PEST SURVEY CARD

APPROVED: 27 November 2019

doi:10.2903/sp.efsa.2019.EN-1749

Pest survey card on *Anoplophora chinensis*

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Abstract

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

Keywords: plant pest, survey, risk-based surveillance, *Anoplophora chinensis*, *Anoplophora malasiaca*, black and white citrus longhorn, citrus long-horned beetle

Requestor: European Commission

Question number: EFSA-Q-2019-00266

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Acknowledgements: EFSA wishes to acknowledge the Netherlands Food and Consumer Product Safety Authority (NVWA) and the Julius Kuehn Institute (JKI) in Germany for the support provided to this scientific output in the context of grant GP/EFSA/ALPHA/2017/02. EFSA also wishes to thank Massimo Faccoli for reviewing this document as well as the working group members that contributed to its preparation. EFSA also wishes to thank especially Maria Chiara Rosace (trainee in the Animal and Plant Health Unit-Plant Health Team) for her support to the finalisation and publication of this survey card.

Suggested citation: EFSA (European Food Safety Authority), Hoppe B, Schrader G, Kinkar M and Vos S, 2019. Pest survey card on *Anoplophora chinensis*. EFSA supporting publication 2019:EN-1749. 22 pp. doi:10.2903/sp.efsa.2019.EN-1749

ISSN: 2397-8325

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Introduction

The information presented in this pest survey card was summarised from the European and Mediterranean Plant Protection Organization (EPPO) datasheet on *Anoplophora malasiaca* and *A. chinensis*, the EPPO Global Database and the Centre for Agriculture and Bioscience International (CABI) datasheet on *A. chinensis* (online) and other documents.

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

i. Pest-specific documents:
   a. The pest survey card on *Anoplophora chinensis*
ii. General documents:
   a. The general survey guidelines
   b. The RiBESS+ manual[1]
   c. The statistical tools RiBESS+ and SAMPELATOR[2].

1. The pest and its biology

1.1. Taxonomy

Scientific name: *Anoplophora chinensis* (Forster, 1771)

Synonym(s): *Anoplophora malasiaca* (Thomson, 1865), *Anoplophora malasiaca malasiaca* (Samuelson, 1965), *Anoplophora perroudi* (Pic, 1953), *Anoplophora sepulchralis* (Breuning, 1944), *Callophora afflicta* (Thomson, 1865), *Callophora luctuosa* (Thomson, 1865), *Calloplophora abbreviata* (Thomson, 1865), *Calloplophora malasiaca* (Thomson, 1865), *Calloplophora sepulcralis* (Thomson, 1865), *Cerambyx chinensis* (Forster, 1771), *Cerambyx farinosus* (Houttuyn, 1766), *Cerambyx pulchricornis* (Voet, 1778), *Cerambyx sinensis* (Gmelin, 1790), *Lamia punctator* (Fabricius, 1777), *Melanauster chinensis* (Forster), *Melanauster chinensis* (Matsumura, 1908), *Melanauster chinensis macularius* (Kojima, 1950), *Melanauster chinensis* var. *macularia* (Bates, 1873), *Melanauster chinensis* var. *macularis* (Matsushita, 1933), *Melanauster chinensis* var. Sekimacularius (Seki, 1946), *Melanauster macularius* (Kolbe, 1886), *Melanauster malasiacus* (Aurivillius, 1922), *Melanauster perroudi* (Pie, 1953).

Common names of the pest: black and white citrus longhorn, citrus longhorn beetle (CLB); citrus long-horned beetle; citrus root cerambycid; mulberry white-spotted longicorn; white-spotted longicorn beetle

Taxonomy: Class: Insecta, **Order:** Coleoptera, **Family:** Cerambycidae, **Subfamily:** Lamiinae **Genus:** *Anoplophora*, **Species:** *chinensis*

According to the revised taxonomy of the genus *Anoplophora* (Lingafelter and Hoebeke, 2002) *Anoplophora malasiaca* was placed in synonymy with *A. chinensis*. This decision was based on shared and similar characteristic features. For example, the variation in colour and size of elytral macula and the presence or absence of hair on the pronotum are not sufficient to justify a taxonomic separation into two distinct species. Earlier uncertainty resulted from taxonomists using colour variation to

[1] https://zenodo.org/record/2541541/preview/ribess-manual.pdf
[2] 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

distinguish specimens from different regions of China, Japan and South-East Asia (compare CABI (online) for details). In some papers *A. malasiaca* is even reported as a subspecies of *A. chinensis*. Since *A. chinensis* is a single taxonomic entity, the synonymous *A. malasiaca* or *A. chinensis malasiaca* have to be considered when screening literature predating 2002.

1.2. EU pest regulatory status

Anoplophora chinensis is regulated under Council Directive 2000/29/EC[3] in Annex I Part A/I, banning its introduction into the EU. Commission Implementing Decision 2012/138[4] lays down measures to prevent the introduction into and the spread of *A. chinensis* within the EU: (a) specific import requirements for plants for planting; (b) requirements for the movement of plants within the EU; and (c) the requirement to perform annual surveys for the presence of *A. chinensis*.

Implementing Decision 2012/138 also sets out emergency measures to be taken once the beetle has been detected and confirmed (via molecular identification). It requires the installation of a demarcated area, comprising: a) an infested zone where the presence of *A. chinensis* has been confirmed; and b) a buffer zone with a radius of at least 2 km beyond the boundary of the infested zone. The exact delimitation of the zones should be based on sound scientific principles, the biology of the pest, the level of infestation, and the particular distribution of the host plants in the area concerned.

