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Pest survey card on *Xylosandrus crassiusculus*

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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. Its purpose is to guide the Member States in preparing data and information for *Xylosandrus crassiusculus* surveys. These are required to design statistically sound and risk-based pest surveys, in line with current international standards. *Xylosandrus crassiusculus* is an ambrosia beetle within the subfamily Scolytinae and is a clearly distinguished taxonomic entity. The pest is present on all continents. Within the EU, *X. crassiusculus* is currently present in Italy, France and Spain and transient in Slovenia. Although it is currently reported in Mediterranean areas, the pest appears able to become established in many countries and regions of the Union territory. The ambrosia beetle is highly polyphagous and able to attack several broadleaf trees species. However, in Europe only three tree species have been reported as infested: *Ceratonia siliqua*, *Cercis siliquastrum* and *Castanea sativa*. The beetle may attack both thin branches of living trees (or saplings) and freshly cut wood. Trade of plants for planting, timber and wood products, as well as wood packing material are major pathways for the introduction of *X. crassiusculus* into the EU and this is also confirmed by multiple interceptions at points of entry. Hence, international airports and seaports and surrounding areas with trading activities are considered risk locations and risk areas. In addition, as a lot of information on attacks on saplings in nurseries is available from North America, large nurseries with international trade may also be considered risk locations. Trapping is recommended when the females emerge and start attacking new hosts. Their flight activity is linked to warm daily temperatures and can start in early spring and last until October.

Keywords: Asian ambrosia beetle, granulate ambrosia beetle, plant pest, risk-based surveillance, survey

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Introduction

The information presented in this pest survey card was summarised from the European and Mediterranean Plant Protection Organisation (EPPO) Global Database and the Centre for Agriculture and Bioscience International (CABI) datasheet on *Xylosandrus crassiusculus* and other documents.

The objective of this pest survey card is to provide the relevant biological information needed to prepare surveys for *X. crassiusculus* in EU Member States (MSs) following the methodology described in EFSA (2018). It is part of a toolkit that is being developed to assist MSs with planning a statistically sound and risk-based pest survey approach in line with International Plant Protection Convention standards (ISPMs) and guidelines for surveillance (FAO, 2016a, b, 2018). 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 *Xylosandrus crassiusculus*[1].
ii. General documents:
   a. The general survey guidelines
   b. The RiBESS+ manual[2]
   c. The statistical tools RiBESS+ and SAMPELATOR[3].

1. The pest and its biology

1.1. Taxonomy

Scientific name: *Xylosandrus crassiusculus* (Motschulsky)

Class: Insecta **Order**: Coleoptera **Family**: Curculionidae **Subfamily**: Scolytinae **Genus**: *Xylosandrus* **Species:** *Xylosandrus crassiusculus*

Synonym(s): *Dryocoetes bengalensis* Stebbing, *Phloeotrogus crassiusculus* Motschulsky, *Xyleborus bengalensis* Stebbing, *Xyleborus crassiusculus* (Motschulsky), *Xyleborus declivigranulatus* Schedl, *Xyleborus ebriosus* Niisima, *Xyleborus mascarenus* Hagedorn, *Xyleborus okoumeensis* Schedl, *Xyleborus semigranosus* Blandford, *Xyleborus semiopacus* Eichhoff, *Xylosandrus semigranosus* (Blandford), *Xylosandrus semiopacus* (Eichhoff)

EPPO Code: XYLBCR

Common name: Asian ambrosia beetle, Granulate ambrosia beetle

Xylosandrus crassiusculus (Figure 1) is an ambrosia beetle within the subfamily Scolytinae. After revision, the previously distinctive taxonomic family, the Scolytidae, are now treated as a specialised subfamily within the Curculionidae.

[1] The Pest Survey Card will be updated in the form of Story Map that will be available in the Plant Pests Story Maps Gallery available online: https://efsa.maps.arcgis.com/apps/MinimalGallery/index.html?appid=f91d6e95376f4a5da206eb1815ad1489
[2] https://zenodo.org/record/2541541/preview/ribess-manual.pdf
[3] https://shiny-efsa.openanalytics.eu/

Figure 1: Adult of *Xylosandrus crassiusculus* (Source: Javier E. Mercado, Bark Beetle Genera of the US, USDA APHIS PPQ, Bugwood.org)

Conclusions on taxonomy

Xylosandrus crassiusculus is a clearly distinguished taxonomic entity.

1.2. EU pest regulatory status

Xylosandrus crassiusculus is regulated as a non-European Scolytidae, which are listed as Union quarantine pests in Annex IIA of Commission Implementing Regulation (EU) 2019/2072[4].

There are no specific import requirements in relation to this pest. Import requirements are laid down in Annex VI of Commission Implementing Regulation (EU) 2019/2072 which prohibits certain plants, plant products and other objects from being imported from certain third countries. Among them are also potential host plants of *X. crassiusculus*.

In addition, further control measures are implemented in Part A of Annex XI of Commission Implementing Regulation (EU) 2019/2072 that lists plants, plant products and other objects, as well as the respective third countries of origin or dispatch, for which phytosanitary certificates are required for their introduction into the Union territory. As an example, this is of concern for the import of plants for planting, wood and wood products of the genera *Acer*, *Quercus*, *Betula*, *Populus*, *Malus*, *Pyrus*, *Prunus*, etc.

The general requirements for surveys of quarantine organisms in EU territory are laid down in Regulation (EU) 2016/2031[5].

[4] Commission Implementing Regulation (EU) 2019/2072 of 28 November 2019 establishing uniform conditions for the implementation of Regulation (EU) 2016/2031 of the European Parliament and the Council, as regards protective measures against pests of plants, and repealing Commission Regulation (EC) No 690/2008 and amending Commission Implementing Regulation (EU) 2018/2019. OJ L 319, 10.12.2019, p. 1–279.

[5] Regulation (EU) 2016/2031 of the European Parliament of the Council of 26 October 2016 on protective measures against pests of plants, amending Regulations (EU) No 228/2013, (EU) No 652/2014 and (EU) No 1143/2014 of the European Parliament and of the Council and repealing Council Directives 69/464/EEC, 74/647/EEC, 93/85/EEC, 98/57/EC, 2000/29/EC, 2006/91/EC and 2007/33/EC. OJ L 317 23.11.2016, p. 4-104.

Overview on EU regulatory status

In the EU, *Xylosandrus crassiusculus* is regulated as a non-European Scolytidae which are listed as Union quarantine pests. There are currently no specific import requirements in place that may mitigate the risk of the introduction of *X. crassiusculus* into EU territory.

