Pest survey card on non-European *Monochamus* spp.

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**Abstract**

This pest survey card was prepared in the context of the EFSA mandate on plant pest surveillance (M-2017-0137) at the request of the European Commission. The purpose of the document is to assist the Member States to plan annual survey activities of quarantine organisms using a statistically sound and risk-based pest survey approach, in line with current international standards. The data requirements for such an activity include the pest distribution, its host range, its biology and risk factors, as well as available detection and identification methods. This document is part of a toolkit that consists of pest-specific documents, such as the pest survey cards, and generic documents relevant for all pests to be surveyed, including the general survey guidelines and statistical software such as RiBESS+.

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**Keywords:** plant pest, survey, risk-based surveillance, non-European *Monochamus* species, pine sawyer beetle, sawyer beetle

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Introduction

The information presented in this pest survey card was summarised from the pest categorisation of non-EU *Monochamus* spp. (EFSA PLH Panel, 2018) and other scientific documents.

The objective of this pest survey card is to provide the relevant biological information needed to prepare surveys for non-European *Monochamus* spp. in EU Member States (EFSA, 2018). This document is part of a toolkit that is being developed to assist Member States with planning a statistically sound and risk-based pest survey approach in line with International Plant Protection Convention guidelines for surveillance (FAO, 2016). The toolkit consists of pest-specific documents and generic documents relevant for all pests to be surveyed:

i. Pest-specific documents:
   a. The pest survey card on non-European *Monochamus* spp.¹

ii. General documents:
   a. The general survey guidelines
   b. The RiBESS+ manual²
   c. The statistical tools RiBESS+ and SAMPELATOR³.

1. The pest and its biology

1.1. Taxonomy

**Scientific name:** *Monochamus* spp. Dejean  
**EPPO Code:** 1MONCG  
**Class:** Insecta, **Order:** Coleoptera, **Family:** Cerambycidae, **Genus:** *Monochamus*  
**Common name of the pest:** pine sawyer beetle; sawyer beetle

Scope of the survey card

*Monochamus* spp. colonise weakened or dead trees for breeding and therefore are not expected to have a direct negative economic impact should new *Monochamus* spp. be introduced into the EU. However, several species are known to be vectors of the pine wood nematode (PWN) *Bursaphelenchus xylophilus*, which is the causative agent of pine wilt disease. Thirteen species in the *Monochamus* genus have already been identified as vectors of *B. xylophilus* in North America, Asia, and Europe (Figure 1), while other species should be considered as potential vectors (Akbulut and Stamps, 2012; Akbulut et al., 2017). Non-EU *Monochamus* spp. could become new vectors of *B. xylophilus* within the EU territory. There are about 150 non-European *Monochamus* spp. (see Section 1.4), while only a few species are known to occur in the EU. Given the scope of this pest survey card, it is important to be able to determine whether a collected specimen is additional to those already present and breeding in Europe. In general, species identification of newly identified *Monochamus* spp. is complicated and should be performed by a specialist.

The pest survey card will mainly focus on the non-EU *Monochamus* spp. that attack conifer species and which could potentially become vectors of *B. xylophilus* within the EU. These include 16 non-EU

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species, nine of which are known to be vectors of *B. xylophilus* and seven others that colonise conifers and which may potentially be vectors of the nematode. *Monochamus* spp. that attack deciduous trees have not been considered because they will not be associated with PWN. The host plants include 21 conifer species (EFSA PLH Panel, 2018).

![Figure 1: The Japanese pine sawyer (*Monochamus alternatus*) and Carolina pine sawyer beetle (*Monochamus carolinensis*) are vectors of *Bursaphelenchus xylophilus* in Asia and North America, respectively (Sources: Steven Valley, Oregon Department of Agriculture, Bugwood.org; Royal Tyler, Pro Pest and Lawn Store, Bugwood.org)](image)

1.2. **EU pest regulatory status**

Non-European populations of *Monochamus* are listed in Annex II, part A, of Commission Implementing Regulation (EU) 2019/2072. *Monochamus* beetles are vectors of *Bursaphelenchus xylophilus* and could thus provide a pathway for introduction of the nematode. With regard to risks of importation and establishment of PWN in Europe, this could occur through successful breeding and expansion of non-European *Monochamus* spp. or by transmission of the nematode to susceptible conifer hosts followed by association and further spread of the nematode by native *Monochamus* spp. In the latter situation, *M. galloprovincialis, M. saltuarius*, *M. urussovi*, *M. sutor* and *M. sartor* may become vectors of PWN even though, as native European species, they are technically outside the scope of this survey card. Nevertheless, these European *Monochamus* spp. will be picked up by the applied survey methodology and can thus be used to survey for the presence of PWN. *B. xylophilus* is listed in Annex II, Part B of Commission Implementing Regulation (EU) 2019/2072 (Figure 2). Part B of Annex II contains a list of the Union quarantine pests that must not be introduced into, moved within, or held, multiplied or released in, the Union territory, but are nevertheless known to occur in the Union territory. *B. xylophilus* is also listed as a priority pest under Commission Delegated Regulation (EU) 2019/1702.

