PEST SURVEY CARD

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Pest survey card on *Geosmithia morbida* and its vector *Pityophthorus juglandis*

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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 *Geosmithia morbida* and *Pityophthorus juglandis* surveys. These are required to design statistically sound and risk-based pest surveys, in line with current international standards. *Geosmithia morbida* and its vector *P. juglandis* are clearly defined taxonomic entities and the combined activity of the fungus and the insect causes the disease complex thousand canker disease on the plant genera *Juglans* and *Pterocarya*. The pest and its vector originate in North America and currently have a restricted distribution in the EU, limited to the northern parts of Italy. However, they are potentially able to become established everywhere in the EU where their host plants occur. Currently, the spread capacity of *P. juglandis* is unknown, but the insect vector may cover large distances by passive dispersal or human-assisted spread. Risk locations include entry points (e.g. seaports, airports), loading stations, storage facilities and wood processing companies that deal with heat-treated wood, bark or woodchips of the genera *Juglans* and *Pterocarya* originating from countries where the fungus and its vector occur. Trapping is the recommended method for detecting the vector in the early stages of an epidemic. Following insect trapping, a specific tree inspection should be carried out, looking for external symptoms (e.g. penetration and exit holes, cankers and wilting). The trapping should start when the mean air temperature exceeds 18°C. Morphological identification of the pathogen and its vector should be performed by experts. Molecular assays are also available for both fungal and vector identification.

Keywords: plant pest, risk-based surveillance, survey, TCD, thousand cankers disease, walnut twig beetle, WTB

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Introduction

The information presented in this pest survey card was summarised from the European and Mediterranean Plant Protection Organization (EPPO) Pest Risk Analysis on thousand cankers disease (EPPO, 2015), the Thousand Cankers Disease Survey Guidelines for 2020 of the United States Department of Agriculture (Seybold et al., 2019), the EPPO Global Database datasheets on *Geosmithia morbida* and *Pityophthorus juglandis* (EPPO, 2020a,b) and other documents.

The objective of this pest survey card is to provide the relevant biological information needed to prepare surveys for *G. morbida* and its vector *P. juglandis* 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 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 *Geosmithia morbida* and its vector *Pityophthorus juglandis*[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

The pests under scrutiny are the fungus *Geosmithia morbida* and its associated vector *Pityophthorus juglandis*, which cause the disease complex thousand cankers disease (TCD). TCD is the result of the combined activity of *G. morbida* and its vector *P. juglandis*.

Scientific name: *Geosmithia morbida* M. Kolařík, Freeland, C. Utley & Tisserat 2010

Synonym(s): none

EPPO Code: GEOHMO

Common name: none

Phylum: Ascomycota **Subphylum:** Pezizomycotina **Class**: Sordariomycetes **Subclass:** Hypocreomycetidae **Order**: Hypocreales **Family:** Bionectriaceae **Genus**: *Geosmithia* **Species**: *Geosmithia morbida*

Scientific name: *Pityophthorus juglandis* Blackman, 1928

Synonym(s): none

EPPO Code: PITOJU

Common name: walnut twig beetle (WTB)

Class: Insecta **Order**: Coleoptera **Family:** Curculionidae **Subfamily**: Scolytinae **Genus**: *Pityophthorus* Eichhoff, 1864 **Species**: *Pityophthorus juglandis*

[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://websso-efsa.openanalytics.eu/auth/realms/efsa/protocol/openid-connect/auth?response_type=code&client_id=shiny-efsa&redirect_uri=https%3A%2F%2Fshiny-efsa.openanalytics.eu%2Fsso%2Flogin&state=d6f7f997-d09f-4bb0-afce-237f192a72d5&login=true&scope=openid

No teleomorph (sexual stage) of *G. morbida* is known (EPPO, 2015).

Currently, there is no evidence of an effective transmission of the fungus by beetles other than *P. juglandis* (Kolařík et al., 2017; Moore et al., 2019).

Conclusions on taxonomy

Geosmithia morbida and its vector *Pityophthorus juglandis* are clearly defined taxonomic entities. The combined activity of the two pests causes the disease complex thousand canker disease (TCD).

1.2. EU pest regulatory status

Geosmithia morbida and its vector *Pityophthorus juglandis* are Union quarantine pests listed in Annex II part B of Commission Implementing Regulation (EU) 2019/2072[4].

Special requirements for the import and movement within the Union territory of plants for planting and wood of the genera *Juglans* and *Pterocarya* are laid down in Annexes VII and VIII of Commission Implementing Regulation (EU) 2019/2072.

Plants for planting, other than seeds, *in vitro* material and naturally or artificially dwarfed woody plants for planting, originating from all third countries and belonging to the genera *Juglans* L. are also included in the list of high-risk plants under Commission Implementing Regulation (EU) 2018/2019[5].

The general requirements for surveys of quarantine organisms within EU territory are laid down in Regulation (EU) 2016/2031[6].

Overview of EU regulatory status

Geosmithia morbida and its vector *Pityophthorus juglandis* are both Union quarantine pests. Detailed import requirements for plants for planting and wood of the genera *Juglans* and *Pterocarya* are laid down in EU legislation.

1.3. Pest distribution

Pityophthorus juglandis is native to the south-western United States and Mexico and was then introduced to the north-western and eastern USA. The known distribution of *Geosmithia morbida* (Figure 1) overlaps with its vector distribution, but *P. juglandis* has additionally been reported from Chihuahua State, Mexico, where the fungus was not detected (Wood and Bright, 1992; EPPO, 2020b; Figure 2). In 2013, the disease complex TCD was first recorded in the EU in north-eastern Italy (Vicenza province). Until 2019, the disease additionally spread within Italy into Piedmont, Veneto, Lombardy, Tuscany and Emilia-Romagna (EPPO, 2019), and the vector *P. juglandis* was also detected

[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] Commission Implementing Regulation (EU) 2018/2019 of 18 December 2018 establishing a provisional list of high risk plants, plant products or other objects, within the meaning of Article 42 of Regulation (EU) 2016/2031 and a list of plants for which phytosanitary certificates are not required for introduction into the Union, within the meaning of Article 73 of that Regulation. OJ L 323, 19.12.2018, p. 10–15.