Commission Implementing Decision 2018/1137[5] specifies commodities that are transported or supported/protected with wood packaging material from China or Belarus. These 'specified commodities' may be identified via Combined Nomenclature (CN) codes and need to be inspected at determined control frequencies.

1.3. Pest distribution

Anoplophora chinensis originates from eastern Asia; according to Haack et al. (2010), the beetle is widely spread in China, Korea and Japan and also present or occasionally reported in Indonesia, Malaysia, Myanmar, the Philippines, Taiwan and Vietnam. CLB has been introduced in the United States, but according to the EPPO global database, it was successfully eradicated in four outbreak areas. In Europe, CLB is present in Italy (2000), and considered transient and under eradication in Croatia (2007, 2015), France (2004, 2018), Switzerland (2016) and Turkey (2014) (Figure 1). Further outbreaks that were successfully eradicated occurred in Denmark (2011–2015), Germany (2008–2017), the Netherlands (2008–2010) and additional interceptions have been reported from Guernsey (2008, not part of the UK) and the UK (e.g. 2005). Hérard and Maspero (2019) report another interception of larval frass in Lithuania in 2008. In total they investigated 115 European reports of detection (59 interceptions and 56 infestations, of which 49 were found in Italy where four infestations are still active in the regions of Lazio (Rome), Tuscany (Prato and Pistoia) and Lombardy).

[3] Council Directive 2000/29/EC of 8 May 2000 on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community. OJ L 169, 10.7.2000, p. 1–112.

[4] Commission Implementing Decision of 1 March 2012 as regards emergency measures to prevent the introduction into and the spread within the Union of *Anoplophora chinensis* (Forster) OJ L 169, 10.7.2000, p. 1.

[5] Commission Implementing Decision (EU) 2018/1137 of 10 August 2018 on the supervision, plant health checks and measures to be taken on wood packaging material for the transport of commodities originating in certain third countries OJ L 169, 10.7.2000, p. 1.

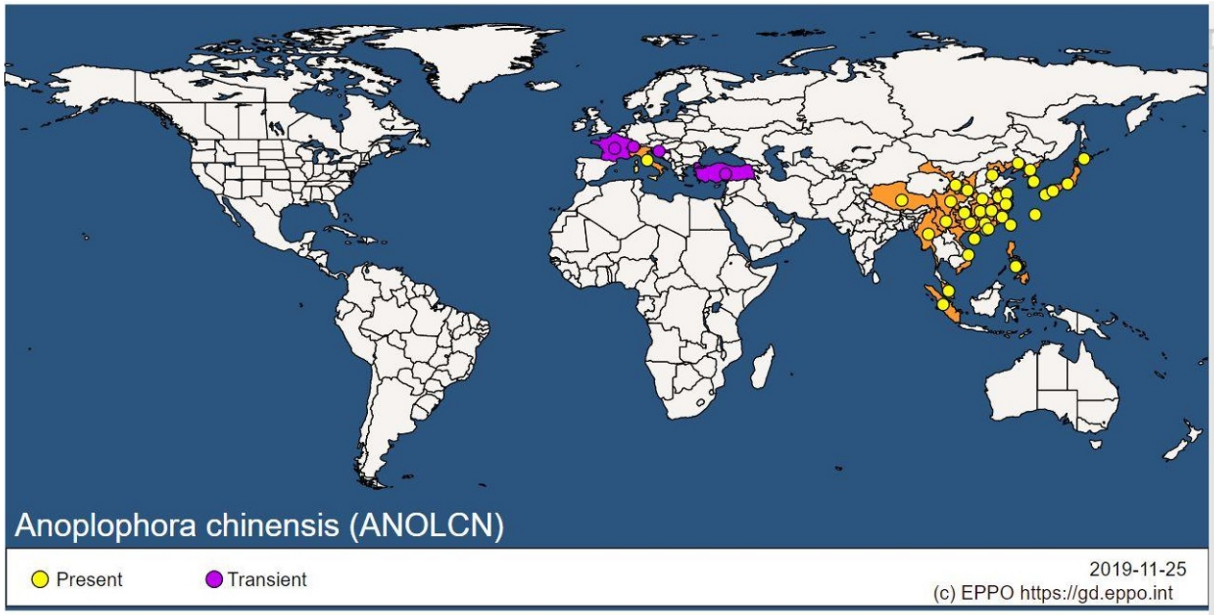

Figure 1: Global distribution of *Anoplophora chinensis* (Source: EPPO global database, https://gd.eppo.int)

1.4. Life cycle

The life cycle of CLB (Figure 2) is similar to that of the Asian longhorn beetle (ALB), *A. glabripennis*, except for the spots where oviposition and larval development take place on the infested trees. CLB usually lay eggs close to the base of the trunk or on roots emerging above ground, while oviposition rarely also occurs on higher parts of the host trees (van der Gaag et al., 2010) as typically occurs with ALB. CLB larvae develop downwards and many also migrate into the roots (Hérard et al., 2005).

The beetle has a 1–2-year life cycle both in its native area (Adachi, 1994) and in southern Europe (Hérard and Maspero, 2019). According to observed evidence and degree day calculations, Baker and Eyre (2006) and van der Gaag et al. (2008) stated that in temperate regions, CLB has a longer life cycle. Under the UK climatic conditions, at least a 3-year life cycle can be expected (Macleod A., FERA, UK, pers. comm., 2008 in van der Gaag et al., 2010).