According to Annex VI of the Commission Implementing Regulation (EU) 2019/2072, the import of plants of the tree genera *Abies, Cedrus, Chamaecyparis, Juniperus, Larix, Picea, Pinus, Pseudotsuga* and *Tsuga* is prohibited from most non-EU countries. Plant species of these genera are hosts of both the non-European *Monochamus* spp. and *B. xylophilus*. Specific import requirements are laid down in Annex VII of Commission Implementing Regulation (EU) 2019/2072, depending on the origin and characteristics of the material, for imports of the wood of conifers (Pinales) (points 76, 78, 79 and 80) and for imports of chips, particles, sawdust, shavings, wood waste and scrap obtained from the wood of conifers (Pinales) (points 77 and 81), and isolated bark of conifers (Pinales) (point 82). The specific import conditions for the introduction of wood packaging material are laid down in article 43.

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of Regulation (EU) 2016/2031 itself. The above-mentioned measures are partly directed towards mitigating the risks of introduction of non-European populations of *Monochamus* spp. and *B. xylophilus*.

1.3. Life cycle

*Monochamus* beetles feed on conifers and target weakened, dying or freshly cut trees for oviposition. *Monochamus* spp. associated with PWN mainly colonise *Pinus* spp., but they can also attack conifers of the genera *Abies* and *Picea* (Akbulut et al., 2017). Feeding on the living bark of young twigs of healthy trees is required to cover the period from emergence as new (callow) adults and needed for sexual maturation (maturation feeding). For example, *M. carolinensis* requires 10–14 days of maturation feeding after emergence (Akbulut and Stamps, 2012). Individual or small groups of eggs are laid in a slit or a pit that the female beetles chew in the bark of a dead or stressed host tree. Oviposition preferences depend on the *Monochamus* species involved, occurring either on trunks, large branches, wood debris, timber and cutting waste. In Europe, each *Monochamus* spp. shows preferences for *Picea* spp. or *Pinus* spp. as well as for the position on the tree for oviposition. This is illustrated schematically in Figure 3.

The larval stages of *Monochamus* are initially present in the cambial zone, but later instars of *Monochamus* migrate to the wood for completion of their life cycle (Figure 2) and bore oval galleries, initially deeper into the wood and later towards the surface, to form a pupal chamber lined by wood shavings arising from larval chewing. Mortality among larvae is generally high, resulting from a combination of resource availability, intraspecific competition and cannibalism during larval development (Akbulut et al., 2004; Akbulut et al., 2008).

Figure 2: The life cycle of *Monochamus* spp.
Figure 3: Preferences of European Monochamus spp. on Pinus spp. and Picea spp. for oviposition (Source: Hugh Evans, Forest Research, UK)
Monochamus spp. can have a life cycle that is either multivoltine (multiple generations per year), univoltine (one generation per year) or semivoltine (one generation takes more than one year to complete) (Akbulut and Stamps, 2012), depending on the species and the climatic conditions. In Portugal and France, most M. galloprovincialis larvae take one year to complete their life cycle (Koutroumpa et al., 2008; Naves et al., 2008), while in some areas of southern Portugal and Madeira, a bivoltine life cycle (two generations per year) has been reported (Firmino et al., 2017). Eventually, pupation occurs at the upper end of a larval gallery (Akbulut et al., 2017) in a pupal chamber close to the surface of the wood, with a final moult to the new (callow) adult stage. The newly formed adults spend a few days (e.g. 12–13 days at 25°C for M. alternatus) in the pupal chamber before emerging through a round hole chewed in the bark.

After emergence, the exact timing and length of the flight period varies between species and is influenced by climatic conditions in the area, so this will have to be determined ultimately on a case-by-case basis but will generally cover part of the period from April to October (further information on the appropriate timing of the surveys are reported in Section 2.1.2). The variation in the emergence time is also related to the number of generations per year (Akbulut et al., 2017). Adults will live for one to five months depending on the species; there is wide within- and between-species variation in longevity in the genus Monochamus (Akbulut et al., 2017). Temperatures will also have a significant impact on the lifespans of the beetles (Zhang et al., 2008; Jikumaru and Togashi, 2000) as well as the quality of the nutritional sources consumed during the larval and adult stages (Akbulut et al., 2017).

As Monochamus spp. prefer to attack weakened or dead trees they are not considered to have a large impact by themselves in either the adult or larval stages. However, several Monochamus spp. are known to be vectors of the PWN (B. xylophilus). Currently, 13 species (Table 1) have been recorded as vectors (EFSA PLH Panel, 2018). In its native range, M. carolinensis and M. scutellatus are the major vectors of B. xylophilus (Akbulut and Stamps, 2012). In Asia, the main vector is M. alternatus (Nakamura-Matori, 2008), while M. galloprovincialis (Figure 4) is the only known vector in the EU (Sousa et al., 2001). Adult beetles can transmit dispersal juveniles of B. xylophilus during oviposition (in stressed, dying or recently dead trees) and via the feeding scars that are made by the beetles during maturation feeding on living trees (Linit, 1988).

<table>
<thead>
<tr>
<th>Species</th>
<th>Present in the EU</th>
<th>Absent in the EU</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. galloprovincialis</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>M. urussovii</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>M. saltuarius</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>M. sutor</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>M. alternatus</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>M. carolinensis</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>M. marmorator</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>M. mutator</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>M. nitens</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>M. notatus</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>M. obtusus</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>M. scutellatus</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>M. titillator</td>
<td></td>
<td>x</td>
</tr>
</tbody>
</table>
1.4. Pest distribution

The genus *Monochamus* comprises between 120 (Rossa et al., 2016) and 163 (Cesari et al., 2004) species, although there are some discrepancies regarding the total number of species/subspecies reported to be included in this genus (EFSA PLH Panel, 2018). Nevertheless, *Monochamus* spp. are distributed across large parts of the world (Figure 5) and have different trophic specialisations. There are two catalogues which provide information on the global presence of *Monochamus* spp.: Titan (Museum National d'Histoire Naturelle) and the Photographic Catalogue of the Cerambycidae of the World (California Department of Food and Agriculture). Most species in the online catalogues are reported from Asia and Africa, fewer from North America and Europe, and only one was reported from South America (*M. blairi* being native to Colombia). No species are present in Australia.

