European and Mediterranean Plant Protection Organization Organisation Européenne et Méditerranéenne pour la Protection des Plantes

#### Phytosanitary procedures Procédures phytosanitaires

## PM 3/79 (1) Consignment inspection for *Anoplophora chinensis* and *Anoplophora glabripennis*

#### Specific scope

This Standard describes the procedures for the inspection of consignments, including wood packaging material and round wood from broad-leaved trees for infestation with *Anoplophora chinensis* or *Anoplophora glabripennis*.<sup>1</sup>

#### Introduction

Anoplophora glabripennis, the Asian longhorn beetle, currently listed as an EPPO A1 List pest, is native to China, Democratic Republic of Korea and Republic of Korea. Anoplophora chinensis, the citrus longhorn beetle, is an EPPO A2 List pest and native to China, the Democratic Republic of Korea, the Republic of Korea and Japan. Both pests are highly polyphagous and are known to attack various genera of host plants, including Alnus spp., Acer spp., Betula spp., Citrus spp., Fagus spp., Platanus spp. and Prunus spp. (Lingafelter & Hoebeke, 2002; Herard et al., 2009), many of these hosts are widely distributed in the EPPO region. Lists of host trees are included in EPPO Standard PM 9/15 Anoplophora glabripennis: procedures for official control (EPPO, 2013a) and EPPO Standard PM 9/16 Anoplophora chinensis: procedures for official control (EPPO, 2013b).

For details on the biology of *A. glabripennis* and *A. chinensis* see the relevant EPPO Datasheets (EPPO, 1999a,b).

For both species, the larvae bore a gallery in the cambium layer and later in the wood, weakening the trees, making them susceptible to secondary fungal infections and damaging them seriously or even killing them. The adults can also cause damage by feeding on leaves, petioles and bark. Damage to ornamental trees and to the fruiting shoots of fruit trees (for *A. chinensis*) may result in important eco-

#### Specific approval and amendment

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nomic losses (Lieu, 1945; Kojima & Hayashi, 1974). Both pests are recognized as quarantine pests by the European Union (EU) and by other EPPO countries (see EPPO Global Database, https://gd.eppo.int/, for an updated list of existing regulations on these two pests). There have been several interceptions at import. In addition, outbreaks in some European countries have had major consequences, for example the cutting down of all host trees in large areas. Most outbreaks of both species have been eradicated, but despite eradication efforts the pests are still present in some areas. From the wide distribution of A. glabripennis in China it may be supposed that the pest could also establish in several European countries. Also the major host trees of both Anoplophora species are widely distributed in many European countries. As eradication is difficult and expensive, the focus should be on prevention of introduction of both pests.

The most important pathway for the introduction of *A. glabripennis* is the import of wood packaging material from areas where it is native (EPPO PM 9/15 (1) A. glabripennis: *procedures for official control*, EPPO 2013a). All forms of untreated wood from areas where the pest is present can pose a potential risk, as all interceptions for *A. glabripennis* with imports in the EU in the years 2000 to 2012 were on wood packing material (EUROPHYT, 2014; http://ec.europa.eu/food/plant/plant\_health\_biosecurity/ europhyt/index\_en.htm). The outbreaks in various European countries have generally been ascribed to infested wood packing material and could be related to neighbouring stone traders or importers of granite. Phytosanitary procedures for consignment inspection of wood packing material are described in Part 1 of this Standard.

<sup>&</sup>lt;sup>1</sup>This Standard forms part of a new series of EPPO Inspection Standards and will be reviewed by the end of 2017. Comments to be taken into account during that review should be sent to the EPPO Secretariat at hq@eppo.int.

The most important pathway for the introduction of *A. chinensis* is the import of host plants for planting from areas where *A. chinensis* is present. In almost all reported cases, detections have been associated with nurseries importing woody host plants for planting, often *Acer* spp. from Asia, particularly China (EPPO, 2013b). Phytosanitary procedures for consignment inspection of plants for plant-ing are described in Part 2 of this Standard.