1.3. Pest distribution

According to EPPO (2009), *Xylosandrus crassiusculus* is native to Asia, especially the tropical and subtropical southern parts and countries. It was then introduced to tropical regions in both West and East Africa (CABI, 2019) and it is currently present in some parts of North, Central and South America (Anderson, 1974; Kirkendall and Ødegaard, 2007; Douglas et al., 2013; Haack et al., 2013; Fletchmann and Atkinson, 2016; Ranger et al., 2016).

In the EU, *X. crassiusculus* was reported for the first time in 2003 in Italy (Pennacchio et al., 2003). The pest was first found in various locations in Tuscany (EPPO, 2013), then in 2007 in Liguria region on various carob trees (*Ceratonia siliqua*) (Tinivella et al., 2010), in Veneto region in broadleaf forests (EPPO, 2010; Faccoli et al., 2011) and in Friuli Venezia Giulia region in 2011 (Bernardinelli et al., 2011). In 2012, it was found again in Liguria on *C. siliqua* about 20 km from the first observation in 2007 (EPPO, 2013). Between 2016 and 2018, the pest was also found on *C. siliqua* in Lazio region (Francardi et al., 2017) and on Japanese hybrids of chestnut *Castanea sativa* in Piedmont region (Dutto et al., 2018; EPPO, 2020a).

In France, the first report of *X. crassiusculus* dates back to August 2014, when adults and larvae were detected on carob trees in Provence-Alpes-Côte d'Azur region (EPPO, 2014; Nageleisen et al., 2015). It was later confirmed in Occitanie and Nouvelle-Aquitaine regions in 2018 and 2019, respectively (EPPO, 2019a).

In 2016, the pest was found in Spain, in Valencia region, on carob trees (Gallego, 2017).

In 2017, a total of 121 beetles were trapped in Slovenia (EPPO, 2018).

Finally, four *X. crassiusculus* specimens were trapped at three import locations of wood packaging material in the Netherlands (NVWA, 2018).

In Italy, France and Spain the pest is now considered to be established, whereas in the Netherlands it has only been intercepted and in Slovenia is currently transient (EPPO, online).

With the reports from Australia (in 2017) and New Zealand (in 2019), *X. crassiusculus* appears to be present on all continents (EPPO, 2017, 2019b).

Figure 2 provides an overview of the current global distribution of *X. crassiusculus* (EPPO, online).

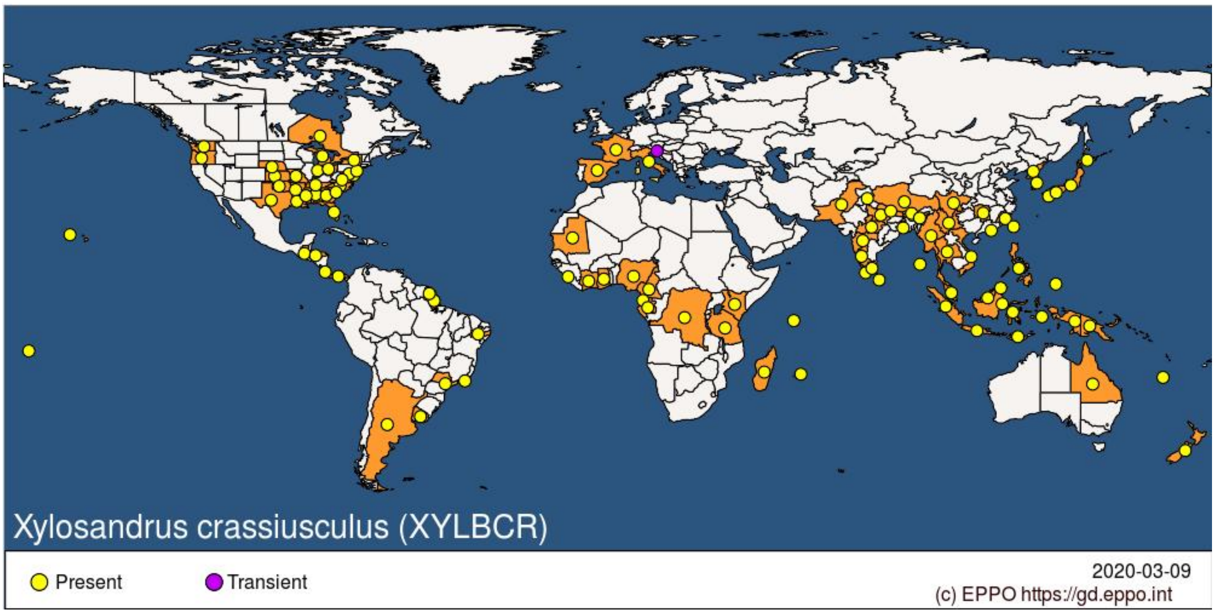

Figure 2: Global distribution of *Xylosandrus crassiusculus* (Source: EPPO Global Database, https://gd.eppo.int/)

Conclusion on pest distribution

Xylosandrus crassiusculus is reported as present on all continents. Within the EU, its presence has been confirmed in Italy, France and Spain.

1.4. Life cycle

Ranger et al. (2016) provide a comprehensive overview of the biology and ecology of *Xylosandrus crassiusculus* in the US.

Adults overwinter in galleries. In their observation on closely related *X. germanus*, Reding et al. (2013) discovered close relationships between maximum daily temperatures of 20°C and the beginning of the spring flight activity, which may also be anticipated for *X. crassiusculus*. In Europe, the beginning of the spring flight activity was observed with a mean air temperature of around 18°C (Faccoli, personal communication), when the mated females leave the overwintering sites and bore into the twigs, branches or trunks of new potential woody host plants and start to excavate a system of tunnels in the wood or pith, introducing a symbiotic ambrosial fungus, and produce a new brood. Mated females deposit eggs in rearing chambers excavated in the distal parts of the galleries, which provide suitable conditions for the breeding of ectosymbiotic fungi. The beetles create galleries of varied size and irregular form in the wood (Atkinson et al., 2017), which may result in loss of mechanical stability. Like all ambrosia beetles within the tribe Xyleborini, *X. crassiusculus* feed on a specific fungus, in this case *Ambrosiella roeperi* (Harrington et al., 2014), which is introduced and cultivated within the rearing chamber (Atkinson et al., 2017). Females carry spores within their mycangia, a morphological structure to vector spores, which is located between the pro- and mesothorax. Ranger et al. (2016) emphasise that females only oviposit after the feeding fungus is successfully cultivated within the breeding gallery. Secondary colonisation of other fungi may also be observed. Ranger et al. (2016) report the presence of *Fusarium* spp. that infest sap secretion at the entrance holes.