Cesari et al. (2004) provide an analysis of the taxonomy and phylogeny of the five *Monochamus* spp. that are present in the EU (*M. galloprovincialis*, *M. saltuarius*, *M. sutor*, *M. sartor* and *M. urussovi*) all of which are also widely distributed across Asia.

*Monochamus* spp. are of phytosanitary concern mainly because of their roles as vectors of *B. xylophilus*. In the native range of PWN the main known vectors include *M. carolinesis* (Figure 5a) and *M. scutellatus* (Figure 5b), whereas in invaded areas, *M. alternatus* in China, Japan and Korea (Figure 5c) and *M. galloprovincialis* in Portugal and Spain (Figure 5d) have taken on the role of vectors.

**Figure 4:** On the left the black pine sawyer (*Monochamus galloprovincialis*) (Source: Fabio Stergulc, Università di Udine, Bugwood.org); on the right the small white-marmorated long-horned beetle (*Monochamus sutor*) (Source: Stanislaw Kinelski, Bugwood.org)
non-European *Monochamus* species survey card

**a**

Monochamus carolinensis (MONCCA)

![World map showing distribution of Monochamus carolinensis](image)

(c) EPPO https://gd.eppo.int

**b**

Monochamus scutellatus (MONCST)

![World map showing distribution of Monochamus scutellatus](image)

(c) EPPO https://gd.eppo.int

**c**

Monochamus alternatus (MONCAL)

![World map showing distribution of Monochamus alternatus](image)

(c) EPPO https://gd.eppo.int
Figure 5: Global distribution of the main known vectors of PWN (a) *Monochamus carolinensis*, (b) *Monochamus scutellatus*, (c) *Monochamus alternatus*, and (d) *Monochamus galloprovincialis*. The countries or states in which the pest is reported have an orange colour and are marked with a yellow dot (Source: EPPO Global Database, https://gd.eppo.int)

According to the EUROPHYT database (accessed on 19 November 2019), interceptions of known or potential vectors included *M. galloprovincialis* (known vector), *M. sutor* or *M. sartor* (potential vectors) which are already present in the EU, or *M. alternatus* (known vector) not present in the EU. There have been 21 interceptions of *Monochamus* spp., confirmed as *M. alternatus*, recorded in the EUROPHYT database between 1999 and 2019. All interceptions of *M. alternatus* were made on wood packaging material or dunnage, mainly from China.

### 1.5. Host range and main hosts

All *Monochamus* spp. indigenous to temperate regions attack species of Pinaceae. Many genera belonging to this plant family are widespread across the EU (Figure 6). In general, the currently known vectors of *B. xylophilus* are mainly associated with *Pinus* spp., but some may also utilise the genera *Picea*, *Abies*, *Larix*, *Pseudotsuga* and *Tsuga* (EFSA PLH Panel, 2012).

In the EU, *M. galloprovincialis* preferentially attack pine species, especially *Pinus pinaster*, *P. sylvestris* and *P. nigra*, and occasionally also attack *Picea* spp. (Brelih et al., 2006). The other four *Monochamus* spp. have a host range which includes *Pinus*, *Picea*, *Abies*, *Larix*, *Pseudotsuga* and potentially *Cedrus* (EFSA PLH Panel, 2012). Should additional *Monochamus* spp. be introduced into the EU, the (potential) host range of *B. xylophilus* may be widened. Besides the above-mentioned genera, vectors of *B. xylophilus* in the genus *Monochamus* can also attack trees of the coniferous genera *Juniperus*, *Chamaecyparis*, *Cryptomeria* and sometimes *Tsuga* (Evans et al., 1996). Although of no concern with regard to transmission of PWN, other *Monochamus* spp. feed on broad-leaved trees, such as *M. leuconotus*, which is a pest of *Coffea arabica* in South Africa (Schoeman et al., 1998). Other species like *M. sutor* can also affect Betulaceae (e.g. *Betula platyphylla*) other than Pinaceae (CABI, online).

The cover percentage of coniferous forests in Europe is shown in Figure 6.
1.6. **Environmental suitability**

Climatic comparison for establishment of non-EU *Monochamus* at the genus level is not feasible, but it can be reasonably assumed that several regions in the EU will have eoclimatic conditions that are highly similar to those of the current distributions of several *Monochamus* spp. As reviewed by Estay et al. (2014), climatic variables, especially temperature and, to a lesser extent, rainfall (which affects the distribution of the host trees) are important factors that influence the population dynamics of *Monochamus* spp. and their host trees. Climate warming is likely to increase the potential spread of pine wilt disease across Europe, potentially increasing the likelihood of vector establishment, although there is no evidence that any *Monochamus* spp. have become established outside their native ranges (Robinet et al., 2011). For the non-EU *Monochamus* spp. that occur in temperate regions of the world (particularly those present in northern Asia and North America, especially Canada) no constraints on climatic conditions are expected (Akbulut et al., 2017) and since suitable hosts are distributed across the EU territory, biotic and abiotic conditions are favourable for establishment (EFSA PLH Panel, 2018).