#### Phytosanitary inspections

ISPM no. 5 *Glossary of phytosanitary terms* (IPPC, 2009), defines inspection as 'Official visual examination of plants, plant products or other regulated articles to determine if pests are present and / or to determine compliance with phytosanitary regulation'.

The general background for carrying out import inspections is included in ISPM no. 20 *Guidelines for a phytosanitary import regulatory system* (FAO, 2004) and ISPM no. 23 *Guidelines for inspection* (FAO, 2005). Information on phytosanitary inspection of consignments is given in EPPO Standard PM 3/72 *Elements common to inspection of places of production, area-wide surveillance, inspection of consignments and lot identification.* 

The procedures described in this Standard refer to consignment inspection in an EPPO importing country. General elements of this inspection procedure may apply to inspection in both the exporting and the importing country. General instructions for inspection are given in the main text. Appendix 1 gives additional details of symptoms and instructions for inspectors and Appendix 2 provides a summary of instructions for inspectors.

In the exporting country inspections should first be carried out at the place of production and additionally before export as long as the whole consignment is still accessible, for example before packing or loading.

Inspections of consignments in the importing country may be done at the point of entry or at an approved point of final destination, depending on the possibilities for carrying out efficient inspections and provided that the consignments remain under official control. When inspection is carried out at the point of final destination there should be no risk of escape of adult beetles during transport.

General information on monitoring, control and eradication of both pests is given in EPPO Standard PM 9/15 (1) Anoplophora glabripennis: *procedures for official control* (EPPO, 2013a) and EPPO Standard PM 9/16 (1) Anoplophora chinensis: *procedures for official control* (EPPO, 2013b).

This Standard is focused on two *Anoplophora* species. However, when an unfamiliar pest or a pest from the EPPO Alert List is detected during inspection of consignments, the procedures specified in EPPO Standard PM 5/2 *Pest risk analysis on detection of a pest in an imported consignment* should be followed to allow the NPPO to make a decision about what phytosanitary action to take.

#### Part 1 Phytosanitary procedures for consignment inspection of wood packaging material, dunnage and wood from broad-leaved trees

#### Consignments concerned

#### (1) Wood packaging material

The term 'wood packaging material' covers a range of different products. This Standard refers to wood packaging material originating from or having been fabricated in countries where *A. glabripennis* is known to occur. It covers all forms of wood packaging material made of raw wood as defined by ISPM no. 15 *Regulation of wood packaging material in international trade* (FAO, 2009).

In accordance with ISPM no. 15 the Standard does not apply to:

- raw wood of 6 mm thickness or less
- wood packaging material constructed entirely from processed wood produced under the use of glue, heat and pressure, or a combination thereof, such as plywood, compressed wood, veneer
- barrels for wine or spirits that have been heated during fabrication
- gift packages for wine, cigars or similar consignments, made from wood that has been treated or fabricated in a way that it is free from pests
- · sawdust, chipped wood and fine wood shavings
- wooden components that are permanently connected to containers or means of transport.

Wood packaging material can be differentiated according to its usage in:

- (a) Wood packaging material in use used for stowing or securing consignments during transport such as:
  - crates
  - boxes
  - packing cases
  - pallets
  - cable drums
  - spools/reels.
- (b) Wood packaging material not in use representing a consignment (wood) itself (e.g. a load of pallets).
- (c) Dunnage wood in different sizes used for load-security (e.g. in containers).

In general, wood packaging material of these types originating from any country without specific agreements (such as those that exist inside the EU and Eurasian Economic Commission) should be debarked, treated and marked according to ISPM no. 15.

#### (2) Wood from broad-leaved trees

#### This includes:

- round wood from host species with and without bark
- sawn wood from host species, with and without bark
- other types of wood (e.g. wood packaging material as consignment from host species) from countries

where A. glabripennis and A. chinensis are known to occur.

#### Lot identification

General information about the definition of a  $lot^2$  is given in ISPM no. 31 *Methodologies for sampling of consignments* (FAO, 2008). According to that definition a lot to be sampled should be a number of units of a single commodity identifiable by its homogeneity in various factors, for example origin, packing facility or exporter.

