# **PEST SURVEY CARD**



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# Pest survey card on Anoplophora glabripennis

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# Abstract

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

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**Keywords:** plant pest, survey, risk-based surveillance, *Anoplophora glabripennis*, Asian longhorn beetle, Asian long-horned beetle

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## Introduction

The information presented in this pest survey card was summarised from the European and Mediterranean Plant Protection Organization (EPPO) National Regulatory Control System for *Anoplophora glabripennis* (EPPO, 2013), the EPPO Global Database and the Centre for Agriculture and Bioscience International (CABI) datasheet on *A. glabripennis* (online) and other documents.

The objective of this pest survey card is to provide the relevant biological information needed to prepare surveys for *A. glabripennis* 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 (IPPC) 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 glabripennis*
- ii. General documents:
  - a. The general survey guidelines
  - b. The RiBESS+ manual<sup>1</sup>
  - c. The statistical tools RiBESS+ and SAMPELATOR<sup>2</sup>.

## 1. The pest and its biology

#### **1.1.** Taxonomy

Scientific name: Anoplophora glabripennis (Motschulsky, 1853)

**Synonym(s):** Cerosterna glabripennis Motschulsky, 1853; Cerosterna laevigator (Thomson, 1857); Melanauster nobilis (Ganglbauer, 1890); Melanauster luteonotatus (Pic, 1925); Melanauster angustatus (Pic, 1925); Melanauster nankineus (Pic, 1926); Melanauster laglaisei (Pic, 1953).

**Common names of the pest:** Asian longhorn beetle (ALB), Asian long-horned beetle, Starry sky beetle and Sky Ox beetle (the two latter used for various species within the Genus *Anoplophora* in Asia)

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

According to an intense revision, the genus *Anoplophora* currently comprises 36 species (Lingafelter and Hoebeke, 2002). *Anoplophora glabripennis* is currently considered to be a single taxonomic entity, although older classifications (Wu and Jiang 1998, only accessible in Chinese), as referred to in the EPPO datasheet, include *A. glabripennis* as part of *glabripennis complex*, together *A. freyi*, *A. flavomaculata* and *A. coeruleoantennatus*.

<sup>&</sup>lt;sup>1</sup> <u>https://zenodo.org/record/2541541/preview/ribess-manual.pdf</u>

<sup>&</sup>lt;sup>2</sup> <u>https://websso-efsa.openanalytics.eu/auth/realms/efsa/protocol/openid-connect/auth?response\_type=code&client\_id=shinyefsa&redirect\_uri=https%3A%2F%2Fshiny-efsa.openanalytics.eu%2Fsso%2Flogin&state=d6f7f997-d09f-4bb0-afce-237f192a72d5&login=true&scope=openid</u>

# **1.2. EU pest regulatory status**

The Asian longhorn beetle is regulated under Council Directive 2000/29/EC<sup>3</sup> in Annex I Part A/I, banning its introduction into the EU. Commission Implementing Decision (EU) 2015/893<sup>4</sup> lays down measures to prevent the introduction into and the spread of *A. glabripennis* within the EU: (a) specific import requirements for plants for planting and wood; (b) requirements for the movement of plants, wood and wood packaging material within the EU; and (c) the requirement to perform annual surveys for the presence of *A. glabripennis*.

Implementing Decision 2015/893 also sets out emergency measures to be taken once the beetle has been detected and confirmed (via molecular identification) (Figure 13). It requires the installation of a demarcated area, comprising: a) an infested zone where the presence of *A. glabripennis* 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 (EU) 2018/1137<sup>5</sup> 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 glabripennis* is endemic to China, where it is present in most of the provinces (absent only in Qinghai, Xinjiang and Xizang (CABI, online), and to the Korean peninsula (Lingafelter and Hoebeke, 2002). In addition, specimens have been recorded in Japan, but they are not considered to be established in the area (Lingafalter and Hoebeke, 2002). The beetle was officially detected in the USA in 1996 and since then it invaded several federal states in the east and in Canada. Further, ALB has been detected in Lebanon, where three specimens were found in 2015 and 2016 (Moussa and Cocquempot, 2017). A finding of many specimens in Turkey in 2014 in the European part of Istanbul (Ayberk et al., 2014) was later recalled due to morphological misidentification (Arslangündoğdu and Hizal, 2019).