1.7. **Spread capacity**

1.7.1. **Natural spread**

Host trees of several *Monochamus* spp. are abundant throughout the EU, but the extent to which host availability limits the natural spread depends on the host preferences of the specific species involved. Detailed studies of the flight capabilities of *M. alternatus* in Japan and China indicate that most flights
are very local (up to 100 m), but that flights of longer distances (various estimates of between 1.8 and 3.3 km) can also take place (Shibata, 1986; Togashi, 1990). It is also important to note that flights are over short distances when there is a local abundance of host trees, whereas longer distance flights take place when trees are absent, which fits with the observation of > 10 km by Mas et al. (2013). Mark–release–recapture experiments with field-based and laboratory-reared *M. galloprovincialis* have been conducted in Europe under extensive forest cover over consecutive years (Etxebeste et al., 2016), and showed that most recaptures occur close to the release point with a median dispersal of 233–532 m, while 99% of the dispersing *M. galloprovincialis* beetles did not disperse further than 2,344–3,496 m. Even longer recapture distances for individual beetles have been reported in experiments in fragmented forests (8.3 km in Galiego et al., 2012; 13.6 km in Mas et al., 2013). Limited data have been published on the flight capabilities of other non-European *Monochamus* spp., but it seems reasonable to assume that their spread characteristics are similar to those of *M. alternatus* and *M. galloprovincialis*. Wind direction and strength can also be a factor affecting beetle dispersion, flight distance and time (Weiss et al., 2019).

In the context of the pest prioritisation project conducted by EFSA (2019), a panel of experts estimated the yearly spread rate of *B. xylophilus* through the Expert Knowledge Elicitation procedure. As the nematode spreads naturally mainly via *Monochamus* spp. vectors, the spread rate of *B. xylophilus* could also be used as a proxy for the spread of *Monochamus* spp. (including non-European species). The median of the maximum distance expected to be covered in one year by *B. xylophilus* was estimated to be approx. 4.5 km, ranging from 100 m (5th percentile) to 14 km (95th percentile).

The indicated probabilities of spread help to create a basis for the survey design. At a local level, the potential spread rate of non-European *Monochamus* spp. is required to delimit the areas of interest for the surveys around the potential areas of introduction of the pest.

For detection survey, when the purpose is to detect the pest, it would be sufficient to focus on the area within the median radius of 4.5 km from the risk locations. In case of a positive finding, the upper range of the distribution should be considered in the delimiting survey (14 km for 95th percentile), as the objective of the survey is to define the boundaries of the area where the pest is contained and where eradication will be applied. This means that there is less than 5% chance for the pest to be found beyond 14 km from the focus of the outbreak.

### 1.7.2. Human-assisted spread

Human-assisted spread of non-European *Monochamus* spp. may occur principally through the movement of infested wood or possibly via transport and subsequent planting of host plants. Reflecting this most likely pathway for international movement, all interceptions of non-European *Monochamus* spp. currently listed in the EUROPHYT database (accessed on 19 November 2019) were made on infested wood. When non-European *Monochamus* spp. are introduced via *B. xylophilus*-infested wood or living trees are infested as well, this poses a high risk for spread of pine wilt disease (Robinet et al., 2009).

### 1.8. Risk factor identification

Identification of risk factors and their relative risk estimation is essential for performing risk-based surveys. A risk factor is a biotic or abiotic factor that increases the probability of infestation by the pest in the area of interest. The risk factors that are relevant for surveillance need to be characterised by their relative risk (and should have more than one level of risk for the target population) and the proportion of the overall target population to which they apply. The identification of risk factors needs to be tailored to the situation in each Member State. This section presents an example of a risk factor for non-European *Monochamus* spp. and is not necessarily exhaustive.

For the identification of risk areas, it is first necessary to identify the activities that could contribute to the introduction or spread of non-European *Monochamus* spp. These activities should then be
connected to specific locations. Risk areas can be defined around these locations. Their size depends on the spread capacity of the target pest and the availability of host plants around these locations.

With regard to the host species of non-European *Monochamus* spp., the current legislation (see Annex VI of Commission Implementing Regulation (EU) 2019/2072) prohibits the import of plants (other than fruit and seeds) from the genera *Abies*, *Cedrus*, *Chamaecyparis*, *Juniperus*, *Larix*, *Picea*, *Pinus*, *Pseudotsuga* and *Tsuga* from most non-EU countries. Although not as important as wood packaging or sawn wood as a pathway for *Monochamus* spp., the rules close this potential pathway for those host plants that are also hosts of *B. xylophilus* or that host the *Monochamus* spp. that are known vectors of *B. xylophilus*. The pathway for many other tree genera is open, particularly broad-leaved species. Although non-European *Monochamus* spp. that attack broad-leaved trees are regulated quarantine pests, we do not consider that their importance is such as to require their incorporation into the risk factors.

For determining the risk areas, it would be sufficient to focus on the area within the median radius of 4.5 km from the risk locations (Section 1.7.1.). For delimiting surveys, the distance from the risk locations (using the values of 7.5 km for the 75th and 14 km for the 95th percentiles) could be used as a risk factor for prioritising the survey efforts closer to the outbreak focus where the pest is more likely to be found. The relative risks of the corresponding areas can be calculated using the estimated distribution values.