- (1) Wood packaging material
- (a) Wood packing material in use. The concept of a lot is difficult to apply to wood packaging material. For practical reasons a lot for inspection should normally be defined by the trading units concerned, for example identical commodities with the same origin or from the same exporter. Lots can also be defined by origin of the packaging material from the same producer if this can be established.
- (b) Wood packaging material not in use. A lot should consist of a trading unit from the same exporter or from the same place of origin or the same producer.
- (c) Dunnage. For dunnage it is not possible to define a lot, as it does not consist of units and hardly any information about movements of dunnage is available to the plant protection service. Wood used for dunnage is often of low quality. If possible a lot might be defined by all dunnage used to secure a consignment from the same place of origin or the same exporter.

#### (2) Wood from broad-leaved trees

Wood (especially round wood) is often transported in bulk or a container while sawn wood is usually traded as bundled packs. A lot should consist of a trading unit that is homogeneous in its place of production and species or variety.

#### Selection of material for inspection

General information is defined in ISPM no. 31 *Methodologies for sampling of consignments* (FAO, 2008). For detection of *Anoplophora* spp. in the consignments, visual inspection will be the first measure.

- (1) Wood packaging material
- (a) Wood packaging material in use. Selection of material for inspection will depend on the type of wood packaging material as well as on the size of a lot and the type of transport. In general, this will be easier at final points of destination than at points of entry, because the cargo

will be unloaded and the plant protection service will have easy access to all units. However, in case of infestation, the risk of escaping beetles increases with time of transport.

The quantity of selected items for inspection and the frequency of inspections should depend on the consignment. Specific commodities or commodities from specific origins that have been the cause of previous interceptions should be inspected more thoroughly and more often. If possible at least 90% of the items in a lot should be inspected for the selected consignments. As this might be difficult to achieve in practice, the general impression of the wood packaging material can be used as a reference. Wood packaging material from third countries has to be treated and marked according to ISPM no. 15 (FAO, 2009), so missing marks, but also low wood quality, damp wood, fungal growth or similar signs clearly require more intensive inspection. Sawdust or other signs of insect activity are also signs that require an increase in the number of samples of the selected material.

A risk register of consignments should be developed for determination of consignments that should be subject to more thorough inspections and higher inspection frequencies.

- (b) Wood packaging material not in use. Wood packing material not in use represents a consignment and should be treated like other consignments consisting of wood (see point 2 below).
- (c) Dunnage. Dunnage often consists of wood of low quality; therefore, if possible, all wood used for dunnage in a movement should be selected for inspection. Preferably, this should happen at such places where containers or other means of transport are completely unloaded. Sometimes dunnage is used to secure containers on ships and is often disposed of afterwards. If possible such dunnage should also be selected for inspection.

#### (2) Wood from broad-leaved trees

Large consignments of wood are easier to inspect at the place of destination, where they will be unloaded, than at the place of entry. However, if they are unloaded at the place of entry (e.g. for repacking) inspections should be held there. According to ISPM no. 31 Methodologies for sampling of consignments (FAO, 2008), the number of items (e.g. logs or planks) to be inspected will depend on the overall size of the consignment, the selected level of confidence and the level of infestation to be detected, which for Anoplophora spp. should be as small as feasible. Thorough inspection of 460 items selected at random (logs or planks) provides at least 99 % confidence of detecting visible symptoms in 1% of the items in large consignments, provided that infestation is distributed uniformly within the consignment. Selection of items for inspection may be targeted to areas where a general overview of the consignment shows signs of insect activity or other risk factors. The

<sup>&</sup>lt;sup>2</sup>In this Standard on inspection, it is necessary to refer to 'lots' in order to define units on which a report can be written.

wood packaging material accompanying consignments of wood should also be inspected.

#### Specific measures - visual inspections

Visual inspections are used to confirm that the consignments comply with the requirements of the importing country. Generally, consignments of wood or wood packaging material should be checked with regard to their overall quality. For low-quality consignments visual inspection should be more intensive. Handling and inspection of material should be carried out in a way which avoids the possible escape of adult beetles in case of infestation. If visual inspections are performed on consignments that are still in a container, it should be ensured in advance that the container is free from any toxic gases. Visible surfaces, including the floor or walls of containers or storage rooms, should be examined for adult beetles. If necessary, consignments should be unloaded to enable an examination.