Ranger et al. (2016) further refer to 1–6 laid eggs per day over a prolonged period. They could not provide evidence of the period of full development, but the closely related *X. germanus* was observed to develop from egg to adult within 15–18 days on tea roots, and even longer on an artificial diet. Kovach (1986) observed emerging adults 48–62 days after the tree infestation. The eggs, larvae, and pupae are found together in the tunnel system excavated by the female adult. The number of progeny appears to be highly variable. As reported by Ranger et al. (2016) and CABI (2019), different brood sizes, ranging from 8 (on average) to 100 individuals, have been observed. Haplodiploidy is also commonly observed in *X. crassiusculus*. Male individuals develop from unfertilised eggs and only possess half the chromosomes of fertilised diploid females (8 vs. 16 chromosomes). Female-to-male ratios of 10:1 are reported for progeny. The rare males are reduced in size and unable to fly (Atkinson et al., 2017). Parthenogenesis and inbreeding between siblings occur before the females emerge to invade a new host.

According to CABI (2019), in tropical regions, breeding is continuous throughout the year, which results in overlapping generations, and the presence of all life stages throughout the year.

In Europe, the flight activity and tree attacks by the pest were documented in the first week of April (Dutto et al., 2018), which also coincide with the mass trapping. In Lazio region (Circeo National Park, Italy), adults of *X. crassiusculus* are still flying at the end of September (Contarini et al., 2020), suggesting the presence of three generations per year. In Spain, recently infested plants were found in October (Gallego et al., 2017), also supporting the presence of a third generation per year.

Figure 3 illustrates the life cycle of the pest.

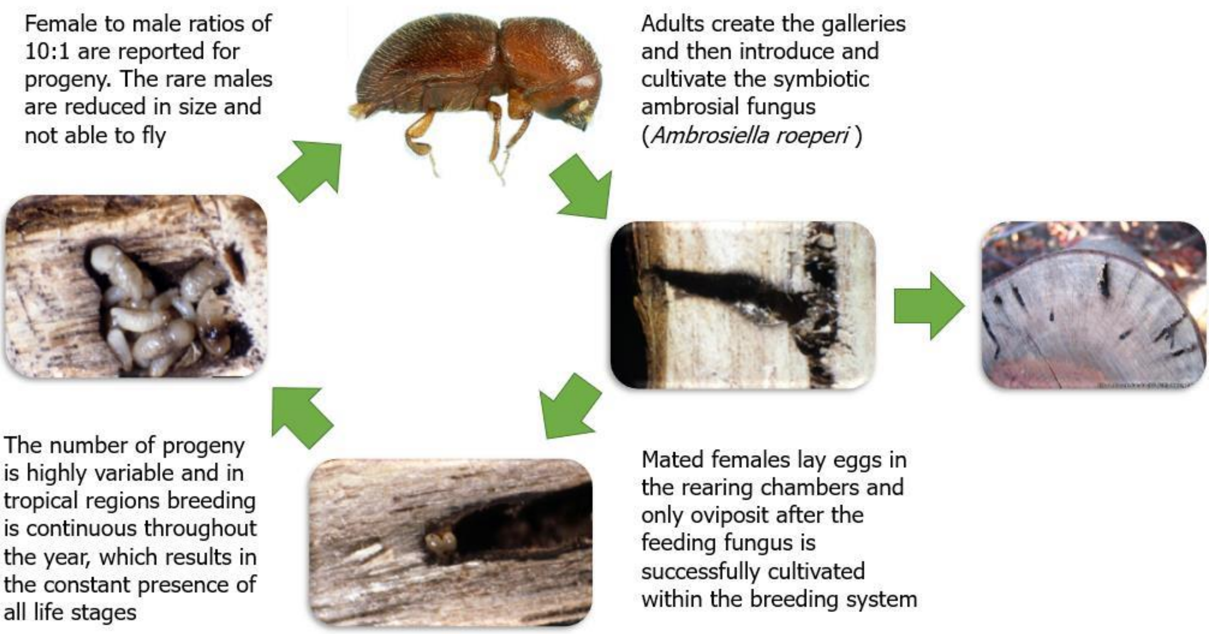

Figure 3: Life cycle of *Xylosandrus crassiusculus* (Source: (top) Natasha Wright, Cook's Pest Control, Bugwood.org; (left, first on the right and bottom) Lacy L. Hyche, Auburn University, Bugwood.org; (second on the right) EPPO Global Database, Courtesy of Dr Andrea Minuto, Centro di Saggio, CERSAA, Albenga, Italy)

Conclusion on life cycle

The adults and young instars of *Xylosandrus crassiusculus* live inside the galleries created in the host plants, thus in general are not visible. Early detection should be performed with trapping in the period of flight activities, which starts in spring when the air temperature exceeds 18–20°C. In the EU, *X. crassiusculus* remains active until October, producing two or three generations per year depending on the latitude.

1.5. Host range and main hosts

Xylosandrus crassiusculus is a highly polyphagous pest, which apparently attacks a wide variety of broadleaf woody plants, but also shrubs and economically important crops. The pest preferentially infests stressed or dying trees (Dutto et al., 2018), colonising both the trunks and branches.

According to CABI (2019), 'it may be expected that almost any non-coniferous crop, plantation or ornamental tree in a particular area can be attacked.' Schedl (1963) listed a total of over 120 host plant species: 94 species in 28 families in Africa and 63 species in 34 families outside Africa. Since then, many additional species have been added to this list (Wood and Bright, 1992). In their datasheet of the Alert list, EPPO (2009) points out that the pest has been reported in economically important crops and forest tree species in tropical areas; fruit and nut crops and forest and ornamental woody species in more temperate areas.

Ranger et al. (2016) further highlight attacked ornamental plants in nurseries, comprising *Cornus* spp., *Gleditsia triacanthos*, *Styrax japonicus*, *Magnolia* spp., *Acer* spp., *Quercus* spp. and *Cercis* spp.