[6] 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.

in Friuli Venezia Giulia (EPPO, 2016). The EPPO pest risk analysis (EPPO, 2015) provides a comprehensive overview of the distribution and spread history of TCD up to the year 2014.

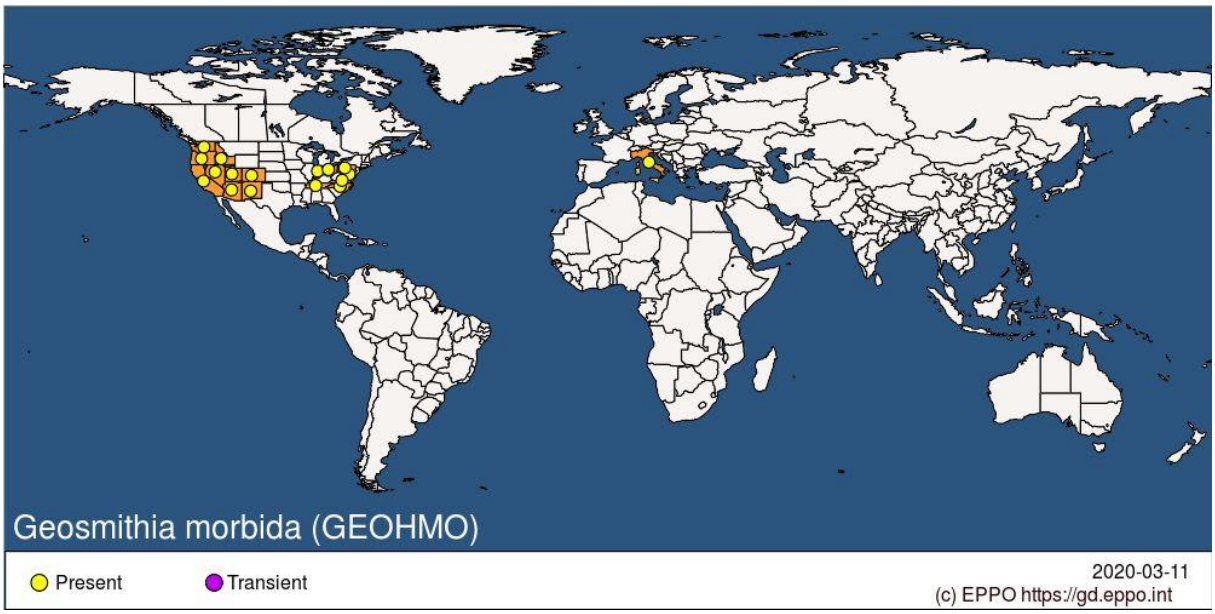

Figure 1: Global distribution of *Geosmithia morbida* (Source: EPPO Global Database, https://gd.eppo.int)

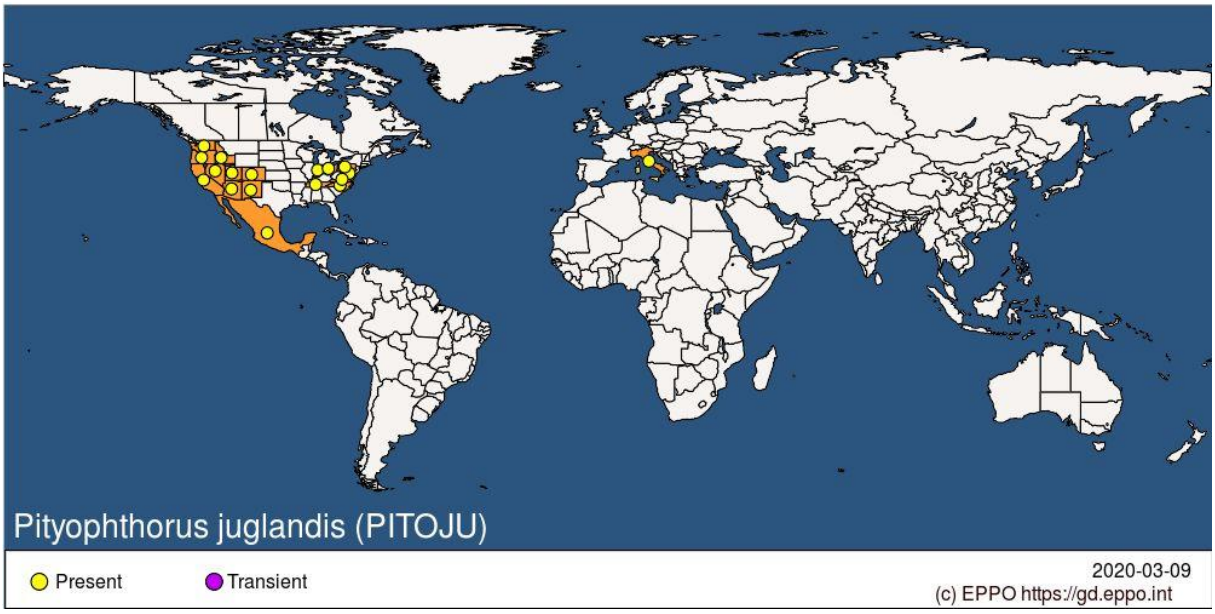

Figure 2: Global distribution of the vector *Pityophthorus juglandis* (Source: EPPO Global Database, https://gd.eppo.int)

Conclusion on pest distribution

Pityophthorus juglandis and the associated fungus *Geosmithia morbida* are currently present in the EU in the northern part of Italy. Surveys in most Member States would be detection surveys aimed at substantiating pest freedom. If there is a new outbreak, delimiting surveys should be conducted in that area to define the boundaries of the infested zone and to demarcate the area.