Depending on the temperature, adults emerge between April–May and August (sometimes later). According to CABI (online) adults live between 30 (in China) and 70 days (in Japan). Adults then conduct maturation feeding for 10–15 days on twigs and the veins of leaves, before mate-finding and copulation occurs (Haack et al., 2010). However, maturation feeding and nutritional feeding of adults continue for the entire adult life, making the deposition distributed over time. Mating occurs from May to August on trunks and main branches at least 0.6 m above the ground (CABI, online). To oviposit single eggs, females cut a T-shaped slit in the bark close to the ground. First instar larvae hatch (depending on temperature, which has to be between 20°C and 30°C) about 10 days after oviposition. Young larvae begin feeding below the bark and later migrate deeply into sap- and hardwood. According to Hérard et al. (2006), since most of the larvae tunnel downwards, reaching the roots, 90% of the *A. chinensis* population can be found below ground level; this is a notable difference in larval behaviour in comparison with *A. glabripennis* which, instead, bores galleries and tunnels only in the upper part of the trunk and main branches. Larval feeding further exposes frass that deposits around the base of trees, which clearly indicates infestation. Larvae pupate in a chamber within the wood in late spring–summer, in many cases in the upper part of the feeding areas. Emergence holes are circular, with a mean diameter of 10–15 mm, usually slightly larger in females than males, larger than those of ALB, and located approximately 25 cm below the oviposition site (Haack et al., 2010).

Figure 2: Life cycle of *Anoplophora chinensis*: **1)** Summer to mid-autumn: adult beetles emerge from infested trees and start **2)** maturation feeding on leaves and twigs before copulation takes place. **3)** After mating in late summer until mid-autumn, females lay eggs (above 100 eggs possible) under the bark. **4)** First instar larvae develop 2–3 weeks after oviposition. Depending on climatic and feeding conditions, larvae develop over a 1–2-year period. **5)** During the winter of either the following year or the year after, the larvae pupate in chambers. (Sources: Björn Hoppe using pictures 1, 2 (top), 3 and 4 courtesy of Thomas Schröder (BMEL Federal Ministry, Bonn, Germany); picture 2 (bottom) mating couple and picture 5 pupa downloaded from EPPO global database courtesy of Matteo Maspero, Fondazione Minoprio, Como (IT))

1.5. Host range and main hosts

Anoplophora chinensis is a polyphagous pest and can attack plants of more than 20 families. Many of them are widespread in the EU, e.g. the following genera: *Acer* spp., *Platanus* spp., *Betula* spp., *Fagus* spp., *Corylus* spp., *Rosa* spp., *Malus* spp., *Pyrus* spp., *Prunus* spp., *Populus* spp., *Ulmus* spp. and *Salix* spp.. It is also regarded as a serious pest of fruit trees, especially *Citrus* spp. in China.

A. chinensis has a wider host range in Asia compared to *A. glabripennis*, which includes conifers in the genera *Cryptomeria* spp. and *Pinus*. As for Europe, *A. chinensis* has been found to complete its life cycle on species belonging to the genera *Acer* spp., *Aesculus* spp., *Alnus* spp., *Betula* spp., *Carpinus* spp., *Citrus* spp., *Cornus* spp., *Corylus* spp., *Cotoneaster* spp., *Crataegus* spp., *Fagus* spp., *Lagerstroemia* spp., *Liquidambar* spp., *Malus* spp., *Platanus* spp., *Populus* spp., *Prunus* spp., *Pyrus* spp., *Quercus* spp., *Rhododendron* spp., *Rosa* spp., *Salix* spp., *Sorbus* spp., and *Ulmus* spp. (Haack et al., 2010). In Europe, *Acer* spp. has been indicated as the most typically infested genus, followed by *Betula* spp.and *Corylus* spp.

According to Maspero et al. (2005), in Italy CLB primarily attacks species of *Acer* (48%), *Platanus* spp. (15%), *Betula* spp. (14%), *Carpinus* spp. (7%) and *Fagus* spp. (5%). Damage has also been found on species of *Aesculus* spp., *Corylus* spp., *Cotoneaster* spp., *Crataegus* spp., *Lagerstroemia* spp., *Malus* spp., *Populus* spp., *Prunus* spp., *Rosa* spp., *Quercus* spp. and *Ulmus* spp.

Sjöman et al. (2014) reviewed literature on host tree preferences for ALB and CLB and identified 108 suitable host species (73 genera) for *A. chinensis*.

Implementing Decision 2012/138 requires annual surveys to be performed on host plants. The target population is composed of all the host plants of the pest within the survey area. When conducting a detection survey, a preference should be given to the inspections of specified plants as laid down in Implementing Decision 2015/893.

Implementing Decision 2012/138 defines specified plants as plants with a minimum stem or root collar diameter of 1 cm or more comprising the following species and genera: *Acer* spp., *Aesculus hippocastanum*, *Alnus* spp., *Betula* spp., *Carpinus* spp., *Citrus* spp., *Cornus* spp., *Corylus* spp., *Cotoneaster* spp., *Crataegus* spp., *Fagus* spp., *Lagerstroemia* spp., *Malus* spp., *Platanus* spp., *Populus* spp., *Prunus laurocerasus*, *Pyrus* spp., *Rosa* spp., *Salix* spp. and *Ulmus* spp.

1.6. Climatic and environmental suitability

According to EFSA (2019), the climate is suitable for the establishment of CLB across the whole of the EU (except the north of Sweden and the north of the UK) (Figure 3).

