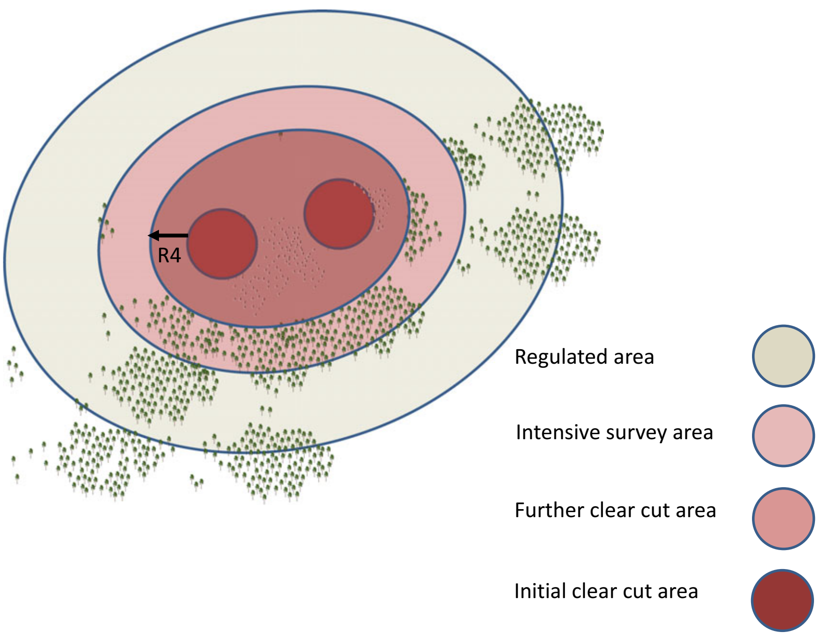

Fig. 7 In situations where pine wilt symptoms are not likely to occur after an initial clear-cut with a radius of a least 50 m has been completed a further clear-cut with a width of at least 50 m (R4) should be carried out. [Colour figure can be viewed at wileyonlinelibrary.com]

soon as possible. Samples should be taken from each tree within 50 m of the infested area and from a representative sample of the other trees. If any sample tests positive, a further delimiting survey should be carried out and a new clear-cut area established.

Old dead standing or lying trees which can (based on expert judgement) be determined to be unsuitable for oviposition or completion of the *Monochamus* life cycle but which are of value for biodiversity need not be removed.

The aim of the measures applied within the regulated area is to locally eradicate the nematode and to prevent its spread to other areas, to limit spread within the regulated area and, for a wider and more dispersed infestation, to eradicate the nematode by continually removing foci of infestation. The methods for preventing spread to other areas and for reducing infestation levels are described in Appendix 3.

5. Spread models

A number of spread models have been developed in recent years. Some spread models may be used to justify variation from the radii set out above for regulated and clear-cut zones, including decreases below the suggested minima, if the following conditions are satisfied: the model should be published in a peer-reviewed journal, parameters should be estimated based on the latest information available, an uncertainty analysis should be provided and the rationale for establishing a radius value other than the one recommended in this Standard should be published.

6. Planting of host species

Planting of susceptible plant species in a regulated area subject to eradication measures should be prohibited, with the aim of reducing the presence of susceptible plants for maturation feeding/oviposition.

7. Treatment

Micro-injection of emamectin benzoate has been investigated as a treatment to protect trees from *B. xylophilus.* It appears to be active against both the vector and the nematode. In Japan, tests on *Pinus thunbergii* and *Pinus densiflora* showed an absence of symptoms for 3 years in 91% of the trees treated with emamectin benzoate (measured at 10 g m^{-3}) and subjected to annual inoculations of the nematode (Takai *et al.*, 2003). In Portugal, no mortality was observed 26 months after the treatment of healthy trees (*P. pinaster*) located in a forest heavily infested with the nematode, whereas one-third of the control trees died during this period (Sousa *et al.*, 2013). Where emamectin benzoate has the appropriate regulatory authorization to be used in this way treatments could be applied in place of all or part of the recommended clear-cut areas. However, injection of emamectin benzoate may also have negative effects on tree health and non-target organisms and these effects should be taken into account (Kuroda & Kenmochi, 2016).

8. Verification of pest eradication

Bursaphelenchus xylophilus can be considered eradicated when the following conditions are fulfilled: 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 after the last finding.