For wood packaging material visual inspections are important to check for the official marks confirming that the wood has been treated according to ISPM no. 15 (FAO, 2008). For pallets it might be useful to lift them up (e.g. using a forklift truck) to look at the lower surface (especially at the central wooden part) or the lowest pallet in a stack, while respecting national rules on operator safety. Any indications of wood-infesting pests may suggest either that the wood has not been treated correctly or that it has been re-infested since the treatment. Detected pests should be identified to species level, if possible, to decide what measures are appropriate.

Visual inspection should focus on:

- the existence of mobile insect stages
- boreholes
- · shavings or frass
- larval galleries and grub holes (peeling off residual bark increases the chance of detection).

#### Part 2 Phytosanitary procedures for consignment inspection of plants for planting including bonsai

#### Consignments concerned

Plants for planting of all host trees should be inspected when coming from regions where *A. chinensis* is present, especially those with a stem diameter of 1 cm or more.

#### Lot identification

General information about the definition of a lot is defined in ISPM no. 31 *Methodologies for sampling of consignments* (FAO, 2008). According to that definition a lot (see footnote on previous page) to be sampled should be 'a number of units of a single commodity identifiable by its homogeneity in various factors', for example origin, packing facility or exporter.

For plants for planting, a lot should consist of plants from the same place of origin, plants of the same species or variety and if possible of the same growing stage or size. In uneven consignments lots should consist of at least plants from the same place of origin (e.g. a nursery).

#### Selection of material for inspection

General information on sampling is given in ISPM no. 31 *Methodologies for sampling of consignments* (FAO, 2008).

For detection of *A. chinensis* in plants for planting, visual inspection will be the first measure combined with destructive sampling of selected material.

According to ISPM no. 31 (FAO, 2008), the number of plants to be inspected will depend on the overall size of the consignments, the desired level of confidence and the level of infestation to be detected. Appendix 2 of ISPM no. 31 provides tables with sample sizes for various levels of confidence and detection. For Anoplophora spp. the level of confidence should allow reliable detection of a level of infestation which is as low as possible. The intensity of inspections and selected number of plants to be inspected should allow a comprehensive inspection of the consignment and should be adapted to the risk associated with the introduction of A. chinensis with plants for planting (EPPO, 2012). For example, from a lot of 10 000 plants, 4600 plants need to be inspected to give 99% confidence of detecting an infestation present in 0.1% of the plants, provided the infestation is uniformly distributed and the plants are selected at random.

#### Specific measures

#### Visual inspections

Visual inspections are used to confirm that the consignments comply with the import requirements of the importing country. Generally, consignments of plants for planting should be checked with regard to the vitality of the trees. Handling and inspection of the plants should be carried out in a way which avoids the possible escape of adult insects in case of infestation. If visual inspections are performed on plants that are still in a container, it should be ensured in advance that the container is free from any toxic gases. All visible surfaces, including the floor or walls of containers or storage rooms, should be examined for adult beetles. If necessary, plants should be unloaded to enable an examination.

For plants for planting, visual inspections should include the stem and roots of trees. Signs of infestations with *A. chinensis* are included in Appendix 1 and further described in EPPO Standard PM 9/16 (1) Anoplophora chinensis: *procedures for official control* (EPPO, 2013b).

#### Destructive sampling

Destructive sampling is a common method for detecting infestation with *Anoplophora* spp. in trees (plants for planting), especially as infestations might not always be detected from the outside by visual inspection of the tree. The method used has to ensure the finding of any possible infestation with *Anoplophora* spp. present in the sample.

#### Acknowledgements

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### Appendix 1 – Specific procedures – identification and typical symptoms

Identification of adult beetles of both species is mainly on the basis of morphological features; polymerase chain reaction (PCR) tests are currently under development.