As the beetle has a very broad range of host plants, their accessibility is not a limiting factor for its establishment and spread in the EU. According to CABI (online), CLB poses a serious risk, especially to *Citrus*-growing countries in the Mediterranean area. Infestations in Italy and France on a broad variety of tree species emphasise the combination of suitable environmental conditions and host tree availability. Nevertheless, the different habitats where the host plants grow might be a discriminating factor affecting CLB establishment and spread. The same plant species may grow in natural forests, in agricultural areas or can be cultivated in urban areas as ornamental trees. In the countries of introduction, CLB infestations are usually limited to urban trees that are isolated, growing in small groups or rows, in small rural stands or along forest edges, while the species has never been found in natural forests (Haack et al., 2010; Hérard and Maspero, 2019).

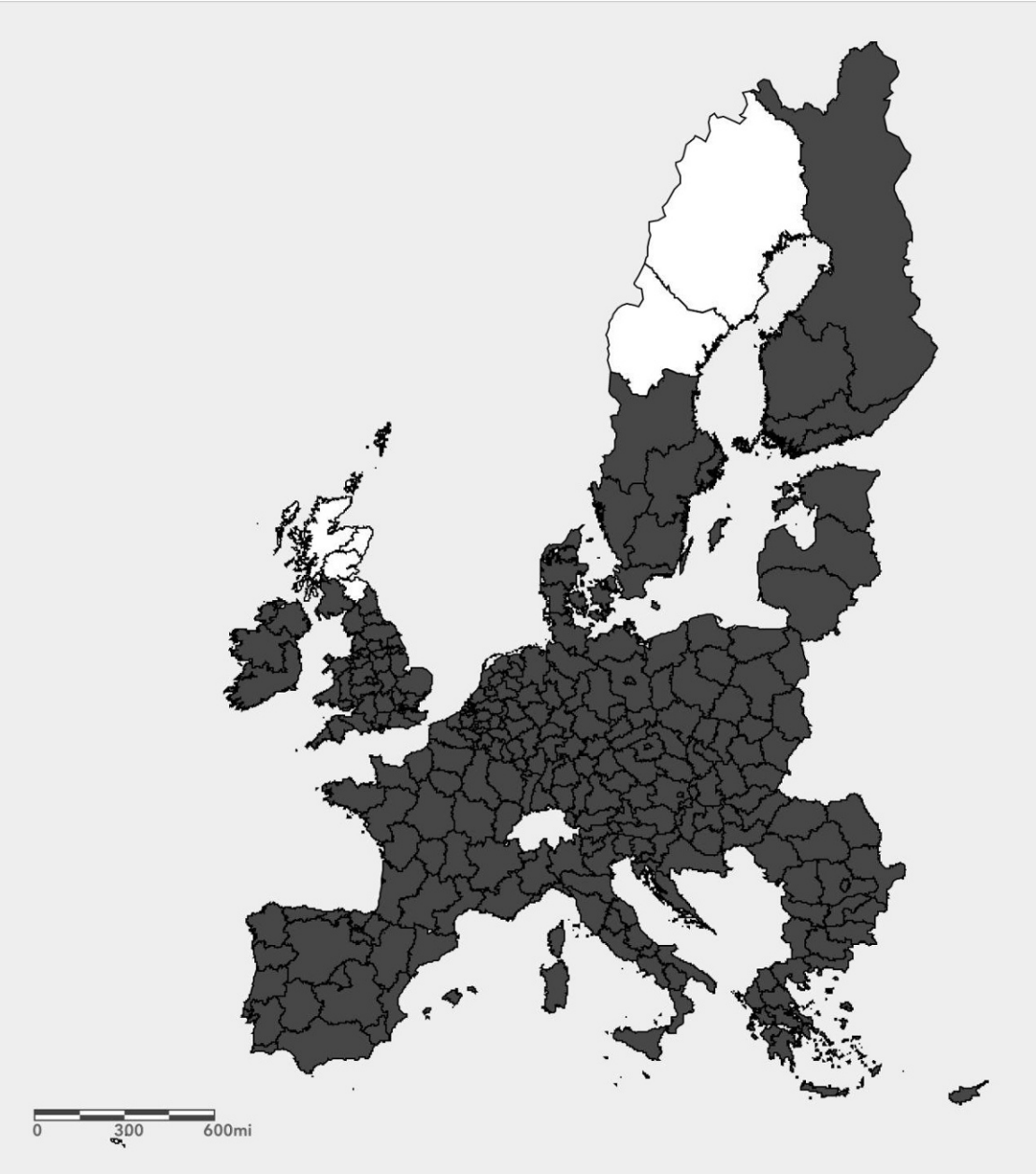

Figure 3: The potential distribution of the pest in the EU NUTS2

1.7. Spread capacity

Relevant literature on the beetle's flying behaviour is rather limited (van der Gaag et al., 2010). According to EPPO (2013) spread capacity is low (less than 50 m), as most adults remain in the vicinity of their tree of emergence. As mentioned above, since the potential host tree range is broad, it is likely that CLB does not need to fly long distances to find suitable host trees. Nevertheless, Adachi (1990) referred to their own unpublished data on marked adults, which were recaptured at distances of 2 km. In addition, males revealed a higher rate of tree-to-tree movement.

A geographic study carried out in Italy (Cavagna et al., 2013) shows that all new infestations of *A. chinensis* can be found within 500 m of the previously infested trees in urban areas and within 663 m in agricultural areas.

Following an Expert Knowledge Elicitation, EFSA (2019) estimated that the maximum distance of natural spread in one year is about 194 m (with a 95% uncertainty range of 42–904 m). The specific scenario considers a population with a 2-year cycle based on average EU conditions.