1.4. Life cycle

Thousand cankers disease is the result of the combined activity of the fungus and the insect vector. No teleomorph (sexual stage) of *Geosmithia morbida* has been identified (EPPO, 2015) and asexual reproduction of the fungus is by conidia produced in conidiophores (CABI, 2019). Adult *Pityophthorus juglandis* carry the spores of the fungus passively and introduce it into the trees when the insects burrow into the bark to feed on the phloem. The vector can introduce the fungus into the tree even via galleries which are then abandoned ('tasting' or gustation probing) (Audley et al., 2017). *Pityophthorus juglandis* has no mycangium (structure to transport symbiotic fungi) but the elytras (wing covers) of emerging beetles are heavily contaminated externally with spores of *G. morbida* (Newton et al., 2009; Cranshaw and Tisserat, 2012). The fungus develops and reproduces asexually within and around the galleries made by the beetle in the phloem and it does not move systemically within the tree. Small round to oval cankers develop around the feeding galleries; these are only visible if the bark is removed (Tisserat et al., 2009). The galleries of *P. juglandis* are 2.5–5 cm long (Graves et al., 2009). The cankers are often scattered every 2–5 cm. In small-diameter branches galleries could sometimes cause the bark to crack. In thicker barked branches and the trunk, the cankers are often initially limited to the phloem and the outer bark and do not reach the cambium. As the cankers develop, the infected plant tissues macerate and become dark brown to black (Tisserat et al., 2009). In addition, the cankers can merge as a result of extensive tunnelling of the beetle due to multiple repeated attacks and may finally cause dieback of branches and twigs. Trunk cankers in the advanced stages of decline can exceed 2 m vertically from the bottom and cover half or more of the trunk circumference (Tisserat et al., 2009).

Pityophthorus juglandis infests the trunks and branches of all sizes of mature trees, but generally not those smaller than 1.3 to 2.0 cm in diameter (Tisserat et al., 2009; EPPO, 2015). The trees are initially colonised by males (Cranshaw and Tisserat, 2012). The beetles prefer the warmer side of the tree and tend to colonise the base of small branches in rough areas of bark on the underside of the small branches (Newton et al., 2009). Unmated males bore into the bark through a small circular penetration hole in bark roughness, create a circular nuptial chamber under the bark and produce aggregation pheromones (Cranshaw and Tisserat, 2012; Seybold et al., 2013). Usually a couple of females join the male for mating and form egg galleries (one for each female) in which they lay the eggs (Cranshaw and Tisserat, 2012). In Italy, three to eight (usually four or five) females join the male (Faccoli et al., 2016). Egg galleries develop orthogonally to the wood fibres (Faccoli, 2015). The number of new adults per egg-laying female varies from five in less suitable hosts (*J. microcarpa*) to 40 in highly suitable hosts (*J. nigra*) per generation (Hefty et al., 2018). The larvae tunnel meandering galleries roughly vertical to the egg gallery and develop under warm conditions within four to six weeks (Cranshaw and Tisserat, 2012), and within six to eight weeks in temperate climates similar to Boulder, Colorado, such as parts of central Europe (Newton et al., 2009). The species goes through at least three larval stages (Nix, 2013). Pupation occurs at the end of the larval galleries. Adults emerge through very small round exit holes. The peak flight activity in Colorado occurs in mid-July to late August. The first emergence of adults was observed in late April in Boulder, Colorado (Cranshaw and Tisserat, 2012). Emerging adults are likely to remain near their natal host or colonise a new host close by to reproduce, as the flight capacity and propensity of adults is limited. The number of generations of beetles is increased by warmer temperatures (Cranshaw and Tisserat, 2012). The number of generations per year varies between one and three depending on the latitude. In Colorado, the beetles probably produce two or three generations per year (Tisserat et al., 2009). Even in cold winters, larvae and beetles have been observed in infested logs, suggesting that there are overlapping generations and continuous breeding. A small proportion of beetles are able to survive temperatures below -23°C for several days. Larvae and adult beetles can easily be found within and under the bark of symptomatic branches (Cranshaw and Tisserat, 2012). In Italy, two partially overlapping

generations occurred. *Pityophthorus juglandis* overwinters in northern Italy as mature larvae, pupae or adults. Individuals overwintering as adults emerge when the air temperature is favourable (in Italy in the second half of May). Overwintering larvae have to complete their development before emergence. Therefore, this cohort emerges four to five weeks later. In northern Italy the development time for one generation takes about 12 weeks (Faccoli et al., 2016). Accordingly, there are four peaks of adult emergence in Italy: in the second half of May, at the beginning of July, and the following generation at the beginning of September and at the beginning of October. Trapping of adults can begin when the mean air temperature exceeds 18°C (Faccoli et al., 2016).

The external symptoms of TCD are not usually visible until the tree is considerably damaged. The period between the first infestation and the development of visible external symptoms of the tree may be several years, depending on many factors. Stressed trees show earlier external symptoms than well-sited trees. External symptoms are crown dieback, abnormally thin crowns, and wilted individual branches with yellowing or wilted leaves that remain on the tree. The best season for external examination is summer, when wilting symptoms will be most noticeable.

The life cycle of *G. morbida* and its vector is shown in Figure 3.