As major hosts, EPPO (online) lists *Acacia* spp., *Alnus* spp., *Aucoumea klaineana*, *Camellia sinensis*, *Carica papaya*, *Carya illinoinensis*, *Ceratonia siliqua*, *Cocos nucifera*, *Coffea arabica*, *Cornus* spp., *Diospyros kaki*, *Eucalyptus* spp., *Ficus carica*, *Hibiscus* spp., *Koelreuteria* spp., *Lagerstroemia indica*, *Liquidambar styraciflua*, *Magnolia* spp., *Malus domestica*, *Mangifera indica*, *Populus* spp., *Prunus avium*, *Prunus domestica*, *Prunus persica*, *Quercus* spp., *Salix* spp., *Tectona grandis*, *Theobroma cacao* and *Ulmus* spp.

It is noteworthy that according to Kavčič and de Groot (2017) only three tree species (*Ceratonia siliqua*, *Cercis siliquastrum* and *Castanea sativa*) have been reported as infested in Europe so far.

Between 2015 and 2020, *X. crassiusculus* was intercepted in the EU several times in wood packaging material from China and in infested roundwood with bark of *Juglans nigra* from the US (EUROPHYT, online).

Conclusion on host range and main hosts

Xylosandrus crassiusculus is a highly polyphagous pest, which can infest a wide variety of broadleaf woody plants, shrubs and economically important crops. Currently in the EU it has been found only on *Ceratonia siliqua*, *Cercis siliquastrum* and *Castanea sativa*. Surveys in EU Member States should target host species in plantations, orchards and nurseries, and focus on EU entry points of imported wood and wood products originating in countries where the pest occurs.

1.6. Environmental suitability

Xylosandrus crassiusculus is a highly polyphagous pest, and the availability of host plants in the EU is not a limiting factor for its establishment.

The ambrosia beetle has successfully spread from Asia into several parts of the world, comprising various climate zones (e.g. tropic, subtropics and temperate zones). The pest is considered to be established in several countries of the Mediterranean Basin, and could become established throughout the Mediterranean area (EPPO, 2020b).

Overwintering as adults inside the woody host tissues, *X. crassiusculus* is protected from low winter temperatures. Mizell and Riddle (2004) reported survival of *X. crassiusculus* in hosts at temperatures of -7°C in Florida. DEFRA (2015) concluded that establishment outdoors seems to be moderately likely with low confidence. The cool summer temperatures in the UK (which might be consistent with Scandinavia), as calculated by temperature accumulations (degree-days based on a 10°C threshold), could be a limit to the potential establishment (DEFRA, 2015). This indication is based on a comparison with pest reports in Japan (Sapporo) and Canada (Province Ontario) and revealed cooler summer temperatures in the UK. However, the pest occurs in climate types (e.g. Ontario, Canada and Washington State, USA, as most northern areas) that are present in a large part of the EU, throughout Europe up to the south of Scandinavia (EPPO, 2020b).

Another fact that might be considered is the number of generations per year, which is linked to temperature profiles and emergence peaks of female adults. Earlier emergence due to higher average temperatures corresponds to more generations per year.

Conclusion on environmental suitability

Xylosandrus crassiusculus is highly polyphagous and the host availability in the EU would not limit its potential establishment. Although the beetle has currently been reported only in the Mediterranean areas of the EU, the climatic conditions seem suitable for its establishment up to the south of Scandinavia.

1.7. Spread capacity

Natural spread

Detailed information on the flight capability of *Xylosandrus crassiusculus* is not available.

Kavčič and de Groot (2017), considering data on the flight capability of similar species (from Grégoire et al., 2001; Putz, 2014), concluded that *X. crassiusculus* can fly several hundred metres and even several kilometres, and may passively travel over greater distances when wind driven. The confirmed findings in Slovenia in 2017 are probably due to natural spread of *X. crassiusculus* from Italy.

Human-assisted spread

Human-assisted spread within the Union territory is possible via the movement of plants, wood, wood products and wood packaging material. As *X. crassiusculus* may also attack the small stems (less than 5 cm diameter), the transport of infested seedlings, saplings and cut branches is of interest. However, it has also been found in larger timber, especially if freshly cut (CABI, 2019).

Conclusion on spread capacity

Detailed information on the spread capacity of *Xylosandrus crassiusculus* is missing. However, the reports from several EU countries in recent years suggest that it can spread several kilometres per year when assisted by wind or human activities.

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 (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 examples of risk factors for *Xylosandrus crassiusculus* and is not necessarily exhaustive (Table 1).

To identify risk areas, it is first necessary to identify the activities that could contribute to the introduction or spread of *X. crassiusculus*. 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.

Example 1: Import of timber, wood products (with bark) and wood packaging material

Xylosandrus crassiusculus prefers freshly cut debarked timber (DEFRA, 2015; Atkinson et al., 2017). Timber and wood products, but also fresh wood packaging material (Marini et al., 2011) are relevant pathways, and their trade is therefore a risk activity. Points of entry, such as international airports and seaports in EU territory, are considered risk locations, from where *X. crassiusculus* could move on by means of natural spread, but mainly by human-assisted movement (e.g. Rassati et al. (2014) report *X. crassiusculus* at Italian ports in the Veneto region in 2009). Moreover, between 2015 and 2020, *X. crassiusculus* was also intercepted in the EU several times in wood packaging material from China and in infested roundwood with the bark of *Juglans nigra* from the US (EUROPHYT, online).

Example 2: Trade and movement of plants for planting

The import, trade and movement of plants for planting, connected to nurseries and plantations, are considered risk activities. Although there is no documentation on infested nurseries in Europe, observations on the highly damaging potential of *X. crassiusculus* are reported from nurseries in the US. As the pest is already present (or was at least trapped) in five EU Member States, nurseries and garden centres producing or trading in host plants are risk locations. The risk areas are the plantations that surround and are in the vicinity of these risk locations.

Table 1: Examples of risk activities and corresponding risk locations relevant for the surveillance of *Xylosandrus crassiusculus*

Risk activity	Risk locations	Risk areas
Import, processing and storage of timber, wood with bark and wood products of host plants with origin from countries where the pest is known to occur	Entry points (e.g. international airports and seaports, loading stations, storage facilities and companies that process wood and wood products from countries where the pest occurs)	Areas surrounding risk locations where host plants grow
Import and trade of plants for planting (host trees) originating in areas where the pest is known to occur	Nurseries cultivating and trading in potential host trees that originate from countries or regions where the pest occurs	Areas surrounding risk locations where host plants grow
Historical findings and recent interceptions	Locations where previous outbreaks have been reported, or locations where the pest has been intercepted	Areas surrounding risk locations where host plants grow

2. Detection, sampling and identification

2.1. Detection

2.1.1. Visual examination

The goal of the visual examination is to detect the symptoms caused by *Xylosandrus crassiusculus.*

Pest

Except when adult females emerge from their breeding chamber and attack new host trees, the beetles are not visible. However, the finding of Scolytinae in nurseries, plantations and orchards, and also at points of entry, may indicate an infestation that should be further investigated.