1.8. Risk factor identification

The identification of the risk factors and their relative risk estimation is essential for performing a risk-based survey. It needs to be tailored to the situation in each EU Member State. The proportion of the target population for each risk factor needs to be known or estimated by each country. This section presents examples of risk factors in one Member State, but others might be more relevant in other countries.

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 the surveillance are those that have more than one level of risk for the target population. The risk factors that will be considered for the surveys need to be characterised by their relative risk and the proportion of the overall plant population to which they apply. For the delimitation of the risk areas to be surveyed as a priority, it is necessary to first identify the risk activities that could contribute to the introduction or the spread of CLB. Then these activities should be connected to specific locations, also called 'risk locations'. In consideration of the spread capacity of the pest and the availability of host plants around these locations, risk areas can be defined.

According to EPPO (2013), surveys should be pathway-based and the main pathway was identified as plants for planting (including bonsai) with a stem or root collar diameter > 1 cm that are moved internationally. Four out of the five infestations reflected in van der Gaag et al. (2010) were found near a site with a history of plant imports (especially *Acer palmatum*) from eastern Asia, thus representing a risk area. Public and private green spaces, as well as parks and forest edges, located in the vicinity of places of trade in international plants, are another risk area.

Even though no interception of CLB in wood packaging material has been recorded in the EPPO regions (EPPO, 2013), given the fact that larvae develop at ground level and therefore do not end up in processed wood, this pathway may not be excluded.

Example: Import of host plants for planting (including bonsai)

Host plants for planting, including bonsai, are presumed to be the major pathway for the introduction of CLB. Therefore, locations where these plants for planting are stored, traded, or imported, need to be considered as risk locations (Table 1). Nurseries, but also any other commercial garden centres, should therefore be considered for surveillance and monitoring activities. This is also the case for areas (= risk areas) including public and private green spaces and parks, but also forest edges which are in the vicinity of these nurseries and garden centres. Citrus plantations near to places of import and trade are another risk area that should be considered for surveillance and monitoring, especially in regions where the cultivation of citrus trees is of economic relevance. Given that *A. chinensis* has a rather limited ability for natural spread, the size of the risk area corresponds to host tree availability.

Table 1: Example of a risk activity and the corresponding risk location and risk area relevant for surveillance of *Anoplophora chinensis* in EU Member States

Risk activity	Risk location	Risk area
Import, trade and storage of host plants for planting including bonsai	Locations where imported plants are stored, traded, or located (e.g. nurseries and commercial garden centres)	Areas surrounding nurseries and commercial garden centres: e.g. public and private green parks, but also forest edges in the vicinity of industrial areas

2. Detection and identification

2.1. Visual examination

Visual examination is the key element for the detection of *A. chinensis*, focusing both on the pest itself (including its various life stages) or symptoms on infested trees. It should be performed mainly at both trunk and crown level since CLB egg laying and adult emergence occur in the lower part of the trunk, while tree decline symptoms due to CLB infestation also occur in the canopy. It is possible to integrate the visual examination with trapping programmes (see section 2.1.3) but the latter need to be further developed.

2.1.1. Pest

Eggs: Eggs are elongate, subcylindrical, white and about 6 mm in size (Figure 4). They are laid under the bark. During development, eggs turn into a yellowish-brown colour.

The chorion is off-white, turning yellowish-brown closer to hatching (CABI, online).