As reviewed by EFSA PLH Panel (2018), possible pathways of entry include:

- a) conifer wood, whether in the round or sawn;
- b) wood packaging material and dunnage from conifers;
- c) conifer particle wood or wood waste large enough to host the larvae;
- d) coniferous wood products (e.g. furniture).

**Example: Import of wooden packaging material and wood**

Wood packaging material (WPM) is regulated internationally by International Standards for Phytosanitary Measures (ISPM) No 15 (regulation of WPM in international trade) and Article 43 of Regulation (EU) 2016/2031 (specific import conditions for the introduction into the EU of WPM). When ISPM 15 is followed, the risk of introduction of *Monochamus* spp. to new areas via WPM is fully mitigated. However, recurring interceptions of WPM infested with living insects and/or living nematodes and the large volume of the pathway indicate that there are still risks associated with WPM in practice. Thus, trees in the vicinity of sites where WPM is stored could be a target for risk-based surveillance. Very often, these will be located in urbanised non-forest locations.

A similar argument can be made for wood-processing yards (e.g. sawmills, roundwood producers and lumberyards) that process imported wood (EPPO, 2018). For imports of the wood of conifers, special requirements are laid down in Annex VII of Commission Implementing Regulation (EU) 2019/2072, depending on the origin and characteristics of the material. Although, if risk mitigation measures have not been carried out properly, wood and wood products may conceal larvae.

### 2. Detection and identification

#### 2.1. Visual examination

#### 2.1.1. Detection of infested trees

Trees may be weakened or damaged by biotic agents, wind or snow, or suffer from drought stress. Irrespective of the cause of a decline in tree viability, weakened trees are exploited by *Monochamus* spp. for oviposition. In addition, *Monochamus* spp. also exploit recently dead trees or logging residues, which may thus also be a target for surveillance. Remote sensing from aircraft or drones may be used to help target ground-based inspections (JRC, 2015).
2.1.2. Detection of the pest

Although in principle it is possible to detect the presence of Monochamus spp. by examining the infested trees, the recommended method is by trapping free-flying adults with traps baited with a lure known to attract adult beetles of the genus. To this end, Teflon-coated traps (either cross-vane or multi-funnel) and containers for captured beetles should be used (JRC, 2015). Multi-funnel traps consist of a series of connected funnels ending with a cup in which the insects are collected (Figure 7). Cross-vane traps consist of two PVC sheets, a funnel and collection cup. Monochamus beetles should be prevented from escaping, while avoiding saturation of the traps. This can be achieved by using insecticides in the collector cup, frequent collection of the captured beetles, and by adjusting the collector cup so that small non-targeted insects are allowed to escape. Traps should be supplemented with pheromone and kairomone attractants (Pajares et al., 2010). Lure vials are attached in the centre of the trap. Currently, the recommended blend consists of the male aggregation pheromone monochamol (2-undecyloxy-1-ethanol) and the two bark beetle attractants ipsenol and 2-methyl-3-buten-1-ol (Pajares et al., 2010; Ryall et al., 2015; Teale et al., 2011; Rassati et al., 2012; Alvarez et al., 2016). These substances can be complemented by the host volatile α-pinene (Pajares et al., 2010; Teale et al., 2011), but the benefits of incorporating this terpene component into the blend may be outweighed by the detrimental effects of luring natural enemies of bark beetles and other non-target organisms (Alvarez et al., 2016).

The trapping system composed of cross-vane traps and the Galloprotect Pack (monochamol, ipsenol, 2-methyl-3-buten-1-ol and α-pinene) that has been firstly utilised in the EU has also been tested in North America and China and proved to be effective for the detection of local Monochamus spp. (M. carolinensis, M. mutator, M. notatus, M. scutellatus, M. clamator and M. titillator were trapped in North America, and M. alternatus in China). This trapping system can thus also be used as an early detection tool at points of entry or near other high-risk sites for detection of non-European Monochamus spp. (Boone et al., 2019). Using this system, it is not possible to specifically capture non-European Monochamus spp., so the collected sample will contain both local and potentially invasive species.

Trapping can be performed in forest stands or in close proximity to risk locations (see Section 1.8). The attraction range of traps baited with the monochamol, ipsenol, 2-methyl-3-buten-1-ol and α-pinene blend is estimated to be approximately 100 m for M. galloprovincialis (Jactel et al., 2019) while no estimated data on attract were found for other Monochamus species. Monochamol is, nevertheless, attractive for many other Monochamus species, including M. notatus, M. carolinensis and M. scutellatus M. saltuarius and M. urussovi (Ryall et al., 2015). It is recommended that traps should be hung as high as possible (at least 2 m) off the ground, either in the open or, if this is not possible, in the tree canopy at the edge of a clearing. When supportive branches are available these are the preferred location for the trap, while the trap can be stabilised using the trunk. When traps are placed on a risk-location itself (for post-import monitoring) trees are preferred, but not always available. In these situations, a pole or fence can be used. Traps are also recommended to be placed out of reach and out of sight of footpaths. In dense forests, traps should be placed near the edge of a stand (e.g. along tracks or roads) or a gap/clearing (JRC, 2015). Traps should be monitored every 1–2 weeks for dry traps and every 3 weeks for wet traps. The exact timing of the trapping activity varies in the different EU Member States depending on climate and latitude of the area where the survey is performed. The timing of the surveillance activities should obviously match the flight season of the Monochamus spp. (e.g. the most appropriate period in Portugal is between mid-June and mid-August: Naves et al., 2008). The choice of the trapping method may also depend on whether additional target species are integrated into the survey activities. Inclusion of additional target species may then also require application of additional lures. In the area where the insects display a bivoltine cycle, a second generation emerges after the dry season in late-August/September (Firmino et al., 2017). Surveys should then also consider the possible second emergence of adults late in the season. Traps should therefore be placed during two annual sampling periods; the first in early summer (June–July) and the second in late summer (September) (Firmino et al., 2017).
2.2. Sampling
The samples from the collector cups should be gathered at regular intervals of once per week or every two weeks when using dry traps. If not already dead (e.g. when using insecticides in the collector cup) all living specimens should be killed before transport. When wet trapping is used, the intervals between collecting dates can be increased to three weeks (Heijerman and Noordijk, 2017) and trapping thus requires fewer time resources.