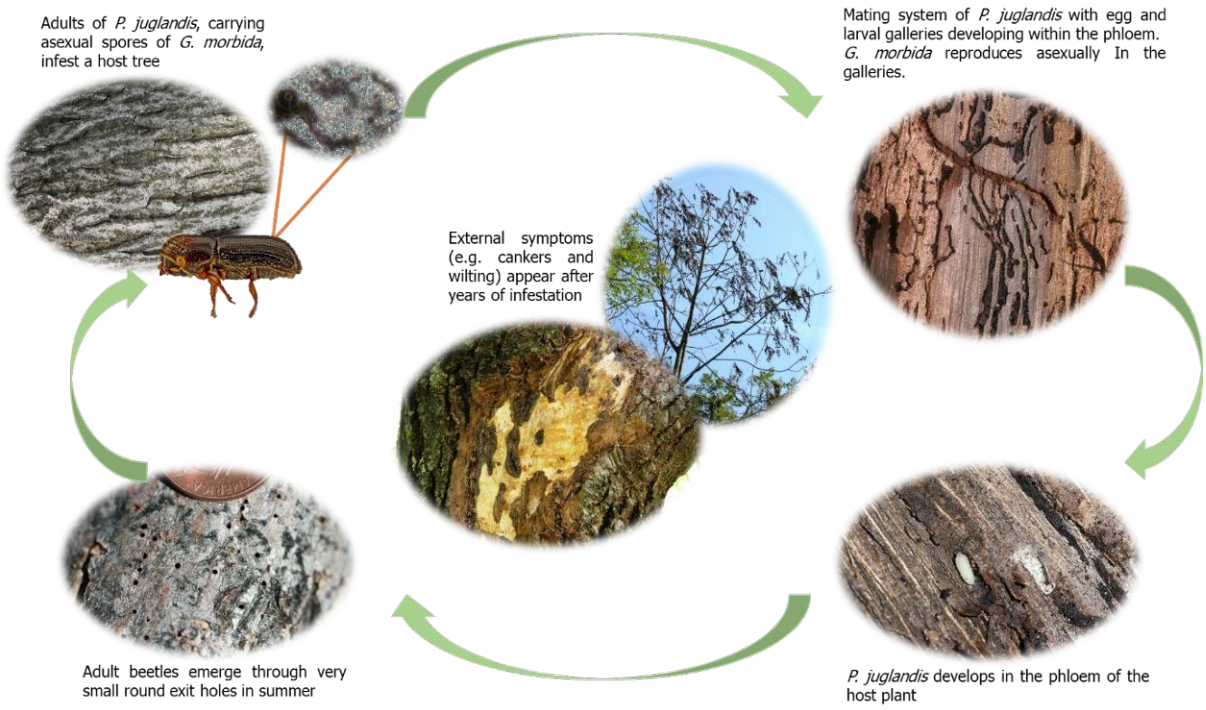

Figure 3: Life cycle of *Geosmithia morbida* and its vector *Pityophthorus juglandis* (Source: Steven Valley, Oregon Department of Agriculture, Bugwood.org; Piero Amorati, ICCroce – Casalecchio di Reno, Bugwood.org; Whitney Cranshaw, Colorado State University, Bugwood.org; Lucio Montecchio, Università di Padova, EPPO Global Database)

Conclusion on life cycle

Pityophthorus juglandis introduces the spores of *Geosmithia morbida* into the trees, where it reproduces asexually within and around the galleries formed by the beetle. Trapping could start when the mean air temperature exceeds 18°C and continue throughout the spring and summer seasons. External symptoms of the trees (e.g. penetration and exit holes, tree dieback, branches wilting and abnormally thinning crowns) would be most easily recognised in early–mid-summer.

1.5. Host range and main hosts

The EPPO pest risk analysis (EPPO, 2015) gives a comprehensive overview of the host plants.

The host plants of *Pityophthorus juglandis* and *Geosmithia morbida* belong to the genera *Juglans* and *Pterocarya* (Juglandaceae). *Carya* spp. (Juglandaceae) are not hosts of the beetle or the fungus (Utley et al., 2013).

The confirmed hosts of *P. juglandis* and *G. morbida* are categorised according to their general susceptibility to TCD in terms of the damage they cause and symptom expression. The susceptibility depends on the vulnerability to *G. morbida* and the ability of *P. juglandis* to find, colonise and breed on the trees (Table 1) (Utley et al., 2013) (Table 1).

Table 1: Level of susceptibility for different host plants (from Utley et al., 2013)

Level of susceptibility	Host plants	Symptoms
Low susceptibility	*Juglans major*	Limited symptoms, rare death of branches or whole trees
Intermediate susceptibility	*Juglans regia*, *Juglans hindsii*, *Juglans cinerea*, *Juglans microcarpa*, *Juglans ailantifolia*, *Juglans mandshurica*, *Juglans illinoinensis*, *Juglans californica*, *Juglans mollis*, *Pterocarya fraxinifolia*, *Pterocarya rhoifolia*, *Pterocarya stenoptera*	Plant death possible but not inevitable, high intraspecific variation, scattered dieback, slow progress of the disease
High susceptibility	*Juglans nigra*	General plant death, large cankers

Juglans regia and *J. nigra* are the major and widespread species in the EU (EPPO, 2015; Montecchio et al., 2016). *Juglans regia* is distributed at latitudes between 10° and 50° north and can be found in most parts of Europe, except the far north (de Rigo et al., 2016). *Juglans nigra* was introduced as a timber tree in most European countries for cultivation (EPPO, 2015). The other known hosts of *G. morbida* and *P. juglandis* are grown as ornamentals in the EU (EPPO, 2015). The pathogen and its vector are known to attack mature host plants, either in plantations for timber and walnut production or in wild, semi-wild and urban areas.