According to the EPPO Global Database, ALB has been detected in 35 European locations in nine different countries. It is present (restricted distribution or few occurrences) in Austria, Finland, France, Germany, Italy, Montenegro and Switzerland (Figure 1). The occurrences in the Netherlands and the UK have been successfully eradicated since its introduction in 2010 and 2012, respectively.

<sup>&</sup>lt;sup>3</sup> 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.

<sup>&</sup>lt;sup>4</sup> Commission Implementing Decision (EU) 2015/893 of 9 June 2015 as regards measures to prevent the introduction into and the spread within the Union of *Anoplophora glabripennis* (Motschulsky) OJ L 169, 10.7.2000, p. 1.

<sup>&</sup>lt;sup>5</sup> 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 glabripennis* (Source: EPPO Global Database, <u>https://gd.eppo.int/</u>)

## 1.4. Life cycle

Anoplophora glabripennis has a 1–3-year life cycle (Figure 2), depending on climatic and feeding conditions (Hua et al., 1992). For example, in Taiwan, ALB has been observed to develop within one year, while in the northern Chinese province Neimenggu, full development requires two years (EPPO, 2004). This may also result in overlapping generations where climate and feeding conditions are favourable for both lengths of cycle. Similar behaviour was observed in Europe, where full development typically requires approximately one year in warmer Italy and two to three years in infested areas located north of the Alps. Under warm climatic conditions, about two to three weeks pass from oviposition to first instar larvae. Sufficient feeding before overwintering then enables larvae to pupate in the following year. Young larvae start feeding in the phloem tissue and create galleries in the cambium. As they mature, older larvae then migrate into the sapwood first and then into the heartwood, creating upward tunnels. The maximum diameter of the tunnel is produced by mature larvae before pupation and corresponds to the width of the emerging holes (10–15 mm in diameter). Pupation takes place in a pupal chamber bored by the larva directly under the bark. When reaching the adult stage, individuals stay in an immobile stage in the pupal chamber for 7 to 10 days, then adults emerge (EPPO, 2013).

Adults emerge between May and October and have a mean lifespan of approximately one month (Li and Wu, 1993; Faccoli et al., 2015), although adult survival for longer than 70 days has also been recorded (Faccoli et al., 2015), with no significant differences between males and females. Eggs start to be laid a week after mating, but egg laying continues for the whole life of the female. The long adult survival (more than one month) associated with the long emerging period for adults (May–October) and the long developing time (more than one year) determine the overlapping of generations and the presence of different developing instars (eggs, young larvae, old larvae) even in the same tree. Moreover, while larvae hatched from eggs laid in spring will be able to produce new adults in the summer of the following year (overwintering once), larvae hatched from eggs laid at the end of summer will not be fully developed within the following year and they will need a second overwintering before producing new adults in the spring of the third year (Dr M Faccoli, Associate professor at University of Padova, personal communication on 13 November 2019).

Faccoli et al. (2015) stated that 90% of the beetles had emerged around 20 July in Italy in three consecutive years (2010–2012). Egg deposition, which occurs mainly in the central and upper part of the trunk and on the main branches, begins after adults terminate the 10–15-day maturation feeding on twigs, petioles and leaf veins (Haack et al., 2010), and continues during the nutritional feeding of

mature females. The female creates small oviposition slits, usually on the eastern side of the trunk or the branches of the host tree. On average, around 30 eggs are laid (Wong and Mong, 1986) separately under the bark, although fecundity of 60 eggs per female is known from northern Italy (Faccoli et al., 2016). Larvae hatch from the eggs about two weeks later.



Figure 2: Life cycle of *Anoplophora glabripennis*: 1) Summer to mid-autumn: adult beetles emerge from infested trees and start 2) feeding on tree leaves and twigs for egg maturation and for adult nutrition. 3) After mating in late summer until mid-autumn, females lay eggs (20–50) under the bark. 4) First instar larvae develop 2–3 weeks after oviposition. Depending on climatic and feeding conditions, larvae develop over a 1–3-year period while creating upward tunnels in the heartwood. 5) During the winter of either the following year or the year after, the larvae pupate in chambers. (Sources: Björn Hoppe using pictures courtesy of Hannes Lemme (LWF Freising, Germany) and Thomas Schröder (BMEL Federal Ministry, Bonn, Germany))

A visual survey would be most successful either when the activity of ALB is high or when there are no leaves on the trees (from the middle or end of October to March), so that symptoms can be detected more easily.