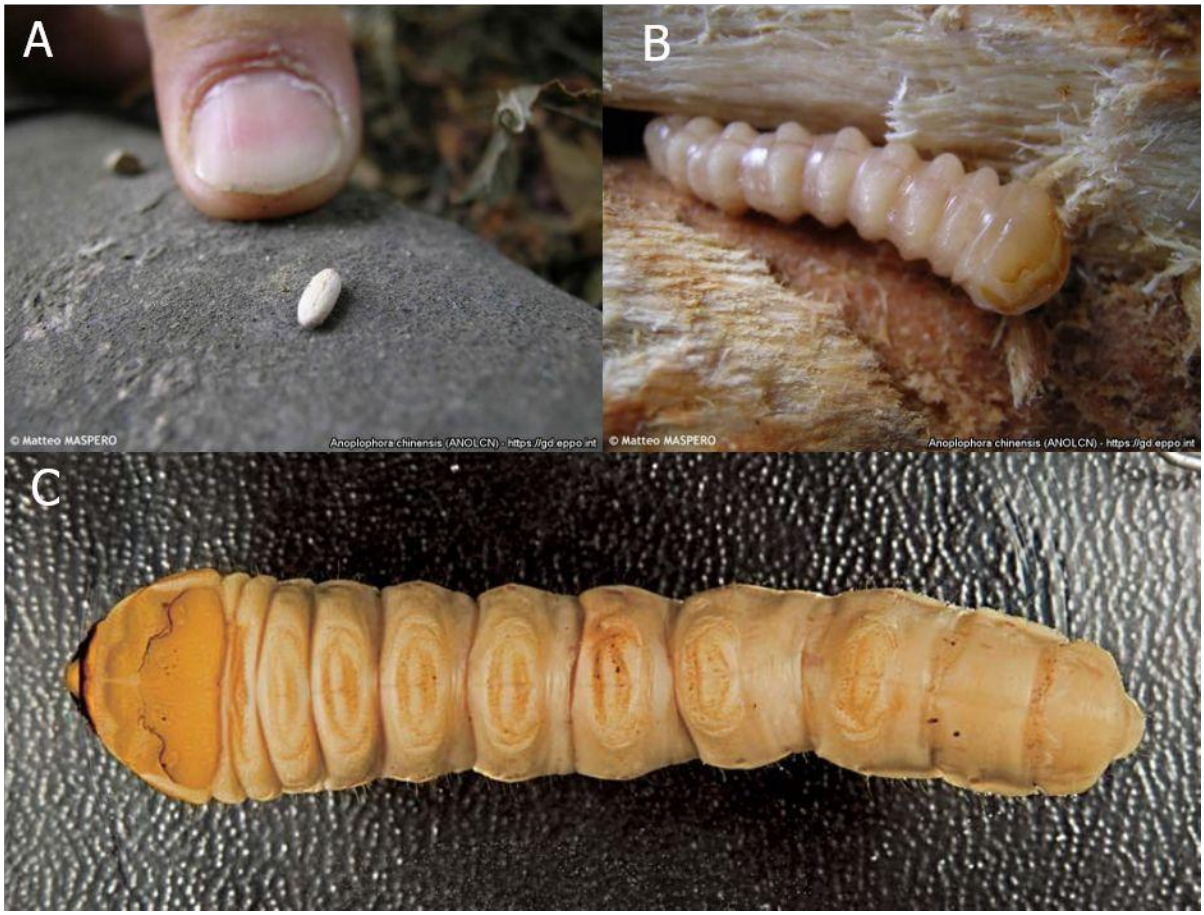

Figure 4: A) Egg of *Anoplophora chinensis*; B) feeding larva in wood; C) larva in dorsal position: divided into head, pronotum (with typical shield) thorax and several abdominal segments (Source: A) and B) from EPPO global database courtesy of Matteo Maspero; C) taken from Pennacchio et al., 2012)

Larvae: The general aspect of the larvae is typical for the subfamily Lamiinae (Figure 4): they have an elongated and cylindrical shape and are cream-coloured; the head is prognathous and usually retracted into the prothorax (Pennacchio et al., 2012). Mature CLB larvae specifically are up to 56 mm long and 10 mm wide at the prothorax. The larva tapers gradually behind the prothorax towards the

end of the abdomen. It is pale yellowish-white and the pronotum has a narrow orange transverse band near the anterior margin and a large, orange, raised area posteriorly.

Pupae: Pupation typically takes place in a pupal chamber at the end of the larval tunnel, in the sapwood below the bark. Pupae are of light-yellow colour and 24–35 mm in length; differences in size are recorded according to the sex, with males usually smaller than females. The shape is typical for cerambycids, e.g. in coiled antennae visible in ventral position.

Adults: Beetles are black and shiny and of typical cerambycid shape (Figure 5). Males (21 mm) and females (37 mm) vary in length. Antennae are 1.7–2 times longer than body length for males and 1.2 times the body length for females. The pronotum has a prominent pointed process on both sides and might display bluish-white hair spots on either side of the pronotum or may also be entirely black. The male has the elytra narrowed distally. The sides of the female elytra are parallel and rounded distally.

According to the *A. chinensis* datasheet published by CABI (online), *A. chinensis* is very similar to *A. davidis* and *A. macularia*. *Monochamus* species larvae are also extremely similar to those of *A. chinensis*. *Anoplophora glabripennis* is another similar species which has a similar geographic distribution and causes similar damage (Topakci et al., 2017). It can be distinguished from *A. chinensis* by the absences of tubercles on the elytra (Figure 5b).

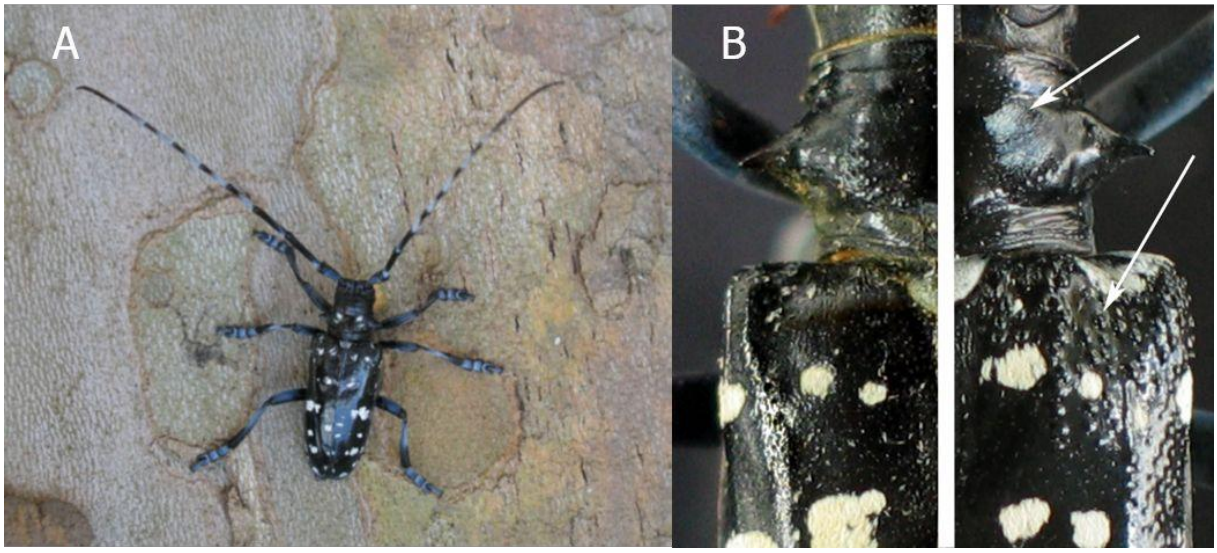

Figure 5: A) Adult of *Anoplophora chinensis* (male); B) Differentiation between ALB (left) and CLB (right): elytra of CLB are grained (white bottom arrow); pronotum with white hair spots, scutellum may appear in white (Source: A) and B) Thomas Schröder)

2.1.2. Symptoms

Severe damage caused by CLB is mainly due to the feeding activities of the larvae within the wood, which weakens and, in many cases, also kills the infested tree. Although the infested trees show a progressive canopy decline and desiccation of the main branches, larval feeding activity is mostly concentrated at the lower and basal area of the trunk.