Conclusion on host range and main hosts

Host plants relevant for the surveillance are trees of the genera *Juglans* and *Pterocarya*. In the EU, the major and most widespread host plants are *J. regia* and *J. nigra*. In areas with mixed *Juglans* spp. plantations, external symptoms will be recognised first on species with a high susceptibility like *J. nigra*.

1.6. Environmental suitability

Pityophthorus juglandis and *Geosmithia morbida* are widespread in the US and they are expected to be able to occur throughout most of that country where host plants are present (Newton et al., 2009). According to EPPO (2015), the climatic conditions in the US where TCD is present occur in a broad area of the EPPO region, including the whole of Europe with the exception of the northern parts of Sweden, Norway and Finland. The environmental suitability for *P. juglandis* and *G. morbida* seems not

to be limited by climatic conditions but it is mainly determined by the presence of host plants (EPPO, 2015).

Due to their origin in the south-western US and Mexico, both species may benefit from the warmer climates in the southern Member States of the EU resulting in faster symptom development (Cranshaw and Tisserat, 2012). In the EU, establishment of TCD and WTB may be facilitated by the extensive use of walnut species for wood and nut production and as ornamental trees.

Conclusion on environmental suitability

Geosmithia morbida and its vector *Pityophthorus juglandis* are potentially able to become established everywhere in the EU where their host plants occur.

1.7. Spread capacity

Natural spread

Currently, the flight capacity of *Pityophthorus juglandis* is unknown (EPPO, 2015). However, in flight mill experiments the mean active flight distance measured in 24 hours was 372 m, and the maximum was about 3.6 km. As the flight capacity and propensity of adults is limited, the natural spread capacity of *P. juglandis* is low, although natural passive dispersal may be favoured by wind (Kees et al., 2017). The spread of *P. juglandis* is expected to be minimal in the early stages of the infestation with a low population density (EPPO, 2015). However, field observations of infested areas in Italy suggest a very high passive dispersal capacity of the disease, which in few years spread from the Veneto region to the whole of northern and central Italy (Faccoli et al., 2016). Further studies on the natural spread potential under natural conditions are still needed.

Geosmithia morbida can release conidia through the beetles' exit holes. However, a successful colonisation of new hosts by the fungus is not expected without the vector (EPPO, 2015; Montecchio et al., 2016).

Human-assisted spread

In the EU, the main pathway for the introduction and spread of *G. morbida* and *P. juglandis* is the transport of fresh wood with bark (timber, logs and firewood) and mature plants for planting from the plant genera *Juglans* and *Pterocarya.* For the fungus, wood chips may also be a pathway but it cannot spread or become further established in other host plants without the presence of the vector. Roots, nuts and seeds of these genera are not pathways for the pest or its vector (Newton et al., 2009; EPPO, 2015).

Conclusion on spread capacity

Currently, the spread capacity of *Pityophthorus juglandis* is unknown. In experimental conditions it can fly approximately 400 m on average in 24 hours. However, in natural conditions it may cover larger distances by passive dispersal or human-assisted spread. The fungus and the vector may have become established several years before the detection of external symptoms (e.g. cankers and wilting); therefore, in an outbreak, the potential spread distance to consider for defining the boundaries of the infested zone has to be set accordingly.

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 an example of a risk factor for *Geosmithia morbida* and its vector *Pityophthorus juglandis* (Table 2).

For the identification of risk areas, it is first necessary to identify the activities that could contribute to the introduction or spread of *G. morbida* and its vector *P. juglandis*. 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 pests and the availability of host plants around these locations.

Example: Import, processing and storage of heat-treated wood, bark or woodchips of the genera *Juglans* and *Pterocarya*

Pityophthorus juglandis is able to re-infest and persist in properly phytosanitarily treated (fumigated, steam-heated), but still fresh, wood with bark and can at least reproduce there (Audley et al., 2016). Therefore, a potential pathway for the introduction of *G. morbida* and its associated vector *P. juglandis* into and within the EU is the import, processing and storage of heat-treated wood, bark or woodchips of the genera *Juglans* and *Pterocarya*.

Table 2: Example of a risk activity and corresponding risk locations relevant for surveillance of *Geosmithia morbida* and its vector *Pityophthorus juglandis*

Risk activity	Risk locations	Risk areas
Import, processing and storage of heat-treated wood, bark or woodchips of the genera *Juglans* and *Pterocarya* with origin from countries where the fungus and its vector occur	Entry points (e.g. ports, airports), loading stations, storage facilities and wood processing companies for wood, bark or woodchips of the genera *Juglans* and *Pterocarya* with origin from countries where the fungus and its vector occur	Areas around risk locations where host plants of the genera *Juglans* and *Pterocarya* grow

2. Detection, sampling and identification

2.1. Detection

2.1.1. Visual examination

Usually, the fungus and its vector are not visible by visual examination of an infested tree. The goal of the visual examination is to detect the symptoms of TCD in the early stages of an epidemic. The symptoms include the penetration and exit holes of the insect vector (larvae and beetles are under the bark), as well as external symptoms on the tree due to fungal proliferation (e.g. cankers and wilting).

The combination of small bark beetles and several cankers around their galleries in the phloem of the plant genera *Juglans* and *Pterocarya* is very specific to the TCD caused by *G. morbida* and *P. juglandis*. Low insect population densities at the beginning of an infestation are difficult to detect. *Pityophthorus juglandis* is one of only a few species of this genus that infests hardwood. Although there are several native *Pityophthorus* species in Europe, none of them is known to infest *Juglans* spp. (Tisserat et al., 2009; EPPO, 2015). A detection of small bark beetles on *Juglans* spp. or *Pterocarya* spp. may indicate an infestation with *P. juglandis*.