In spring–summer (May–September) it is possible to look for nutritional feeding and oviposition sites; symptoms directly produced by adult activities. While in wintertime (October–March) it is easier to search for emerging holes. The canopy is no longer present and it is therefore easier to search for the holes occurring in the upper part of the trees where the eggs have been laid.

#### **1.5.** Host range and main hosts

*Anoplophora glabripennis* is a polyphagous pest and is known to attack a wide range of broad-leafed tree species. It caused severe damage in *Populus* spp. cultivations that were planted during afforestation programmes in northern China in the 1970s and 1980s. These particularly included poplars from the section *Aigeiros* and hybrids with parental plants belonging to this section (e.g. *Populus nigra* L. var. *italica* and *Populus nigra* var. *thevestina*) (Hu et al., 2009), where ALB impressively displayed its damaging potential. However, field trials demonstrated that *Acer* appeared to be the most attractive genus (Gao et al., 1997). Similar host preferences for *Acer* spp. are reported from North America (e.g. Haack et al., 1997) and Europe Tomiczek and Hoyer-Tomiczek, 2007; Faccoli and Favaro, 2016).

The EPPO Global Database (online) lists *Acer negundo*, *A. platanoides*, *A. pseudoplatanus*, *A. saccharinum*, *A. saccharum*, *A. truncatum*, *Aesculus hippocastanum*, *Betula* spp., *Populus* spp. and

*Salix* spp. as major hosts. The entire list comprises 27 species or genera. 'Woody plants', 'deciduous trees', and 'unclassified' lists are not further considered in this pest survey card (see Table 1). In their literature review, van der Gaag and Loomans (2014) identified 34 plant taxa (at species or genus level), grouped into four categories of level of observation which formed the basis for the list of 'host plants' (29) (ALB has been observed) and 'specified plants' (15) (complete life cycle development) of Implementing Decision (EU) 2015/893. Sjöman et al. (2014) at the same time identified host trees belonging to 34 genera (with complete life cycle development) based on a literature survey comprising 35 reviewed papers.

Table 1: Summary of listed host plants as mentioned in: i) the EPPO Global Database, ii) van der Gaag and Loomans (2014) (I to IV categories listed according to life cycle completion in Table 1), iii) Sjöman et al. (2014) (with 0=resistant, 1=host, 2=good host, 3=very good host; according to Appendix 1), and iv) Implementing Decision (EU) 2015/893 (S=specified plants and H=host plants)

	EPPO Global Database	van der Gaag and Loomans (2014)	Sjöman et al. (2014)	Implementing Decision (EU) 2015/893
Acer spp.*	Major	I	3	H+S
Aesculus spp.*	Major	I	3	H+S
<i>Betula</i> spp.	Major	I	3	H+S
<i>Populus</i> spp.*	Major	I	3	H+S
Salix spp.*	Major	I	3	H+S
Ulmus spp.*	Minor	I	3	H+S
Alnus spp.	Minor	III	2	H+S
Carpinus spp.	NA	III	2	H+S
Cercidiphyllum spp.	NA	I	2	H+S
Corylus spp.*	Minor	I	0	H+S
Fagus spp.*	Minor	I	2	H+S
Fraxinus spp.*	Minor	I	2	H+S
Koelreuteria spp.*	Minor	I	3	H+S
Platanus spp.*	Minor	I	2	H+S
Tilia spp.*	NA	III	1	H+S
Albizia spp.*	Minor	I	3	Н
Buddleja spp.	NA	NA	NA	Н
<i>Celtis</i> spp.	NA	IV	2	Н
Elaeagnus spp.*	Minor	I	3	H
Hibiscus spp.*	NA	III	2	H
Malus spp.	Minor	I	1	H
Melia spp.	Unclassified	IV	2	H
Morus spp.*	Minor	III	2	Н
Prunus spp.*	Minor	III	2	Н
<i>Pyrus</i> spp.*	Minor	I, III, IV	2	Н
Quercus rubra spp.	NA	III	2	Н
Robinia spp.	NA	III	2	Н
Sophora spp.	NA	IV	1	Н
<i>Sorbus</i> spp.	Incidental	I	2	Н
Aleurites montana	Unclassified	NA	NA	NA
Broussonetia papyrifera	NA	NA	2	NA
Cajanus cajan	Unclassified	NA	NA	NA
Casuarina spp.	Unclassified	NA	NA	NA
<i>Citrus</i> spp.	Unclassified	NA	NA	NA
Gleditsia spp.	NA	NA	1	NA