Fig. 1 Adult A. chinensis form malasiaca (photo: Dr. T Schröder/JKI).



Fig. 2 Adult A. glabripennis (photo: Dr. T. Schröder/JKI).



Fig. 3 Visual morphological differences between *A. glabripennis* (left) and *A. chinensis* (right) (photo: Dr. T. Schröder/JKI, U. Hoyer-Tomiczek/BFW).

Both beetles are black with irregular white hairy spots on the elytra (the hard exoskeleton which covers their wings), of a large size (25–35 mm) and with noticeable long antennae. The pronotum of *A. chinensis* form *malasiaca* shows white

hair spots, the pronotum of *A. glabripennis* and *A. chinensis* is entirely black. *Anoplophora chinensis* in both forms has rows of polished tubercles at the base of the elytra, whilst the elytra of *A. glabripennis* are plain (Thomas, 2004).



Fig. 4 Adult A. glabripennis leaving the host tree.



Fig. 5 Egg of A. chinensis.



Fig. 6 Head of larvae of A. chinensis.



Fig. 7 Head of larvae of A. glabripennis.

Table 1 gives a short overview of general symptoms of infestation with *Anoplophora* spp. on relevant consignments. More information is provided in EPPO Standards PM 9/15 (1) and PM 9/16 (1) (EPPO, 2013a,b).

Table 1 Typical symptoms of infestation by Anoplophora spp.

Symptom	Consignment	Location
Sap oozing from oviposition sites (Fig. 8)	Plants for planting, including bonsai	Mainly around the lower part of the trunk or below ground level (for <i>A. chinensis</i> ) or on the trunk and on branches or in branch forks (for <i>A. glabripennis</i> )
Wilting or loss of foliage	Plants for planting (bonsai)	Whole plant
Death of aerial parts or branches	Plants for planting (bonsai)	Whole plant
Discoloration and deformation of bark	Plants for planting (bonsai)	Mainly around the lower part of the trunk or below ground level (for <i>A. chinensis</i> ) or on the trunk and on branches (for <i>A. glabripennis</i> )
Frass (wood shavings) (Fig. 9)	Plants for planting (bonsai)	Mainly around the lower part of the trunk or below ground level (for <i>A. chinensis</i> ) or on the trunk and in branch forks (for <i>A. glabripennis</i> )
	Wood packaging material	No specification
	Wood of broad- leaved trees	No specification
Larval galleries and grub holes (Figs 10 and 11)	Plants for planting (bonsai)	Mainly around the lower part of the trunk or below ground level (for <i>A. chinensis</i> ) or on the trunk and on branches (for <i>A. glabripennis</i> )
	Wood packaging material	No specification
	Wood of broad- leaved trees	No specification
Round exit holes (approximately 10–15 mm diameter) (Figs 12–14)	Plants for planting (bonsai)	Mainly around the lower part of the trunk or below ground level (for <i>A. chinensis</i> ) or on the trunk and on branches (for <i>A. glabripennis</i> )
	Wood packaging material	No specification
	Wood of broad- leaved trees	No specification



Fig. 8 Sap oozing from oviposition site on a tree.



Fig. 11 Circular exit hole.



Fig. 9 Frass (wood shavings) on the base of an infested tree.



Fig. 12 Larval galleries with exit holes.



Fig. 10 Larval galleries with frass.



Fig. 13 Exit holes in the crown of a tree.



Fig. 14 Old overgrown exit holes.



Fig. 17 Example 2: destructive sampling of plants for planting for inspection for *A. chinensis*.



Fig. 15 Feeding damage of adult beetles.



Fig. 18 Larvae holes in a sample.



Fig. 16 Example 1: destructive sampling of plants for planting for inspection for *A. chinensis*.

# Specific inspection measures for consignments liable to be infested by *Anoplophora* spp.

#### Destructive sampling

For plants for planting (including bonsai), destructive sampling should involve cutting the stem and any main branches and roots with a diameter of > 10 mm into short pieces (2.5 cm) and splitting them for the whole length, to look for signs of tunnelling or pests.