Penetration and exit holes created by the vector are easy to detect. The shape of the mating system (egg and larval galleries) may help in identifying the beetle species (Faccoli, 2015). All life stages of *P. juglandis* can be found under the bark of infested trees. The typical oval cankers caused by *G. morbida* are located in the phloem around *P. juglandis* galleries and tasting holes on the bark of

branches or on the trunk; cankers are also only visible if a thin layer of the outer bark is removed (Tisserat et al., 2009; Figures 4 and 5). Dark stains of old plant sap may be visible on the bark (Graves et al., 2009; Figure 6). On heavily damaged branches, many small circular entries and exit holes of the beetles are visible (Seybold et al., 2019; Figure 7).

The first external symptom of TCD is yellowing and wilting of foliage on individual branches (flagging) in the upper crown and in general resulting in crown thinning and branch mortality (Montecchio et al., 2016; Seybold et al., 2019) (Figure 8).

Juglans nigra trees died within 3–4 years after the first external symptoms appeared (Kolařík et al., 2011). The symptoms become visible in late spring or early summer, and are more severe in late summer (Seybold et al., 2019).

Visible symptoms on damaged trees may look similar to symptoms caused by other biological and abiotic stress factors (EPPO, 2015; Oren et al., 2018).

Figure 4: Cankers by *Geosmithia morbida* around galleries of *Pityophthorus juglandis* in *Juglans californica* (Source: Whitney Cranshaw, Colorado State University, Bugwood.org)

Figure 5: Tasting holes of *Pityophthorus juglandis* on *Juglans nigra* in California (USA) (Source: Miroslav Kolařík, Institute of Microbiology of the Czech Academy of Sciences)

Figure 6: External dark stains induced by sap weeping on the bark of *Juglans regia* around holes caused by *Pityophthorus juglandis* (Source: Ned Tisserat, Colorado State University, Bugwood.org)

Figure 7: Entry and emergence holes of *Pityophthorus juglandis* on a *Juglans nigra* branch (Source: Troy Kimoto, Canadian Food Inspection Agency, Bugwood.org)

Figure 8: Massive *Pityophthorus juglandis* infestation in a black walnut plantation of north-eastern Italy. Note the strong canopy decline and the stump sucker emission. Vicenza (Italy), 2013 (Source: Massimo Faccoli, Università degli Studi di Padova)

2.1.2. Trapping

Seybold et al. (2011, 2013 and 2019) provide comprehensive guidelines for the detection of *P. juglandis* which are currently applied in the US. The main information in this document is summarised in the section below.

Trapping of adults can begin when the mean air temperature exceeds 18°C (Faccoli et al., 2016) and can be stopped at the end of summer. Approved traps for surveillance of *P. juglandis* are black multi-funnel traps (Figure 9) baited with the aggregation pheromone of *P. juglandis* (commercially available) in a passive slow-release dispenser which should be hung in the middle of the trap (Seybold et al., 2013, 2019). The surveillance with baited traps for *P. juglandis* is effective at low, intermediate and high population densities and therefore suitable for detection of the beetle population in the early stages of an epidemic when no external symptoms on the trees are visible yet.

The active compound of the lure is Prenol (3-methyl-2-buten-1-ol) (Seybold et al., 2011). A few other bark and ambrosia beetle species may be found in the traps because they respond to the same lures, although in small numbers because they do not live on walnut species (Seybold et al., 2013). The wet trap cup should be filled with propylene glycol dissolved in water to a 25–30% solution. Ethylene glycol should not be used, as it has a very high toxicity and ethanol would attract other ambrosia beetles and so it is not recommended either. Dry traps could also be used (Faccoli et al., 2016).

The traps should be placed near to, but never on, host trees, to avoid an infestation by *P. juglandis* of an uninfested tree or branch. It is recommended that the traps are installed on poles ~2.5–4.5 m

from the main stem of the tree, 1.5–3 m from living branches and ~3 m above the ground. Traps should be installed in walnut plantations, orchards and around risk locations.

The effective attractive range of the traps is about ~20 m (Ginzel, 2015). The traps should be checked every 7–14 days. Under constant air temperatures of 30°C, the lure should last for two months. Under field conditions, with lower mean air temperatures, the lure should be replaced after three months.

In northern Italy, the lifespan of the dispenser that contains the lure, is about 90 days (Faccoli, personal communication).

In the EU, Faccoli et al. (2016) describe the trapping activity carried out under north Italian environmental conditions. No information is available on the capacity of the lure to also attract European bark beetles; therefore, the trapped beetles should always be identified by expert entomologists.

Figure 9: A four-unit funnel trap in place at the top of a pole (left) and a close-up of the same trap (right) (Source: Steven J. Seybold, USDA Forest Service, courtesy of University of California Statewide IPM Program. Pictures from Seybold et al., 2013)

Conclusions for detection methods

The pest *Geosmithia morbida* and its vector *Pityophthorus juglandis* are not usually detectable through a visual examination of host plants, and external symptoms may become visible only after several years of infestation. Therefore, trapping is the recommended method for detecting the vector. Following insect trapping, a specific tree inspection should be carried out looking for external symptoms on the host plant (e.g. penetration and exit holes, cankers and wilting). In the US and Italy, trapping of *P. juglandis* has proven to be effective even at low population densities. Traps should be placed near host trees (when the mean air temperature exceeds 18°C) and at the risk locations.

2.2. Sampling

Suspicious beetles caught in the traps should be taken to the laboratory for species identification. The catches should be stored cool for transport or in 70% ethanol for long-term conservation (Seybold et al., 2013). Trees showing symptoms of TCD or trees close to traps where *Pityophthorus juglandis* was caught, should be sampled to confirm the presence of *Geosmithia morbida* and *P. juglandis* (see Section 2.3). Only trees suspected to be infested with *P. juglandis* should be sampled; Seybold et al. (2019) provide a comprehensive guide to sampling suspicious trees. The essential information is summarised here.