Hedysarum spp	NA	IV	1	NA
Hippophae spp.	NA	IV	1	NA
Liquidambar spp.	NA	IV	0	NA
Liriodendron tulipifera	NA	IV	1	NA
Rosa spp.	NA	IV	1	NA
Toona sinensis spp.	NA	IV	0	NA
Vitis vinifera	NA	NA	1	NA

\* refers to those genera reported at species level in the original sources (e.g. the EPPO Global Database lists eight *Acer* species). Those species have been grouped at genus level in order to homogenise the information reported in the current table. Where hosts were listed as single entities in the original sources, the entry remained at species level. The colour gradient indicates the preference of host plants to be included in the survey. **NA:** Not Applicable

As ALB in many cases attacks various species within the same genus, it seems reasonable to focus on the genus level when performing the survey.

Concluding from very detailed reports of European infestations sites (e.g. Tomiczek and Hoyer-Tomiczek, 2007; Faccoli and Favaro, 2016), *Acer* spp., *Aesculus* spp., *Betula* spp., *Populus* spp., *Salix* spp., and *Ulmus* spp. represent the significant majority form all infested tree genera in Europe.

Implementing Decision 2015/893 requires annual surveys to be performed on all host plants listed in the legislation. Of course, the list of plants included in the survey depends on their availability in the survey area. 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 and in particular for the genera mentioned in the paragraph above.

#### **1.6.** Climatic and environmental suitability

As the beetle has a very broad range of host plants, their availability is not a limiting factor for its establishment and spread in the EU. Nevertheless, the different habitats where the host plants grow might be a discriminating factor affecting ALB 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.

As reviewed by Faccoli et al. (2016) *Anoplophora glabripennis* infestations are often confined to urban isolated trees in the countries where the pest has been introduced. Also, in countries where the species is native from, e.g. South Korea, the ALB is distributed along riparian habitats, not in forests (Williams et al., 2004). Faccoli et al. (2016) also report that although trees growing in forests have a lower nitrogen concentration, considering the number of laid-eggs and the survival of the larvae, ALB tree colonisation and breeding performance is not influenced by the tree characteristics *per se.* The authors suggest that the limited biotic factors occurring in the forest's habitats may be the reason of the absence of *A. glabripennis* from these areas. For instance, generalist predators that may negatively affect the presence of the ALB, are more easily found in natural ecosystems rather than urban areas (Pan, 2005; Li et al., 2007; Huang et al., 2008).

Concerning climatic suitability, ALB is widely distributed in arid, cool and warm temperate as well as tropical zones in China. The EU Member States have arid, cool and warm temperate regions (MacLeod et al., 2002). Thus, host plants are present and widely spread in European areas that are climatically suitable for *A. glabripennis*, except in the most northern EU Member States (Figure 3).





## **1.7.** Spread capacity

ALB has not been observed to fly long distances. Though flight mill experiments revealed distances of 14 km covered by adult beetles (Javal, 2017), under natural conditions and assuming host tree availability, a maximum annual active spread rate of 300 m is realistic (Smith et al., 2004; Favaro et al., 2015). Although some individuals can move farther than 2,000 m, Favaro et al. (2015) report that about 80% of the annual ALB dispersal ranges between 0 and 300 m from the closest infested trees. As the beetles infest healthy trees, they are also capable of completing their maturation feeding on the same tree, without the need to fly far.

Following an Expert Knowledge Elicitation, EFSA (2019) estimated that the maximum distance of natural spread of *A. glabripennis* in one year is about 150 m (with a 95% uncertainty range of 28–

860 m). The specific scenario considers a population with a two-year cycle based on average EU conditions.

Although beetles are capable flyers, they might not tend to move due to the large local host tree availability in most cases.

#### **1.8.** Risk factor identification

The identification of the risk factors and their relative risk estimation is essential for performing a riskbased 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 EU countries.

A risk factor is a biotic or abiotic factor that increases the probability of introduction and spread of 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 ALB. 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 untreated fresh wood packaging material made with broad-leaf wood (mainly poplar), in particular, that associated with imports of stone or tiles from East Asian countries where *A. glabripennis* is native.