2.3. Laboratory testing and pest identification
After Monochamus beetles have been taken to the laboratory, the species should be identified. Currently, two online catalogues (Titan, http://titan.gbif.fr/ and the Photographic Catalogue of the Cerambycidae of the World, http://bezbycids.com/byciddb/wdefault.asp?w=n) provide global information and several national and regional keys are available, but there are discrepancies regarding the total number of recognised (sub)species of the genus (EFSA PLH Panel, 2018), so species identification might be challenging. Initially, it will be required to determine that the collected specimen does not belong to one of the species that occur in the EU (M. galloprovincialis, M. saltuarius, M. sutor, M. urussovi and M. sartor). These Monochamus spp. can be distinguished based on morphological features, complemented by molecular characteristics (Cesari et al., 2004). Note that the distinction between M. galloprovincialis and M. sutor is considered a taxonomic challenge (Koutroumpa et al., 2013) and largely based on male and female genitalia (Wallin et al., 2013); the main morphological characteristics used for their identification are extremely variable within M. galloprovincialis. Molecular data confirm the close relationship of both species (Koutroumpa et al., 2013). Should a collected specimen not belong to one of the EU species, it is necessary to identify the species. Species identification should always be carried out by an expert.
According to the PM 7/129 (1) standard, DNA barcoding protocols can support the morphological identification of several regulated pests (EPPO, 2016), including *Monochamus* spp. The barcoding protocol is based on the cytochrome oxidase I (COI) gene and can be applied on all life stages of the beetle. Sequences of several *Monochamus* spp. are available in databases like BOLD (Barcode of Life Data System, Ratnasingham and Hebert, 2007), but available data concern a subset of mostly North American, European and some Asian species. Nevertheless, there are several examples showing how DNA barcoding can support the identification of *Monochamus* spp. Wu et al. (2017) showed that DNA barcoding can be helpful for the identification of *Monochamus* at species level when applied to larval life stages which are generally difficult to identify below family or genus level. Moreover, Jeon et al. (2015) demonstrated that DNA barcoding can also be used as a tool to characterise intraspecific diversity in *M. saltuarius*.

3. **Key elements for survey design**

The preferential survey strategy for non-European *Monochamus* spp. is by trapping. The inclusion of non-European *Monochamus* populations as regulated and prohibited pests is particularly useful at the point of import into the EU to prevent joint entry of *B. xylophilus* and its vector. Once imported, it is not possible to distinguish between local populations and non-European populations for the four species that are present in the EU. Given that the survey strategy for *B. xylophilus* – by sampling and testing of its vectors – can be co-aligned with surveillance for non-European *Monochamus* spp., this would generally be recommended.

Based on the analyses of the information on the pest–host plant system, the different units that are needed to design the survey have to be defined and tailored to the situation in each Member State. The size of the defined target population and its structure in terms of the number of epidemiological units need to be known. When several pests have to be surveyed in the same plant species or forest area, it is recommended that the same definitions for the epidemiological unit and inspection unit are used for each pest in order to optimise the survey programme as much as possible.

To design a survey on non-European *Monochamus* spp. the following steps will generally be necessary:

1/ Determine the type of survey based on its objectives. For non-European *Monochamus* spp., the type of survey will depend on the pest status (according to ISPM No. 8) in the area of interest. The objective could be to substantiate pest freedom, to increase the likelihood of early detection of a new infestation, to delimit an outbreak area or to determine pest prevalence. The next steps deal with the example of substantiating pest freedom. Confidence level and design prevalence are to be decided by the risk managers before designing the surveys as they reflect the acceptability level of the risk of infestation of the host plants by non-European *Monochamus* spp. The general guidelines for pest surveillance provide some further reflections on the choice of these values and the related consequences in terms of pest surveys.

2/ Define the target population and the epidemiological unit. When determining the target population for surveillance of non-European *Monochamus* spp., it is necessary to select the host plants that are relevant for the survey area. Table 2 shows an example of the definitions of the units needed to design a survey on non-European *Monochamus* spp. by trapping.

3/ Determine the size of the target population.

4/ Determine the inspection unit. In the case of trees, this would be a single pine tree. When using traps, this would be the ensemble of *Monochamus* beetles collected from an individual trap.

5/ Determine the number of inspection units per epidemiological unit. This would be the average number of pine trees per epidemiological unit. Given the range of attraction of the traps, a single trap would suffice to cover one hectare.
Table 2: Example of definitions of the target population, epidemiological unit and inspection unit for surveillance on non-European *Monochamus* spp.