9. Long-term containment strategy

Achieving eradication will not be possible in all circumstances. For example, in situations where the pest has already spread widely before it is detected the relevant authorities may decide that eradication can no longer be achieved and a long-term containment strategy should be adopted and announced. This long-term containment strategy should at least be sufficient to protect neighbouring areas and countries which remain free. Many of the measures outlined above for an eradication strategy will also be appropriate for a long-term containment strategy, because they suppress the pest and reduce the risk of spread. The prevalence of *B. xylophilus* in the infested area in which eradication is no longer being pursued should continue to be monitored and reported. Additional measures should be taken to reduce the risk of spread from this area. These may include:

- surveying and identifying trees with any symptom of decline at least three times per year (at the beginning of spring, midsummer and the end of autumn for instance)
- eliminating all such trees before the next flight period working from the outer limit to the inside
- trapping of the vector from the middle to the outer limit of the demarcated area.

To reduce the risk of natural spread these measures should also include a buffer zone of at least 20 km around the edge of the area, in which as a minimum the containment and eradication measures set out in this Standard continue to be taken. In relation to other pathways, the measures should be at least as stringent as those set out in EPPO Standard PM 8/2 (1).

Enquiries

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

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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 the biological characteristics of both the vector *Monochamus* species and *B. xylophilus*. These criteria target those trees and wood (such as cut stems, cutting residues material and naturally occurring debris) most likely to be exploited by *Monochamus* species (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[1] are considered. Therefore, the following characteristics should be used when designing a survey strategy: known host range and distribution of *Monochamus* species, areas with recent logging history and areas of commercial forest, wood processing facilities, trees with a state of health (fire, wind and snow damage, etc.) which affect the presence and distribution of *Monochamus* species, points of wood import (including wood packaging), handling or storage. Other *Bursaphelenchus* species in the *xylophilus* group (particularly *Bursaphelenchus mucronatus*) may also be recorded and are valuable for the design of an overall survey strategy (in particular, *B. mucronatus* and *B. xylophilus* have a similar relationship to *Monochamus* species as dispersal vectors). Records of *B. mucronatus* in the survey may confirm the correct choice of sample substrates and help in designing 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. trees damaged by biotic agents, wind- or 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* and 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 for 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.

[1]Sites suitable for oviposition include weakened or freshly dead or felled trees, including those trees situated in storm- or fire-affected areas, as well as logging residues such as tops, branches and any trunk wood with bark remaining after felling.

Surveys should be carried out annually as follows.

In situations where wilt symptoms would be likely to occur the survey should be carried out at a time of year when both potential pine wilt disease symptoms and vector activity are likely to be detected. Visual surveys and tree sampling should 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, but it is mainly *Monochamus* larvae and/or pupae that are found in these 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* species and/or *B. xylophilus* being present and causing introduction into 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* species. 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* species 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, and any other facilities where wood packaging material is stored
- conifer plants for planting: larger trees that are moved internationally may have been used by *Monochamus* species for maturation feeding or, if the trees were already weak, for breeding by the vector. The likelihood of finding either or both *Monochamus* species 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 an area previously free of *B. xylophilus*, emphasis should be on the pathways as they enter the area, or on sampling in woodlands close to the end points of the pathways [see, e.g., Douma *et al.* (2017) for an illustration of a pathway model]. Emphasis should therefore be on areas near to known infested areas, 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 areas not known to have *B. xylophilus*.

Appendix 2 – Procedures for tree sampling

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, *Monochamus 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. It is difficult 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 the presence of *B. xylophilus* in trees is not always associated with wilt symptoms, and the presence of nematodes 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 with a diameter of at least 17 mm, 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 and insect-associated nematodes, including other *Bursaphelenchus* species. 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. It is important to avoid cross-contamination between samples from different geographical locations; use of, for example, a mini-burner to sterilize instruments or alcohol (>70%) to clean instruments and avoiding 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 proved 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 a quarantine 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, or pass filters (milk filters) [e.g. the Baermann funnel technique; see EPPO Standard PM 7/119 (1) *Nematode extraction* (EPPO, 2013a)]. Nematodes recovered in this way can be identified using EPPO Diagnostic Standard PM 7/4 Bursaphelenchus xylophilus (EPPO, 2013b).