Further introduction pathways, which are of lower relevance, include the import of round wood and sawn wood from broad-leafed trees from countries where ALB is known to occur both as a native species or as an introduced species. Surveys should focus on areas where companies involved in the trade or processing of high-risk material are located.

# Example 1: Import of specified commodities in wood packaging material from China, particularly stone imports

The import of specified commodities (according to Commission Implementing Decision (EU) 2018/1137), which are transported by or supported/protected with wood packaging material from China, is most likely to be the pathway for the introduction of the ALB. Therefore, it is more likely to find the pest in locations where the products/commodities are stored or traded or their surrounding areas. Pest infestations in some EU Member States might be linked to imports and the processing of stone (granite) from China.

The arrival site where the stone material is traded or directly processed (e.g. construction works) may be considered as a risk location. Additionally, the vicinity of import and processing locations needs to be considered a risk area. Moreover, wood waste landfill sites where the wood packaging material is accumulated at the end of its working life may also be considered a risk locations, as recorded for many other invasive wood-boring beetles (Rassati et al., 2015).

The introduction of ALB is connected to international trade, therefore, risk areas are more likely to be identified in urban and industrial areas. Risk areas may thus also comprise public and private green parks as well as forest edges in the vicinity of industrial areas.

Given that *A. glabripennis* has a rather limited tendency for natural spread, the size of the risk area corresponds to host tree availability.

#### Example 2: Import of host plants for planting (including bonsai)

Given the fact that host plants for planting (including bonsai) could be another pathway (EPPO, 2013) for the introduction of *A. glabripennis* (even if unusual), nurseries and commercial garden centres that

trade in host plants from regions where ALB is known to be present (both as a native species or where it has been introduced), may be considered risk locations. Risk areas therefore surround these nurseries and commercial garden centres, e.g. public and private green spaces, parks and forest edges.

**Table 2:** Examples of risk activities and the corresponding risk locations and risk areas relevant for

 the surveillance of *Anoplophora glabripennis* in EU Member States

Risk activity	Risk location	Risk area
Import, storage and trading of wood packaging material	Locations where the products/commodities associated with wood packaging material are stored or traded	Areas surrounding locations where the products/commodities associated with wood packaging material are stored or traded
Import 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 *Anoplophora glabripennis*, focusing both on the pest itself (including its various life stages) or symptoms on infested trees. It should be performed at the crown level since this is the place where oviposition and adult emergence occur (and therefore where the main symptoms may be displayed). 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

**Egg:** Eggs are laid under the bark, in the phloem region (Figure 4), are 5–7 mm in size, white and oblong in shape. During development, their colour turns to yellowish-brown.



Figure 4: Deposited eggs after removal of bark (Source: Björn Hoppe)

**Larvae:** These are subdivided into one head segment, three thorax segments and several abdominal segments. The head or mouthpart is brown, while subsequent thoracic and abdominal segments are typically a white–cream colour. The first segment of the thorax is the largest and has a brown sclerotised (i.e. hardened) shield on the dorsal side (Figure 5). The shape of the body tapers from

thorax to abdomen. Young larvae usually measure more than 5 mm, older larvae vary in length from 30 to 60 mm. Larvae have neither legs nor hair or evident bristles.

**Pupa:** Pupation typically takes place in a pupal chamber located in the sapwood immediately under the bark at the end of the larval tunnel. Pupae are white or ivory–white in colour and 30–37 mm in length. The shape is typical of cerambycids, e.g. in the ventral position antenna are visible and shaped as a spiral (Figure 5).

**Adult:** Beetles are black and glossy (Figure 6). Each elytron has about 20 white or yellow patches, although some rare individuals may be completely black, without patches (Dr M Faccoli, Associate professor at University of Padova, personal communication on 13 November 2019). Antennae consist of 11 segments that alternate between blue-white and blue-black (Ric et al., 2006). Males are usually slender and smaller than the females; in males the antennae are clearly longer than the body, while in females the antennae are as long as the insect.

The training guide by Ric et al. (2007) and the German 'Praxishilfe' (guidelines on ALB, Lemme, 2015), provide useful information and guidance on the morphological features of ALB.