<table>
<thead>
<tr>
<th>Definition</th>
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<tr>
<td><strong>Target population</strong></td>
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<tr>
<td><strong>Epidemiological unit</strong></td>
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<tr>
<td><strong>Inspection units</strong></td>
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</table>

6/ Implement the sampling procedure suggested by the reference laboratory within the epidemiological units and estimate its effectiveness in order to determine the overall detection method sensitivity. In the case of traps, the method sensitivity is directly determined by the effectiveness of the trapping combined with the subsequent detection probability of the nematodes within the captured beetles. RiBESS+ can be used to calculate how many inspection units need to be examined or sampled when using a predefined prevalence level (e.g. 1%) to obtain a particular method sensitivity. This method sensitivity is in turn needed to calculate the number of inspection sites (Step 8). Note that a larger number of inspected units will result in a higher method sensitivity, but this will be more laborious per site. However, a higher method sensitivity will result in a lower number of inspection sites in the calculations for Step 8. Vice versa, a low number of inspected units per site will result in low method sensitivity, and consequently a higher number of sites to be visited. In the end, this will need to be balanced.

7/ Define the risk factors. A risk factor affects the probability that a pest will be present in a specific portion of the target population. By including the risk factors identified in Section 1.8, the survey focuses mainly on those areas that are more likely to be infested by the target species. It may not always be possible to identify or include a risk factor in the survey design. Risk factors can only be included when both the relative risk and the proportion of the overall target population to which they apply are known or can be reliably estimated.

8/ Determine the sample size. RiBESS+ can be used to calculate how many epidemiological units need to be surveyed in order to achieve a predefined confidence level (e.g. 95%) and a predefined design prevalence (e.g. 1%), while also including the method sensitivity from Step 6 and the risk factors identified in Step 7. This will, for example, result in the number of epidemiological units where host trees are present that have to be surveyed in order to state with 95% confidence that the prevalence of non-European *Monochamus* spp. in a Member State will be at 1% or below.

9/ Summarise and evaluate. At this stage, it is necessary to evaluate whether the above steps have resulted in a survey design that matches the available resources, meaning that a feasible number of inspections can be performed within an acceptable time frame per inspection, and resulting in a feasible number of samples. If not, available resources should be adjusted, or the survey design should be adjusted. This can be done by going back to Step 2 (adjusting the number of components) or Step 6 (when rebalancing method sensitivity and sample size).

10/ Integrate the pest-based survey into a host-plant or crop-based survey (optional).

11/ Select the survey sites from the list of available locations.

12/ Consider which data are needed and how these data will be reported.

13/ Develop or update the specific instructions for the inspectors.
References


Ryall K, Silk P, Webster RP, Gutowski JM, Meng Q, Li Y, Gao W, Fidgen J, Kimoto T, Scarr T, Mastro V and Sweeney JD, 2015. Further evidence that monochamol is attractive to *Monochamus* (Coleoptera: Cerambycidae) species, with attraction synergised by host plant volatiles and bark beetle (Coleoptera: Curculionidae) pheromones. The Canadian Entomologist, 147, 564-579.