Appendix 3 – Measures in the regulated area

The regulated area, where measures are taken to avoid spread, of radius at least 20 km, consists of the 'infested area' where the pine wood nematode is known to occur and a surrounding buffer zone.

The measures to be applied in the regulated area to susceptible plant species, wood and bark, in order to prevent transfer of *B. xylophilus* and its vectors to other areas, should be at least as stringent as those applied for import, as recommended in EPPO Standard PM 8/2 (1) on commodity-specific phytosanitary measures standard on Coniferae (EPPO, 2014a).

The measures aimed at eradicating *B. xylophilus* are based on the principle of eliminating or preventing the spread of the vector, longhorn beetles of the genus *Monochamus*, that carry this species of nematode. This is done by removal and destruction of potential breeding material for vector before the next flight period of the beetles, thus eliminating the possibility of transfer of nematodes 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 (EPPO, 2016). In addition, in those situations where maturation feeding does not lead to wilt symptoms, an evaluation should be carried out 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*. Where a long-term containment strategy is adopted, plants for planting should not be moved out of the infested area or out of the buffer zone unless they comply with these conditions.

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. Trees should be cut as close as possible to the soil surface and the stump covered with soil.

Larger infested areas

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 any dead or dying coniferous trees (except *Thuja* and *Taxus*) are therefore to be considered as being potentially infested with *B. xylophilus*. All dead or dying coniferous trees should be felled and destroyed 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 left on site/*in situ* during the first flight season after the removal of the dead and dying trees but then removed before the following flight season of *Monochamus* (trap trees). As long as the eradication measures take place, some living coniferous trees without symptoms should be felled at the beginning of 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. Trees should be cut as close as possible to the soil surface and the stump covered with soil. All felled trees should be assessed for the presence of *B. xylophilus*.

Wood

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, according to EPPO Standards PM 10/6, or fumigated with a suitable fumigant
- 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 2.5 cm in any dimension or they should be heat-treated (at 56°C for 30 min), according to EPPO Standards PM 10/6 or fumigated with a suitable fumigant;
- 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, trees infested by the vector should be completely covered with an insecticide-treated net. Wood from trees felled in the winter period (1 November 1–31 March) 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.

Wood from trees in the buffer zone:

- 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 according to EPPO

Standard PM 10/6 *Heat treatment of wood to control insects and wood borne nematodes* (EPPO, 2009c) or

- 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*), or
- can be used for industrial purposes in which the material is processed in a way which eliminates risk of spread 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 chipped to no more than 2.5 cm in any dimension or heat-treated (at 56°C for 30 min), or fumigated with a suitable fumigant
- 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.

Bark

Isolated bark removed from trees in the regulated 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 nearby approved processing facilities at any time of the year.

Wood residues and debris

Wood residues and debris produced during felling in the regulated area should be destroyed completely by burning at or near the place where the tree was felled, or transformed into chips not exceeding 2.5 cm in any dimension. This should be done as soon as possible after felling, and this is especially important in the summer. Wood residues produced during other processing procedures should be destroyed by burning, used as industrial fuel or fumigated with a suitable fumigant. Wood residues can also be transported in closed containers and under official control to nearby approved processing facilities outside the vector flight period, and utilized before the start of the next flight period.

General measures

General measures should aim to decrease the likelihood of build-up and dispersal of *Monochamus* species, 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* species, wood residues including branches should be thoroughly 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 devastating forest fires, which are important ecological factors in enhancing the build-up of populations of *Monochamus* species. The presence of weakened trees, dying trees or trees that have recently died should be kept to a minimum to avoid build-up of populations of *Monochamus* species. Both visual examination of trees for the 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 the emergence of adult vectors that could have completed their development in the logs. The use of trap logs provides information on vector populations can also contribute to population reduction when the logs are destroyed.

Erratum statement 13 November 2018

Owing to a typesetting error at a late stage of production, the number ‘9’ was inadvertently deleted in various parts of the originally published paper. This has now been corrected. The deletions had affected the following elements of the paper:

* the approval and amendment dates on page 1;
* citations of references and Standards on pages 1–3, 11 and 13;
* the final numbered item in Section 4.1;
* the percentage (91%) quoted in the third sentence of Section 7;
* the numbering of Section 9;
* the reference list.

Also in the reference list, the species names in the titles of Hernandez et al. (2011) and Sousa et al. (2013) have now been correctly formatted in italic.