Oviposition: Females cut T-shaped slits using their mandibles. Slits are visible depending on the texture of the bark, more likely on trees with smooth and clear bark (Figure 6). In addition, sap oozing out of freshly cut slits may be observed in the first weeks following oviposition (EPPO, 2016a).

Frass: Larvae typically produce frass, which is deposited initially below the bark and then inside the larval galleries bored within the wood. In some cases, the bark cracks and frass may be observed at the base of the trees (Figure 7). Experience from Italy reveals that more frass may be found on or close to plants with smaller rather than with larger diameters, which might be due to the limited space available for larval galleries in smaller plants (Hérard and Maspero, 2019). But the same authors

further report that plants can be infested without (evident) external signs or symptoms (as has been reported from Netherlands).

Exit holes: These are the result of emerging adults that have completed their life cycle within the wood. Exit holes are perfectly circular and have a mean diameter of about 10–15 mm. They can mainly be observed around the lower part of the trunk, on emerging roots or below ground level.

Maturation feeding: This causes damage to leaves, petioles and the bark of young twigs (Figure 6).

Further symptoms to be observed are wilting foliage and discolouration, and deformation of bark. Larval galleries may not be detected on young living trees where they might remain unnoticed (plants for planting including bonsai). Larval galleries are usually more visible (as an indication of infestation) in processed wood (e.g. wood packaging material).

Figure 6: Symptoms on *Acer* spp: A) and B) Maturation feeding of adult beetles on branches and bark; C) and D) Oviposition slits on the basal area of trees (Source: A) to D) Thomas Schröder)

Figure 7: Symptoms: A) Exit hole on stem base; B) Larval frass which is deposited on the stem base; Larval galleries: C) transverse section and D) longitudinal section (Source: A) to D) Thomas Schröder)

2.1.3. Traps

According to the available literature there is no commercial trapping system available for CLB. In fact, Hansen et al. (2015) identified the same male-produced volatile pheromones 4-(n-heptyloxy)butan-1-ol and 4-(n-heptyloxy) butanal as in ALB, but no practical applications were reported except by Hérard and Maspero (2019), who reported monitoring with traps in Italy without further specification. Nevertheless, CLB monitoring is carried out in northern Italy using multi-funnel traps baited with ALB pheromones, although the trapping performance is very low with, on average, about only one adult trapped per trap per year (Dr M Faccoli, Associate professor at University of Padova, personal communication on 15 November 2019).

2.2. Other methods for detection of *Anoplophora chinensis*

In the event of CLB infestations, the European regulations require monitoring of all specified plants (host plants intended for planting) not intended for chipping. In some European countries (i.e. Italy), visual surveillance is carried out twice a year: in summer to look for oviposition pits and maturation feeding, and in winter to look for the exit holes of adults at the end of the emerging period. Visual inspection is not a completely effective approach and could be supplemented by the use of other detection methods to improve the level of CLB detection in the defined areas. As part of monitoring and surveillance of CLB in infested areas, visual inspection may be complemented by well-trained scent detection dogs (sniffer dogs) adapting the current methodology used for the ALB (Hoyer-Tomiczek et al., 2016). In this respect, the use of sniffer dogs produced positive outcomes in Austria, France, Italy and Germany. In Austria, Hoyer-Tomiczek et al. (2016) conducted experimental trials to quantify the sensitivity and specificity of trained dogs. Their experiments revealed an overall sensitivity of 85–93% (correct positives of all positives) and specificity of 79–94% (correct negatives of all negatives) concluding that dog detection is a feasible complementary method for monitoring and surveying *A. glabripennis*. Hérard and Maspero (2019) reported the use of sniffer dogs on limited time and special scales in Italy. Nevertheless, it has to be considered that training material (e.g. frass samples, living and dead larval material) needs to be continuously provided to maintain sniffer dogs at a high performance level. Like visual surveillance, the proposed inspection frequency of sniffer dogs is two passages per year (early spring and autumn). Specific centres for dog training are now being established in Austria and Switzerland.

2.3. Laboratory testing and pest identification

Pennacchio et al. (2012) provide a useful key for the morphological identification of *A. chinensis* and its separation from sibling species including *A. glabripennis*. In addition, EPPO standard PM 7/129 (EPPO, 2016b) provides protocols for the molecular diagnostics of Arthropods, which include *A. chinensis* and *A. glabripennis*. In order to confirm CLB infestations on plants, it is necessary to collect adults or larvae on which to carry out morphological or molecular analyses. However, obtaining such specimens from infested plants can be a demanding and difficult task. Therefore, a non-invasive molecular diagnostic tool may be useful to confirm the ALB infestation on the host plants even in the absence of insect samples. In this respect, Strangi et al. (2013) propose a protocol of molecular analyses based on polymerase chain reaction (PCR) amplification of DNA samples extracted from recent CLB frass collected from potentially infested host trees. Within the project ANOPLO-diag, funded by the German Federal Ministry of Food and Agriculture, researchers from the Julius Kuehn Institute in Braunschweig are developing, in cooperation with scientists from the Phytopathologic laboratory of the Lombardy Region (Vertemate con Minoprio, Italy), a specific and sensitive molecular diagnostic tool to detect the beetle from frass and wood chips.