Symptoms

Xylosandrus crassiusculus is a well-investigated pest on nursery trees (saplings). Atkinson et al. (2017) report on mass attacks on *Quercus shumardii* that resulted in the death of the plants. According to Ranger et al. (2016), infestations do not always result in dieback, but growth and both aesthetic and economic value may be impacted. Kavčič and de Groot (2017) remark that, especially during the initial phase of colonisation, when symptoms on the canopies are not clearly expressed, the beetles (and the 'hidden' life stages) are difficult to detect.

Adults may infest plants of any size up to 30 cm growing in nurseries, orchards, plantations and forests.

Females bore through the bark, but these entrance holes can be difficult to detect due to their small circular form; 1 mm in diameter (Figure 4).

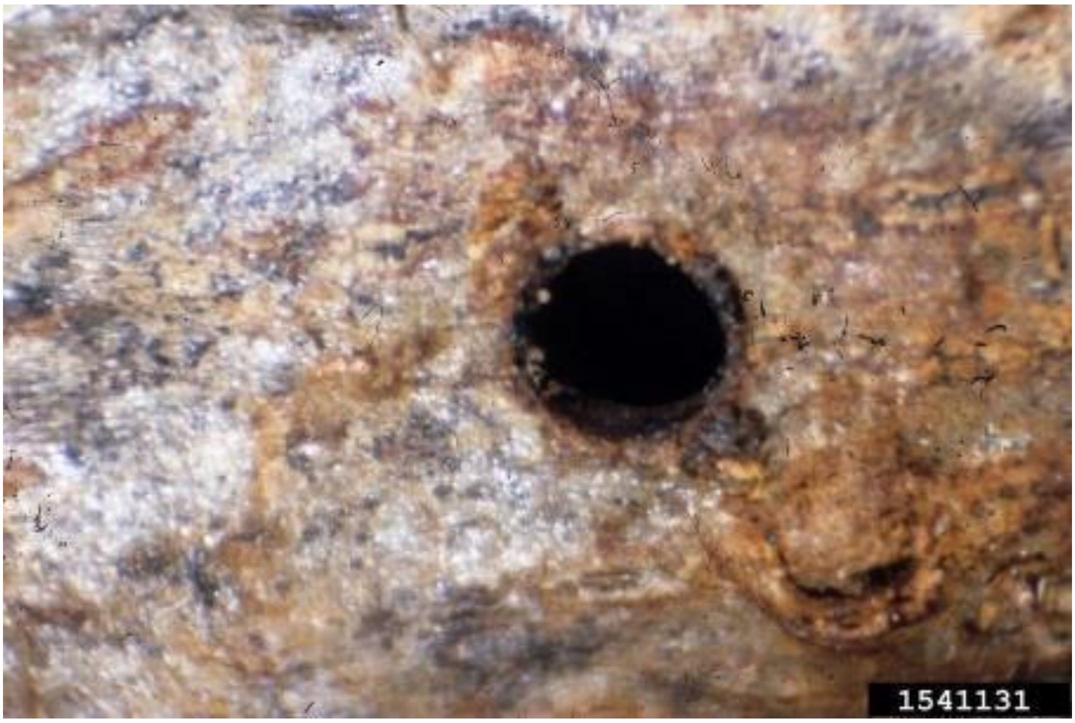

Figure 4: Symptoms on infested trees: entrance hole (approximately 1 mm in diameter) (Source: Lacy L. Hyche, Auburn University, Bugwood.org)

Stem

Similar to other *Xylosandrus* species, the females of *X. crassiusculus* produce typical external symptoms represented by toothpick-like chewings ('sawdust noodles') which are pressed out of the entrance hole when the females create the galleries (Figure 5). These sawdust noodles may be up to 4 cm long, are white in colour and visible only during the first days of the gallery boring. Rain and strong wind can cause the fall of sawdust noodles, making the detection of infested trees more difficult.

In addition, strong sap production that rinses out the entrance hole can also indicate an infestation (Figure 6).

Figure 5: Symptoms of suspicious trees: 'sawdust noodles' as chewed woody material is pushed out from galleries (Source: (top) EPPO Global Database, courtesy of: Dr Andrea Minuto, Centro di Saggio, CERSAA, Albenga, Italy; (bottom) EPPO Global Database, courtesy of: B. Rapa, private consultant)

Figure 6: Symptoms on *Ceratonia silique*: rinsing sap (Source: Julia Cerveró Albert, Vaersa-Generalitat Valenciana)

Foliage

Wilting, the resulting defoliation and dieback of branches, in some cases accompanied by basal sprouting, are also symptoms of *X. crassiusculus* infestation (Figure 7).

Figure 7: Symptoms and sampling of suspicious trees: wilting and defoliation (Source: EPPO Global Database, courtesy of: Dr Andrea Minuto, Centro di Saggio, CERSAA, Albenga, Italy)

Discolouration of wood and galleries

Female beetles tunnel horizontally from the entrance hole into the xylem of stems and trunks. These tunnels are then extended, also in a vertical direction, creating a rearing chamber where eggs are laid (Figure 8). These chambers can be 4–5 cm long and 1–1.5 cm wide (Figure 10).

Dark tissue discolouration induced by *Ambrosiella roeperi* around the entrance hole may be observed (Figure 9). After dissection of potentially infested plants, the impact of secondary fungal pathogens (*Fusarium* spp.) and host defence responses inducing blue stain discolouration may be observed (Figure 10).