The larval distribution of *A. chinensis* will mainly be restricted to wood at ground level or even below ground level. Larger stems may be debarked and cut into smaller pieces of 10–15 cm which are then split and examined to check for larval galleries and larvae inside them. Whenever galleries are seen, further careful splitting should be carried out in order to find the intact larvae, if possible, for identification.

In the case where larval holes, larvae or other signs of infestation with *Anoplophora* are found, appropriate action should be taken, according to legislation or EPPO Standards PM 9/15 (1) and PM 9/16 (1) (EPPO, 2013a,b).

#### Alternative non-destructive methods

There are a number of non-destructive methods of detecting *Anoplophora* species which are at different stages of development and validation. None of these methods have been demonstrated to be fully effective at detecting infestation in consignments or in standing trees.

#### Sniffer dogs

Dogs can be trained to detect different development stages of *Anoplophora* spp. in trees, plants for planting (bonsai) and in wood packing material, as has been shown in the EUPHRESCO project on risk management of *Anoplophora* species (ANOPLORISK). Sniffer dogs can check large lots in a short time. They can also detect infestation in hidden units and indicate where inspectors should look more closely. Results from ANOPLORISK indicate that the time saved by inspecting wood packing material with sniffer dogs compared with a visual investigation by inspectors is about 33%, with a simultaneous increase in the findings ratio of about 50%.

Preliminary results of standardized test series in the follow-up project ANOPLORISK 2 indicate a sensitivity of detection dogs towards *A. glabripennis* frass, woodshavings or larvae of between 75% and 95%.

#### Electronic nose

An electronic nose system is under development; a pump can be used to collect telluric air for analysis of volatile components. (Villa *et al.*, 2012).

#### Sound detectors/vibration detectors

A detection tool is currently being tested in the United Kingdom for its suitability to detect living larvae with piezo-electric sensors (Schofield, 2011).

#### X-rays

Tests with different methods using X-rays (results from ANOPLORISK) showed that computed tomography (CT) could possibly be used to detect infestation by *Anoplophora* spp. in small trees or stems.

## Appendix 2 – Short procedures for inspectors

These short procedures include the main elements of the practical work of inspectors when carrying out inspections of consignments associated with the risk of infestation by A. chinensis or A. glabripennis. It is assumed that documentary checks and identity checks should already have been carried out.

Lot identification and selection of material for inspection has to be performed according to the consignment, the associated risk and possibilities (e.g. if the consignment can be lifted).

If inspections are conducted in a container, inspectors should follow specific security advice:

- (a) Make sure that the container is free from toxic gases or other chemical substances before opening or entering the container. If necessary (e.g. if treatments are listed in the accompanying papers) the container should be checked by specialists to be free of dangerous gases or substances.
- (b) Container doors should be opened carefully in case the consignment has not been secured and also during the flight period of the insects to avoid the escape of beetles.
- (c) Make sure that there is no danger of collapsing consignments, for example piles of pallets.

Inspectors should wear security clothing, reflective vests and if possible helmets and security boots.

Equipment should also include:

- flash lights
- gloves
- dust masks
- knives, chisels, saws, splitting axe
- magnifiers, tweezers;
- utilities to securely store sample material for further identifications (no plastics for larvae and beetles, glass containers with metal lids are more suitable)
- · warning tape
- camera for pictures in case of findings.

Visual inspection of the consignment as a whole can give a first impression of the general appearance and quality of the consignment. Parts of the consignment or wood packaging material of obviously low quality should be inspected with special care. Inspections should not be exclusively visual, as larvae might be hidden inside the wood or stems. Therefore destructive sampling of selected material has to be part of the inspection process, especially for plants for planting. For wood packaging material, wood might be peeled off of patches where insects might hide.

In case of findings of infestation with *A. glabripennis* or *A. chinensis*, actions should be taken according to applicable regulations.

The known host plants are listed in EPPO Standards PM 9/ 15 (1) and PM 9/16 (1) (EPPO, 2013a,b) and in EPPO Global Database, a further description of the biology of both pests can be found in EPPO Datasheets (EPPO, 1999a,b).