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 *Anoplophora chinensis*

	Definition
Target population	All host trees in a Member State
Epidemiological unit	A single homogeneous area that contains at least one individual of a host species
Inspection unit	Individual tree or branch

To design a survey on *Anoplophora chinensis*, the following steps will generally be necessary:

1/ Determine the type of survey based on its objectives. For *A. chinensis*, the type of survey will depend on the pest status (according to International Standards for Phytosanitary Measures (ISPM) No. 8) in the area of interest. The objective could be to substantiate pest freedom, to delimit an outbreak area following an infection or to determine the pest prevalence. The next steps deal with the example of substantiating pest freedom.

2/ Define the target population and the epidemiological unit. When determining the target population for surveillance of *A. chinensis*, the host plants that are relevant for the survey area have to be selected. For example, the target population could be all host trees in a Member State. The epidemiological unit would then be a single homogeneous area that contains at least one individual of a host species. Note that it is recommended that the survey parameters are harmonised among the different pests affecting the same host plants in order to optimise field inspections, which are generally organised per crop visit and not by pest.

3/ Determine the size of the target population.

4/ Determine the inspection unit. In the case of a park, for example, the inspection unit is a single host tree.

5/ Determine the number of inspection units per epidemiological unit. In the case of a park, this is the average number of host trees per epidemiological unit.

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

7/ Define the risk factors. A risk factor affects the probability of a pest to 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 sample size. RiBESS+ can be used to calculate how many epidemiological units need to be surveyed in order to achieve a predefined confidence level (e.g. 95%) and a predefined

prevalence level (e.g. 1%), while also including the method sensitivity from Step 6 and the risk factors identified in Step 7. This will, for example, result in the number of hectares that need to be surveyed in a Member State in order to state with 95% confidence that the prevalence of *A. chinensis* in the host plants will be at 1% or lower.

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

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

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

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

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

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Glossary

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, 2019).
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	Sensitivity of the survey. Is a measure of reliability of the survey procedure (Montgomery and Runger, 2010).
Design prevalence	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, 2019).
Delimiting survey	Survey conducted to establish the boundaries of an area considered to be infested by or free from a pest (ISPM 5: FAO, 2019).
Diagnostic protocols	Procedures and methods for the detection and identification of regulated pests that are relevant to international trade (ISPM 27: FAO, 2016).
Epidemiological unit	A homogeneous area where the interactions between the pest, the host plants and the abiotic and biotic factors and conditions would result in the same epidemiology should the pest be present. The epidemiological units are subdivisions of the target population and reflect the structure of the target population in a geographical area. They are the units of interest, on which statistics are applied (e.g. a tree, orchard, field, glasshouse, or nursery) (EFSA, 2018).
Expected prevalence	In prevalence estimation approaches, it is the proportion of epidemiological units expected to be infected or infested.
Identification	Information and guidance on methods that either used alone or in combination lead to the identification of the pest (ISPM 27: FAO, 2016).
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, 2019).
Inspection unit	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, 2019).

Method sensitivity	The conditional probability of testing positive given that the individual is diseased (Dohoo et al., 2010). The method diagnostic sensitivity (DSe) is the probability that a truly positive epidemiological unit will give a positive result and is related to the analytical sensitivity. It corresponds to the probability that a truly positive epidemiological unit that is inspected will be detected and confirmed as positive.
Pest diagnosis	The process of detection and identification of a pest (ISPM 5: FAO, 2019).
Pest freedom	An area in which a specific pest is absent as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained (ISPM 5: FAO, 2019).
Population size	The estimation of the number of 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 (Cameron et al., 2014).
RiBESS+	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, 2019).
Risk factor	A factor that may be involved in causing the disease (Cameron et al., 2014). It is defined as a biotic or abiotic factor that increases the probability of infestation of the epidemiological unit by the pest. The risk factors relevant for the surveillance should have more than one level of risk for the target population. For each level, the relative risk needs to be estimated as the relative probability of infestation compared to a baseline with a level 1. Consideration of risk factors in the survey design allows the survey efforts to be enforced in those areas where the highest probabilities exist to find the pest should the pest be present.
Risk-based survey	A survey design that considers the risk factors and enforces the survey efforts in the corresponding proportion of the target population.
Sample size	The number of sites that need to be surveyed in order to detect a specified proportion of pest infestation with a specific level of confidence, at the design prevalence (McMaugh, 2005).
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, 2019).
Target population	The set of individual plants or commodities or vectors in which the pest under scrutiny can be detected directly (e.g. looking for the pest) or indirectly (e.g. looking for symptoms suggesting the presence of the pest) in a given habitat or area of interest. The different components pertaining to the target population that need to be specified are: • Definition of the target population – the target population has to be clearly identified • Target population size and geographic boundary. (EFSA, 2018)

Test	Official examinations, other than visual, to determine whether pests are present or to identify pests (ISPM 5: FAO, 2019).
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 (DSp) is the probability that a truly negative epidemiological unit will test negative and is related to the analytical specificity. In freedom from disease it is assumed to be 100%.
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, 2019).

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