According to the *A. glabripennis* datasheet published by CABI (online), *A. nobilis* from north-western China and *A. freyi* from south-western China are two similar species and microscopic characteristics might be needed to distinguish them from *A. glabripennis. Anoplophora chinensis*, the citrus longhorned beetle, is another similar species which has a similar geographic distribution and causes similar damage (Topakci et al., 2017). It can be distinguished from *A. glabripennis* by tubercles on the elytra (Figure 6b).



**Figure 5:** A–F: Impressions of larvae of *Anoplophora glabripennis*. A) brown sclerotised (i.e. hardened) shield on the dorsal side; B) Lateral view – larvae typically without legs; C) and D) Typical shape of the body that tapers from thorax to abdomen; E) and F) Feeding larvae in wood; G) Pupa of *Anoplophora glabripennis* (Source: Hannes Lemme and Thomas Schröder)



**Figure 6:** A) Male adult of *A. glabripennis*. B) Differentiation between ALB (left) and CLB (right): elytra of CLB are grained (white arrow); pronotum with white hairspots, sculetum may appear in white (Source: Hannes Lemme and Thomas Schröder)

#### 2.1.2. Symptoms

Severe damage caused by ALB is mainly due to the feeding activities of the larvae within the wood, which weaken and, in many cases, also kill the infested tree. Most of the symptoms tend to be detected from approximately 1.5 m above the ground up to the middle of the crown (EPPO, 2013).

**Oviposition:** From May to September, females bore rounded oviposition pits into the bark using their mandibles on trees with thin bark. Occasionally, oviposition may be T-shaped as typically recorded for CLB (Haack et al., 2010). Pits are visible depending on the texture of the bark and more likely on trees with smooth bark (Figure 7). On tree species with thicker bark, oviposition pits tend to look more like funnels. In addition, sap oozing out of freshly cut pits may be observed (EPPO, 2016a) (Figure 7). Depending on the tree size, dozens of oviposition pits per tree may be visible (Figure 8). Oviposition pits are visible on the bark but after a few weeks from the oviposition event they dry out, oxidise, change colour and become less visible. It is therefore important to search for these types of symptoms during the oviposition period (from May to September).

**Frass:** Larvae typically produce frass, which is deposited within the larval galleries (Figure 9). If the pressure inside is high (Figure 10), the bark cracks and frass may be observed on leaves, crotches and on the ground around the base of the trees. The presence of large amounts of frass and wood shavings is rather rare.

**Exit holes:** These are the result of emerging adults that have completed their life cycle within the wood (Figure 11). Exit holes are perfectly circular, with a mean diameter of about 10–15 mm (Haack et al., 2010), and usually located above oviposition pits (which might not be observed, Figure 10D) on the upper part of the trunk and main branches. On branches with diameters smaller than 15 cm, oviposition pits and exit holes are often observed to be on opposite sides of the branch.

**Adult feeding:** Adult feeding causes damage to leaves, petioles and also the bark of young branches (1–3 years old) and shoots (e.g. stripped bark) (Figure 2). Feeding sites are visible only for a few weeks after the feeding event because they dry out, oxidise, change colour and become less visible. It is therefore important to search for these types of symptoms during the feeding period (from May to September).



**Figure 7:** Oviposition slits and pits (A–F). C) Funnel is filled with oozing sap (Source: Hannes Lemme and Thomas Schröder)

**Other symptoms:** Further symptoms to be observed are wilting foliage, sectorial crown discolouration, branch desiccation and deformation of bark. Usually crowns remain asymptomatic for the first 3–4 years after ALB infestation. Larval galleries may not be detected on living trees (including plants for planting and bonsai) (compare Figures 9 and 10) but are an indication of infestation of processed wood (e.g. wood packaging material).



**Figure 8:** Quantitative impression on oviposition pits on an approximately 1 m piece of wood from *Acer* spp. (Source: Björn Hoppe)



**Figure 9:** Larval development underneath the bark: (Left) with closed bark, (Right) pressed larval frass visible after removal of the bark (Source: Hannes Lemme and Thomas Schröder)



**Figure 10:** A) to D) Removal of bark for the detection of fresh frass material from *Anoplohopra glabripennis* on *Acer* spp. (Source: Björn Hoppe)



**Figure 11:** Impressions of heavily infested and dying stem parts with typical exit holes caused by *Anoplophora glabripennis* (Source: Hannes Lemme and Thomas Schröder)