Heavily affected branches may be high in the crown of the tree. Branches with little round holes must be peeled carefully by removing thin bark layers with a knife without cutting in the cambium or the wood (Figure 10). Only branches with holes, beetles or cankers should be collected. Seybold et al. (2019) recommend cutting 2–4 different branches with a diameter of 5–10 cm into 15–30 cm long sections with healthy and damaged wood. Possibly exposed beetles or larvae can be stored in 70% ethanol. The branch samples should be placed in closable bags. The tools for sampling must be sterilised before additional sampling.

Figure 10: Peeling off thin layers of bark to detect cankers and beetle galleries (Source: Elizabeth Bush, Virginia Polytechnic Institute and State University, Bugwood.org)

Conclusions for sampling

Trapped beetles should be taken to the laboratory for species identification. Symptomatic trees and trees near the traps where *Pityophthorus juglandis* have been caught should be sampled to confirm an infestation with *Geosmithia morbida* and *P. juglandis*. Sampling of asymptomatic trees or branches will not help to detect the pathogen or its vector.

2.3. Identification

2.3.1. Laboratory testing

Geosmithia morbida

A manual for the isolation, morphological and molecular identification and culture maintenance of *G. morbida* is included in the TCD guidelines from the US Department of Agriculture (Seybold et al., 2019). Incubated on ¼ or ½ PDA++ (potato dextrose agar with streptomycin sulfate and chloramphenicol) at 25°C, colonies of the fungus exceed 20–40 mm in diameter after 3–5 days when hyphae or infected wood chips are plated (Figure 11). *Geosmithia morbida* can be confused with *Penicillium*. However, colonies of *G. morbida* are lobated and various shades of yellow, whereas *Penicillium* has green to grey colonies. Conidia are the best differentiating characteristic from *Penicillium*. *Geosmithia morbida* produces dry conidia on multi-branched verticillate, verrucose conidiophores with similar appearance like the ones of *Penicillium*. The conidia of *G. morbida* are formed in chains, shaped cylindrical to ellipsoid with a mean size of 2.7 µm x 6.5 µm (Seybold et al., 2019; Figure 12), distinguishable from the globose conidia of *Penicillium.* Additional pictures of the colony appearance and microscopy pictures of the fungus are shown in Kolařík et al. (2011).

Figure 11: Sporulating *Geosmithia morbida* in PDA agar (left: top view; right: bottom view) (Source: Ned Tisserat, Colorado State University, Bugwood.org)