## Glossary

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition*</th>
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<tbody>
<tr>
<td><strong>Buffer zone</strong></td>
<td>An area surrounding or adjacent to an area officially delimited for phytosanitary purposes in order to minimise the probability of spread of the target pest into or out of the delimited area, and subject to phytosanitary or other control measures, if appropriate (ISPM 5: FAO, 2019).</td>
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<tr>
<td><strong>Component (of a survey)</strong></td>
<td>A component is a survey entity which can be distinguished based on its target population, the detection method (e.g. visual examination, laboratory testing, trapping) and the inspection unit (e.g. vectors, branches, twigs, leaves, fruits). A pest survey comprises various components. The overall confidence of the survey will result from the combination of the different components.</td>
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<tr>
<td><strong>Confidence</strong></td>
<td>Sensitivity of the survey. Is a measure of reliability of the survey procedure (Montgomery and Runger, 2010).</td>
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<tr>
<td><strong>Design prevalence</strong></td>
<td>It is based on a pre-survey estimate of the likely actual prevalence of the pest in the field (McMaugh, 2005). The survey will be designed in order to obtain at least a positive test result when the prevalence of the disease will be above the defined value of the design prevalence. In ‘freedom from pest’ approaches, it is not statistically possible to say that a pest is truly absent from a population (except in the rare case that a census of a population can be completed with 100% detection efficiency). Instead, the maximum prevalence that a pest could have reached can be estimated, this is called the ‘design prevalence’. That is, if no pest is found in a survey, the true prevalence is estimated to be somewhere between zero and the design prevalence (EFSA, 2018).</td>
</tr>
<tr>
<td><strong>Delimiting survey</strong></td>
<td>Survey conducted to establish the boundaries of an area considered to be infested by, or free from, a pest (ISPM 5: FAO, 2019).</td>
</tr>
<tr>
<td><strong>Detection survey</strong></td>
<td>Survey conducted in an area to determine whether pests are present (ISPM 5: FAO, 2019).</td>
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<tr>
<td><strong>Diagnostic protocols</strong></td>
<td>Procedures and methods for the detection and identification of regulated pests that are relevant to international trade (ISPM 27: FAO, 2016).</td>
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<tr>
<td><strong>Epidemiological unit</strong></td>
<td>A homogeneous area where the interactions between the pest, the host plants and the abiotic and biotic factors and conditions would result in the same epidemiology should the pest be present. The epidemiological units are subdivisions of the target population and reflect the structure of the target population in a geographical area. They are the units of interest, on which statistics are applied (e.g. a tree, orchard, field, glasshouse, or nursery) (EFSA, 2018).</td>
</tr>
<tr>
<td><strong>Expected prevalence</strong></td>
<td>In prevalence estimation approaches, it is the proportion of epidemiological units expected to be infected or infested.</td>
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<tr>
<td><strong>Identification</strong></td>
<td>Information and guidance on methods that either used alone or in combination lead to the identification of the pest (ISPM 27: FAO, 2016).</td>
</tr>
<tr>
<td><strong>Inspection</strong></td>
<td>Official visual examination of plants, plant products or other regulated articles to determine whether pests are present or to determine compliance with phytosanitary regulations (ISPM 5: FAO, 2019).</td>
</tr>
<tr>
<td><strong>Inspection unit</strong></td>
<td>The inspection units are the plants, plant parts, commodities or pest vectors that will be scrutinised to identify and detect the pests. They are the units within the epidemiological units that could potentially host the pests and on which the pest diagnosis takes place (EFSA, 2018).</td>
</tr>
<tr>
<td><strong>Inspector</strong></td>
<td>Person authorised by a national plant protection organisation to discharge its functions (ISPM 5: FAO, 2019).</td>
</tr>
<tr>
<td><strong>Method sensitivity</strong></td>
<td>The conditional probability of testing positive given that the individual is diseased (Dohoo et al., 2010). The method diagnostic sensitivity (DSe) is the probability that a truly positive epidemiological unit will give a positive result and is related to the analytical sensitivity. It corresponds to the probability that a truly positive epidemiological unit that is inspected will be detected and confirmed as positive.</td>
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<tr>
<td><strong>Pest diagnosis</strong></td>
<td>The process of detection and identification of a pest (ISPM 5: FAO, 2019).</td>
</tr>
<tr>
<td><strong>Pest freedom</strong></td>
<td>An area in which a specific pest is absent as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained (ISPM 5: FAO, 2019).</td>
</tr>
<tr>
<td><strong>Population size</strong></td>
<td>The estimation of the number of plants in the region to be surveyed (EFSA, 2018).</td>
</tr>
<tr>
<td><strong>Relative risk</strong></td>
<td>The ratio of the risk of disease in the exposed group to the risk of disease in the non-exposed group (Dohoo et al., 2010).</td>
</tr>
<tr>
<td><strong>Representative sample</strong></td>
<td>A sample that describes very well the characteristics of the target population (Cameron et al., 2014).</td>
</tr>
<tr>
<td><strong>RiBESS+</strong></td>
<td>An online application that implements statistical methods for estimating the sample size, global (and group) sensitivity and probability of freedom from disease. Free access to the software with prior user registration is available at: <a href="https://shiny-efsa.openanalytics.eu/">https://shiny-efsa.openanalytics.eu/</a></td>
</tr>
<tr>
<td><strong>Risk assessment</strong></td>
<td>Evaluation of the probability of the introduction and spread of a pest and the magnitude of the associated potential economic consequences (ISPM 5: FAO, 2019).</td>
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<tr>
<td><strong>Risk factor</strong></td>
<td>A factor that may be involved in causing the disease (Cameron et al., 2014). It is defined as a biotic or abiotic factor that increases the probability of infestation of the epidemiological unit by the pest. The risk factors relevant for the surveillance should have more than one level of risk for the target population. For each level, the relative risk needs to be estimated as the relative probability of infestation compared with a baseline with a level 1. Consideration of risk factors in the survey design allows the survey efforts to be enforced in those areas where the highest probabilities exist to find the pest should the pest be present.</td>
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<tr>
<td><strong>Risk-based survey</strong></td>
<td>A survey design that considers the risk factors and enforces the survey efforts in the corresponding proportion of the target population.</td>
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<tr>
<td><strong>Sample size</strong></td>
<td>The number of sites that need to be surveyed in order to detect a specified proportion of pest infestation with a specific level of confidence, at the design prevalence (McMaugh, 2005).</td>
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<tr>
<td><strong>Survey</strong></td>
<td>An official procedure conducted over a defined period of time to determine the characteristics of a pest population or to determine which species are present in an area (ISPM 5: FAO, 2019).</td>
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</tbody>
</table>
| **Target population** | The set of individual plants or commodities or vectors in which the pest under scrutiny can be detected directly (e.g. looking for the pest) or indirectly (e.g. looking for symptoms suggesting the presence of the pest) in a given habitat or area of interest. The different components pertaining to the target population that need to be specified are:  
  - Definition of the target population – the target population has to be clearly identified  
  - Target population size and geographic boundary. |
<table>
<thead>
<tr>
<th><strong>Test</strong></th>
<th>Official examinations, other than visual, to determine whether pests are present or to identify pests (ISPM 5: FAO, 2019).</th>
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</thead>
<tbody>
<tr>
<td><strong>Test specificity</strong></td>
<td>The conditional probability of testing negative given that the individual does not have the disease of interest (Dohoo et al., 2010). The test diagnostic specificity (DSp) is the probability that a truly negative epidemiological unit will test negative and is related to the analytical specificity. In freedom from disease it is assumed to be 100%.</td>
</tr>
<tr>
<td><strong>Visual examination</strong></td>
<td>The physical examination of plants, plant products, or other regulated articles using the unaided eye, lens, stereoscope or microscope to detect pests or contaminants without testing or processing (ISPM 5: FAO, 2019).</td>
</tr>
</tbody>
</table>

*References*