Figure 8: Symptoms on infested trees: horizontally and vertically structured galleries with an adult female (Source: Lacy L. Hyche, Auburn University, Bugwood.org)

Figure 9: Symptoms on *Ceratonia silique*: visible holes after debarking (Source: Julia Cerveró Albert, Vaersa-Generalitat Valenciana)

Figure 10: Symptoms and sampling of suspicious trees: cross-section of an infested and dissected log displaying rearing chambers in a horizontal direction. The sapwood discolouration (blue stain) can also be observed (Source: EPPO Global Database, courtesy of: Dr Andrea Minuto, Centro di Saggio, CERSAA, Albenga, Italy)

2.1.2. Trapping

Trapping is used to detect the beetle at the early stages of an infestation and to monitor *X. crassiusculus* in nurseries, plantations, orchards and points of entry for wood and wood material from countries where the pest occurs. Depending on the climate, the first flight activity may be observed in spring with a mean temperature of 18–20°C, and the monitoring can continue until October. The most appropriate traps to be used are black multi-funnel traps (Reding et al., 2013). Reding et al. (2010) revealed that traps placed at 0.5 m to <1.7 m above the ground more efficiently capture *X. crassiusculus* than traps placed at a height of 3 m. A 70–95% ethanol solution is the standard attractant for a variety of ambrosia beetles (Dutto et al., 2018; Marini et al., 2011; Rassati et al., 2014). There is a wide variety of commercially available ethanol-based traps. Attraction of *X. crassiusculus* to ethanol baits may be enhanced by the addition of semiochemicals (e.g. conophthorin), but the effect is not always consistent and a benefit is not necessarily added, as reflected by various studies in Ranger et al. (2016). Soapy water is recommended as a killing agent and preservative within the trap. Pennacchio et al. (2003) captured *X. crassiusculus* using multi-funnel traps baited with α-pinene + ethanol and with α-pinene + ethanol + *Ips sexdentatus* pheromone.

If there is a positive finding, the surrounding potential host trees should be inspected for symptoms (e.g. penetration holes and sawdust noodles). Trapped insects should be identified by expert entomologists.

Conclusions for detection methods

The use of black multi-funnel traps is recommended for trapping *Xylosandrus crassiusculus* adults. Trapping can start when daily temperatures reach 18–20°C and continue until autumn. Traps should be placed at the risk locations, in nurseries, plantations, orchards, forests and points of entry of wood and wood materials from countries where the pest occurs.

2.2. Sampling

Symptomatic trees should be inspected to confirm the presence of the ambrosia beetle and the relevant and suspicious plant parts should be sampled. The plant material should be dissected with the purpose of looking for galleries. A lateral cut, close to any observed entrance holes should expose the galleries and system architecture (Figure 10). In addition, discolouration of wood, as caused by associated fungi should be visible and should serve as further sampling material for laboratory testing.

Conclusions for sampling

Symptomatic trees should be inspected and suspicious plant parts should be sampled and scrutinised to confirm the presence of the ambrosia beetle. The collected beetles should be preserved to be identified by expert entomologists.

2.3. Identification

The genus *Xylosandrus* currently comprises 40 species worldwide (Dole and Cognato, 2010). The relevant literature providing a key for differential diagnosis is available at Rabaglia et al. (2006). In addition, a key for identification of the four *Xylosandrus* species currently present in the EU is available in Gallego et al. (2017). Detailed morphological descriptions are also available in Pennacchio et al. (2003), Landi et al. (2017) and Ranger et al. (2016).

The identification of the species based on morphological features should be performed on the observation of adult specimens. The eggs, larvae and pupae have not yet been formally described (Ranger et al., 2016; CABI, 2019).

The females of *Xylosandrus* species can be distinguished from other species in the tribe Xyleborini by the stout body and the tuft of setae at the pronotal base (Figures 11 and 12). Female adults of *X. crassiusculus* have a more reddish-brown pronotum (Figure 11C) compared to the one of *X. germanus* (Figure 12), even though the distal half of the elytra is dark brown and as long as wide (Figures 11B and 11D) (Ranger et al., 2016). The females are stout and squat, cylindrical, and from a dorsal view they are rounded in front and rear, and the prominent pronotum hides the majority of the head (Pennacchio et al., 2003; Rabaglia et al., 2006; CABI, 2019). Females are long between 2.1 and 2.9 mm and are about 1.2 mm wide (Figures 11B and 11D). The elytra are slightly longer than wide and somewhat larger than the pronotum, and the elytral declivity is steep, convex, and densely covered with small granules arranged uniformly on the entire surface that result in an opaque appearance (Ranger et al., 2016).

Adult males do not fly, are smaller in size and remain inside the galleries.