#### 2.1.3. Traps and tree monitoring

The lab of M. Keena (USDA, Hamdon, Cennecticut) substantially contributed to the development of trapping approaches to monitor ALB in invaded landscapes. Plenty of work has been invested in the isolation of pheromone components and to delineate the sensory structures of antennae and identify the respective receptors. Nehme et al. (2014) showed that traps baited with male pheromones and

different combinations of plant-derived volatiles can be used to catch ALB in invaded landscapes. Typically, intercept panel traps are used (Figure 12), which should be hung up in the lower canopy of trees from June until September. Keena recommends a solution in the collection cup at the bottom containing a saturated salt solution with a couple of drops of dish-washing liquid added, which will safely kill the beetles that fall into it (USDA, online). Baits need to be changed at 4–6-week intervals. Trapping is not completely effective because the pheromone in not strongly attractive for the insects (Dr M Faccoli, Associate professor at University of Padova, personal communication on 13 November 2019).



**Figure 12:** Intercept panel trap hang up in a crown as part of a monitoring programme (Source: LfL Sachsen-Anhalt, Germany)

The use of traps is one element of the systemic survey approaches for: a) detection surveys in areas where *A. glabripennis* has not yet been detected; but also for b) delimiting surveys to establish the boundaries of an area considered to be infested by or free from a pest. Crown inspections using binoculars from the ground is of crucial importance for the detection of infested trees. It can be necessary to integrate the survey with deeper inspections by tree climbers especially for tall trees or trees having rough and brown bark (e.g. *Ulmus* spp., *Aesculus* spp.) or branches covered with ivy. Visual inspections directly in the crown are more efficient, but more expensive and time-consuming than regular ground observations (Faccoli and Gatto, 2016). In both cases, inspectors must be well trained to assign the appropriate symptoms to *A. glabripennis*.

Visual inspections should be carried out twice per year: in summer to look for the recent oviposition pits and the bark damage due to maturation feeding, and in winter – without the broad-leaf crowns – to easily check for the presence of emerging holes occurring in the upper part of the canopy.

In case of a finding of *A. glabripennis*, phytosanitary measures are required by Implementing Decision (EU) 2015/893 need to be to be carried out (Figure 13).



**Figure 13:** Time series of felling and processing infested trees. These trees must be felled in accordance with the phytosanitary measures required by Implementing Decision (EU) 2015/893: A) and B) Symptomatic trees (exit holes were discovered during monitoring) have to be felled; C) Additional symptoms (larval frass) become visible during the process; D) to G) Felled tree is dissected into smaller pieces, of which all must be inspected for living larvae, pupae and emerging adults; H) and I) All felled and inspected material must be chipped into pieces of not more than 2.5 cm in thickness and width (Source: Björn Hoppe)

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# 2.2. Other methods for detection of *Anoplophora glabripennis*

In some European countries (e.g. 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 season. Visual inspection is not a completely effective approach and could be supplemented by the use of other detection methods to improve the level of ALB detection in the defined area. As part of surveillance for ALB in infested areas, visual inspections may be complemented by well-trained scent detection dogs (sniffer dogs). In this respect, the use of sniffer dogs produced positive outcomes in Austria, 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*. 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. glabripennis and its separation from sibling species including A. chinensis. In addition, EPPO standard PM 7/129 (EPPO, 2016b) provides protocols for the molecular diagnostics of arthropods, which includes A. chinensis and A. alabripennis. In order to confirm ALB 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 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 CLB frass collected from potentially infested host trees. However, the frass needs to be recent (10-15 days) for it to be usable for the DNA analysis. A similar protocol was also developed for the detection of ALB-infested 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 insect species 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 units needed to design a survey plan 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 glabripennis

	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 of *Anoplophora glabripennis*, the following steps will generally be necessary:

1/ Determine the type of survey based on its objectives. For *A. glabripennis*, 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 infestation area following an introduction 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. glabripennis*, 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 should be 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 needs to be surveyed in a Member State in order to state with 95% confidence that the prevalence of *A. glabripennis* 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	<ul> <li>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: <ul> <li>Definition of the target population – the target population has to be clearly identified</li> <li>Target population size and geographic boundary.</li> </ul> </li> <li>(EFSA, 2018)</li> </ul>
I COL	orneal chaminations, other than visual, to determine whether pests

	are present or to identify pests (ISPM 5: FAO, 2018).
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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