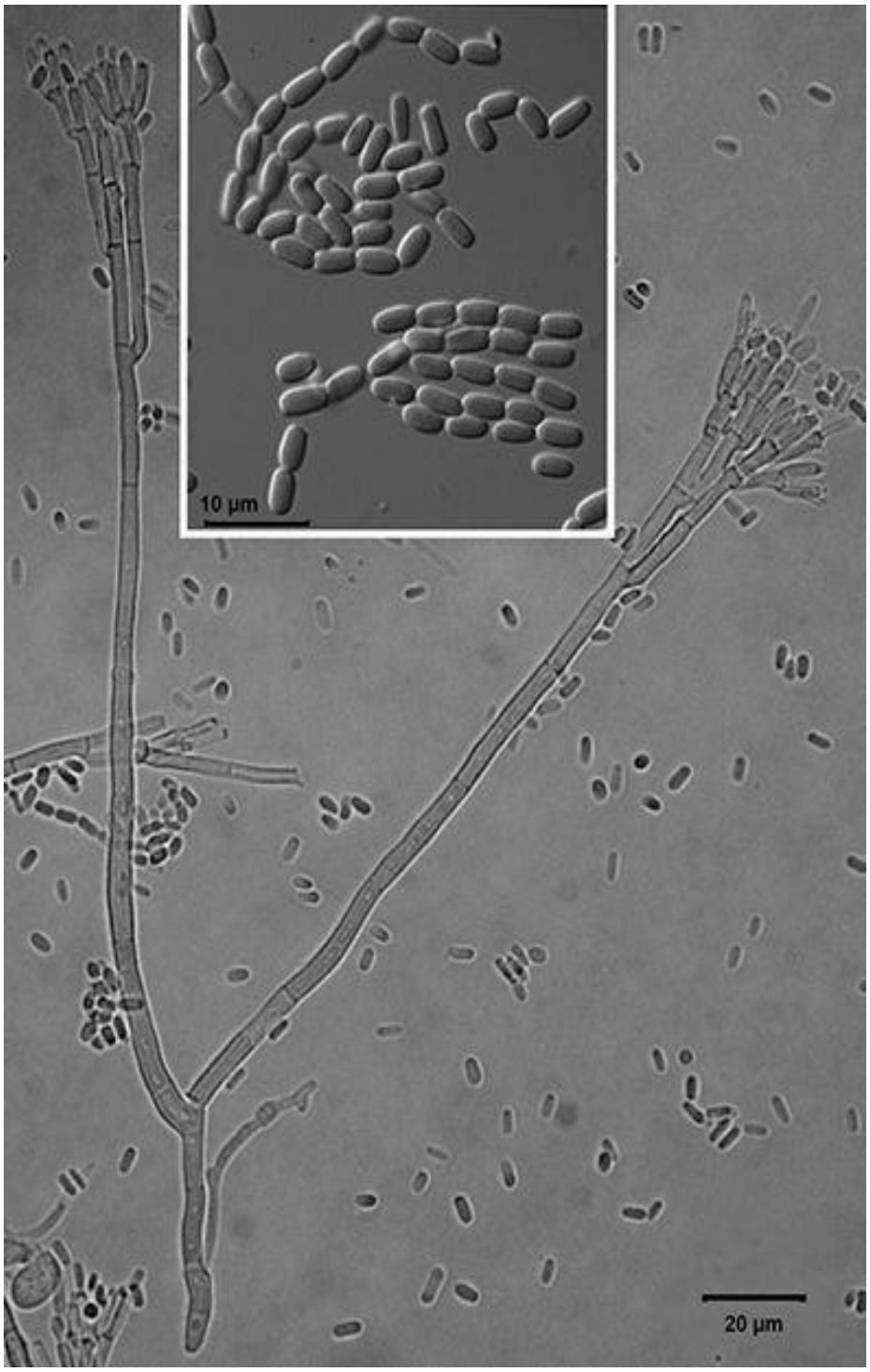

Figure 12: Conidiophores and conidia of *Geosmithia morbida* (Source: Miroslav Kolařík, Institute of Microbiology of the Czech Academy of Sciences)

Morphological identification of the fungus by colony characteristics and microscopy should be performed by expert mycologists. The molecular assay with the *G. morbida*-specific primers GmF3 and GmR13 resulted in 80–90% or better confidence of detecting the fungus on insects (Moore et al., 2019; Seybold et al., 2019). Molecular identification of *G. morbida* from colonies in culture has a very high confidence, as fungal DNA is very concentrated. As the formerly mentioned primers are highly specific to *G. morbida*, there is no need for further sequencing after gel electrophoresis (Seybold et al., 2019). A rapid molecular method to identify *G. morbida* with the species-specific GS 004 microsatellite locus is described by Oren et al. (2018).

Pityophthorus juglandis

Seybold et al. (2013) describe the species as follows:

> WTB is 1.5 to 2 millimetres long, has a relatively narrow body (about three times longer than wide), and has a reddish-brown to brown cuticle (outer 'skin'). The frons of the female WTB contains a round brush of golden setae (short hairs) that are no longer than half the distance between the eyes, whereas the male frons has very sparse setae, sometimes consisting of a narrow brush of short setae immediately above the mandibles. The anterior half of the pronotum is sloped upward from the frons, reaches an apex before the midpoint, and features four to six concentric arcs of asperities (ridges). These arcs of ridges may be discontinuous and overlapping, especially near the median. Small teeth line the anterior edge of the pronotum. The elytra (hardened forewings) have closely spaced punctures and sparse, short setae. The elytral apex is rounded, and the declivity (depression at the rear end) is very shallow and often shiny. The female declivity is smooth, whereas the male declivity features rows of minute granules on the first and third interstrial spaces.

Pictures of the female (Figure 13) and male (Figure 14) of *P. juglandis* are provided below.

The publication by Seybold et al. (2013) contains a comparison picture with other bark and ambrosia beetles that have been caught in WTB traps in North America (Seybold et al., 2013). Some of these species are native to or have been introduced into Europe, like *Cyclorhipidion bodoanum*, *Xyleborinus saxeseni*, *Scolytus rugulosus*, *Scolytus multistriatus, Phloeotribus liminaris, Xyleborus atratus, Xylosandrus crassiusculus* and *Xylosandrus germanus.*

The identification of bark beetles is difficult because of their small size and high diversity and should be done by expert entomologists.

There is no key for the larval stages of *P. juglandis*. The larvae are C-shaped, white and legless. As adults are present all year round, identification of the larval stages is not necessary.

In addition, according to Moricca et al. (2019), molecular identification of *P. juglandis* is possible by amplifying partial sequences of the mitochondrial cytochrome *c* oxidase I (*COI*) using the primer combination LCO1490 and HCO2198, as introduced by Folmer et al. (1994). Referenced sequence material is available at EPPO-Q-Bank[7].

Figure 13: Female of *Pityophthorus juglandis* (lateral view) (Source: Steven Valley, Oregon Department of Agriculture, Bugwood.org)

[7] https://www.eppo.int/RESOURCES/eppo_databases/eppo_q_bank

Figure 14: Male of *Pityophthorus juglandis* (lateral view) (Source: Steven Valley, Oregon Department of Agriculture, Bugwood.org)

Conclusion for pest identification

Morphological identification of *Geosmithia morbida* and its vector *Pityophthorus juglandis* should be performed by expert mycologists and entomologists, respectively. Molecular assays are available for both fungal and vector identification.

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 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 3 shows an example of these definitions.

Table 3: Example of definitions of the target population, epidemiological unit and inspection unit for *Geosmithia morbida* and its vector *Pityophthorus juglandis*

	Definition
Target population	All trees of the genera *Juglans* and *Pterocarya* in a Member State
Epidemiological unit	A single homogeneous area that contains at least one individual tree of the genera *Juglans* and *Pterocarya* (e.g. orchard)
Inspection unit	A single tree or trap

To design a plant pest survey on *Geosmithia morbida* and its vector *Pityophthorus juglandis* 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 *G. morbida* and its vector *P. juglandis*, 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 *G. morbida* and its vector *P. juglandis.* 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 *G. morbida* and its vector *P. juglandis*, 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. They should be single homogeneous areas that each contain at least one individual host plant. For *G. morbida* it is necessary to include two components in the survey design: the vector (detected through trapping) and surrounding host plants when the vector is present.

4/ Determine the inspection unit. For a walnut orchard, for example, the inspection unit is a single walnut tree.

5/ Determine the number of inspection units per epidemiological unit. For a walnut orchard, this is the average number of walnut trees 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 orchards that need to be surveyed are estimated for a Member State in order to state with 95% confidence that the prevalence of *G. morbida* and its vector *P. juglandis* 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 infected 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 the 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 *Phyllosticta citricarpa*, 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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