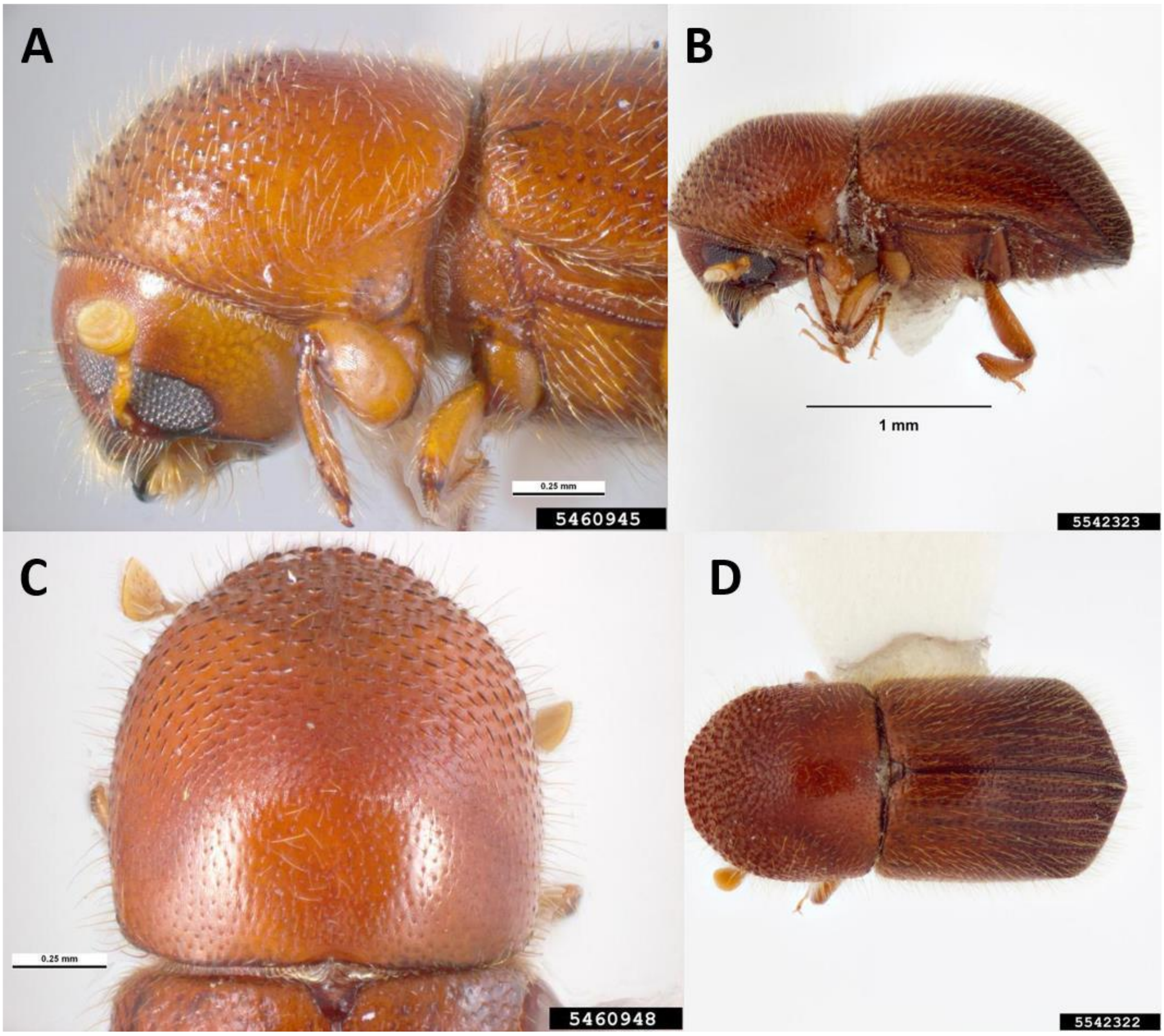

Figure 11: Adult females: A) lateral view of the pronotum (Source: Pest and Diseases Image Library, Bugwood.org; B) lateral view (Source: Luke Tembrock, Museum Collections: Coleoptera, USDA APHIS PPQ, Bugwood.org); C) dorsal view of the pronotum (Source: Pest and Diseases Image Library, Bugwood.org); D) dorsal view (Source: Luke Tembrock, Museum Collections: Coleoptera, USDA APHIS PPQ, Bugwood.org)

Figure 12: Comparison of adult females of A) *Xylosandrus crassiusculus*, B) *Xylosandrus germanus* and C) *Xylosandrus compactus* (all in lateral view). Note the different sizes of the species (Source: Pest and Diseases Image Library, Bugwood.org)

2.3.1. Laboratory testing

According to Landie et al. (2017) molecular identification of *X. crassiusculus* is applicable by amplifying partial sequences of the mitochondrial cytochrome *c* oxidase I (*COI*) using the primer combination LCO and HCO, as described by Simon et al. (1994). Curated referenced DNA sequence material is available at EPPO-Q-Bank.

Identification of the symbiotic fungus *Ambrosiella roeperi* (Harrington et al., 2014) isolated from galleries in the absence of any life stages of *X. crassiusculus* may also serve as an indication for the presence of the ambrosia beetle. The fungus could be isolated and identified by amplifying the fungal ITS region via PCR (White et al., 1990).

Conclusion for pest identification

Keys are available for the identification of adults of *Xylosandrus crassiusculus*, as well as molecular methods. The morphological identification should be performed by expert entomologists.

3. Key elements for survey design

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 crop, it is recommended that the same epidemiological and inspection units are used for each pest in order to optimise the survey programme as much as possible. This would optimise field inspections since they are organised per crop visit and not by pest. Table 2 shows an example of these definitions.

Table 2: Example of definitions of the target population, epidemiological unit and inspection unit for *Xylosandrus crassiusculus*

	Definition
Target population	All host plants in a Member State (e.g. *Ceratonia siliqua*, *Cercis siliquastrum*, *Castanea sativa*)
Epidemiological units	A single homogeneous area that contains at least one individual host plant (e.g. orchard, nursery, forest, hectare)
Inspection units	A single tree or trap

To design a plant pest survey on *Xylosandrus crassiusculus* the general guidelines provide further details on the following steps that will generally be necessary:

1/ Determine the type of survey based on its objectives. For *X. crassiusculus*, the type of survey will depend on the pest status (according to ISPM No. 8 (FAO, 2017)) in the area of interest. The objective could be to substantiate pest freedom, to delimit an outbreak area following an infestation or to determine the pest prevalence. The next steps deal with the example of substantiating pest freedom.

The overall confidence level and design prevalence of the survey have to be decided by the risk managers before designing the surveys as they reflect the acceptable level of the risk of infestation of the host plants by *X. crassiusculus*. The general guidelines for pest surveillance provide further details on the choice of these values and the related consequences in terms of pest surveys.

2/ Define the target population and its size. When determining the target population for surveillance of *X. crassiusculus*, the host plants that are relevant for the survey area have to be selected. The size of the target population should be determined. For example, the target population could be all host trees in a Member State.

3/ Define the epidemiological units. The epidemiological units should be single homogeneous areas that each contain at least one individual host plant.

4/ Determine the inspection unit. For *X. crassiusculus*, the inspection unit would be a single tree or a trap.

5/ Determine the number of inspection units per epidemiological unit. For *X. crassiusculus*, this is the average number of trees or traps per epidemiological unit.

6/ Implement the inspections and, if appropriate, the sampling, following the procedures suggested by the competent authorities, within the epidemiological units and estimate the method effectiveness in order to determine the overall method sensitivity (sampling effectiveness × diagnostic sensitivity). A representative number of plants should be examined and if there are suspicious symptoms they should be sampled. 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 confidence level. This confidence level is in turn needed to calculate the number of sites to be inspected (Step 8). Note that the more units are inspected the higher the confidence will be. The competent authorities need to align the survey efforts with the resources available.

7/ Define the risk factors. A risk factor affects the probability that a pest will be present or detected in a specific portion of the target population. 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 plant population to which they apply are known or can be reliably estimated.

8/ Determine the number of epidemiological units to survey. RiBESS+ can be used to determine the number of epidemiological units to survey in order to achieve the objectives of the survey set at Step 1 in terms of confidence level (e.g. 95%) and design prevalence (e.g. 1%), while also including the method sensitivity from Step 6 and the risk factors identified in Step 7. As a result, considering, for example, fields where host plants are present, the number of fields that need to be surveyed are estimated for a Member State in order to state with 95% confidence that the prevalence of *X. crassiusculus* will be at 1% or below.

9/ Summarise and evaluate the survey design. 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. This adjustment would result in a modified survey design using different input parameters of the statistical tool RiBESS+ (e.g. varying the number of components, method sensitivity, etc.).

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

11/ Allocate the calculated survey effort. In the survey area, the output of RiBESS+ should be allocated proportionally to the host plant population or to the number of epidemiological units. In addition, the survey size should be selected from the list of available locations.

12/ Data collection and survey reporting. Consider which data are needed and how these data will be reported together with the related assumptions.

13/ Plan, develop or update the specific instructions for the inspectors. These activities are not addressed by EFSA and fall within the remit of the competent national authorities.

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General glossary for pest survey

Term	Definition*
Buffer zone	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, 2020).
Component (of a survey)	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.
Confidence	The sensitivity of the survey is a measure of reliability of the survey procedure (Montgomery and Runger, 2010). The term **confidence level** is used in 'Methodologies for sampling of consignments' (ISPM 31: FAO, 2016b).
Delimiting survey	Survey conducted to establish the boundaries of an area considered to be infested by, or free from, a pest (ISPM 5: FAO, 2020).
Design prevalence *analogous to the term **level of detection** used in 'Methodologies for sampling of consignments' (ISPM 31: FAO 2016b)*	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).
Detection survey	Survey conducted in an area to determine whether pests are present (ISPM 5: FAO, 2020).
Diagnostic protocols	Procedures and methods for the detection and identification of regulated pests that are relevant to international trade (ISPM 27: FAO, 2016a).
Epidemiological unit *analogous to the term **lot** used in 'Methodologies for sampling of consignments'*	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 to which statistics are applied (e.g. a

(ISPM 31: FAO 2016b)	tree, orchard, field, glasshouse, or nursery) (EFSA, 2018).
Expected prevalence	In prevalence estimation approaches, it is the proportion of epidemiological units expected to be infested or infested.
Expert knowledge elicitation	A systematic, documented and reviewable process to retrieve expert judgements from a group of experts in the form of a probability distribution (EFSA, 2014).
Host plant	A host plant is a plant species belonging to the host range on which the pest could find shelter, feed or subsist at least for a period of time.
Host range	Species capable, under natural conditions, of sustaining a specific pest or other organism (ISPM 5: FAO, 2020). This definition is limited to an array of host plant species and does not include commodities other than plants or plant parts.
Identification	Information and guidance on methods that either used alone or in combination lead to the identification of the pest (ISPM 27: FAO, 2016a).
Infected versus infested	Infected is used when a pathogen is referred to in relation to its hosts (e.g. the trees are infected by the bacterium). Infested is used when an insect is referred to in relation to its hosts (e.g. the trees are infested by beetles). Infested is used when the pest is mentioned in relation to an area (e.g. an infested zone).
Inspection	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, 2020).
Inspection unit *analogous to **sample unit** used in 'Methodologies for sampling of consignments' (ISPM 31: FAO 2016b*)	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).
Inspector	Person authorised by a national plant protection organisation to discharge its functions (ISPM 5: FAO, 2020).
Method sensitivity *analogous to the term **efficacy of detection** used in 'Methodologies for sampling of consignments'*	The conditional probability of testing positive given that the individual is diseased (Dohoo et al., 2010). The method sensitivity (MeSe) is defined as the probability that a truly positive host tests positive. It has two components: the sampling effectiveness (i.e. probability of selecting infested plant parts from an infested plant) and the diagnostic sensitivity (characterised by the visual inspection

(ISPM 31: FAO 2016b)	and/or laboratory test used in the identification process). The diagnostic sensitivity is the probability that a truly positive epidemiological unit will result positive and is related to the analytical sensitivity. It corresponds to the probability that a truly positive inspection unit or sample will be detected and confirmed as positive. The sampling effectiveness depends on the ability of the inspector to successfully choose the infested plant parts in a host plant. It is directly linked to the sampling procedure itself and on the training of the inspectors to recognise the symptomatology of the pest. Furthermore, symptom expressions are dependent, among other factors, on the weather conditions as well as on the physiological stage of the host plant when the sample is taken.
Pest diagnosis	The process of detection and identification of a pest (ISPM 5: FAO, 2020).
Pest freedom	Pest freedom can be defined, for a given target population, in a statistical framework, as the confidence of freedom from a certain pest against a pre-set design prevalence (threshold of concern).
Population size	The estimation of the number of the plants in the region to be surveyed (EFSA, 2018).
Relative risk	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).
Representative sample	A sample that describes very well the characteristics of the target population (FAO, 2014).
RiBESS+	Risk-based surveillance systems. This is 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 https://shiny-efsa.openanalytics.eu/
Risk assessment	Evaluation of the probability of the introduction and spread of a pest and the magnitude of the associated potential economic consequences (ISPM 5: FAO, 2020).
Risk factor	A factor that may be involved in causing the disease (FAO, 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.
Risk-based survey	A survey design that considers the risk factors and enforces the survey efforts in the corresponding proportion of the target population.
SAMPELATOR	Sample size calculator. This is an online application that implements statistical methods to estimate the sample size for pest prevalence estimation surveys. Free access to the software with prior user registration is available at https://shiny-efsa.openanalytics.eu/
Sample size	The sample size refers to the output of the statistical tools for survey design (RiBESS+ and SAMPELATOR). 'A well-chosen sample will contain most of the information about a particular population parameter but the relation between the sample and the population must be such as to allow true inferences to be made about a population from that sample.' (BMJ, online). The survey sample consists of the required number of 'inspection units' or samples thereof to be examined and/or tested in the survey to retrieve sufficient information on the pest presence or prevalence in the total population. For risk-based surveys, the sample size is calculated on the basis of statistical principles that integrate risk factors. If the examination for pest presence is performed by laboratory testing, at least one sample is taken from each inspection unit. These samples will undergo relevant laboratory testing.
Sampling effectiveness	For plants, it is the probability of selecting infested plant parts from an infested plant. For vectors, it is the effectiveness of the method to capture a positive vector when it is present in the survey area. For soil, it is the effectiveness of selecting a soil sample containing the pest when the pest is present in the survey area.
Specified plant	The plant species known to be susceptible to the pest. For example, for *Xylella fastidiosa*, the list of specified plants, which includes host plants and all plants for planting, other than seeds, belonging to the genera or species, can be found in Annex I of Decision (EU) 2015/789.
Survey	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, 2020).
Target population *analogous to* ***consignment*** *used in 'Methodologies for*	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:

sampling of consignments' (ISPM 31: FAO 2016b)	• definition of the target population: the target population has to be clearly identified; • target population size and geographic boundary. (EFSA, 2018)
Test	Official examination of plants, plant products or other regulated articles, other than visual, to determine whether pests are present, identify pests or determine compliance with specific phytosanitary requirements (ISPM 5: FAO, 2020).
Test specificity	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 is the probability that a truly negative epidemiological unit will give a negative result and is related to the analytical specificity. In freedom from disease it is assumed to be 100%.
Visual examination	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, 2020).

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