PEST SURVEY CARD

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Pest survey card on *Aromia bungii*

European Food Safety Authority (EFSA),
Eduardo de la Peña, Gritta Schrader, Sybren Vos

Abstract

This pest survey card was prepared in the context of the EFSA mandate on plant pest surveillance (M-2017-0137), at the request of the European Commission. Its purpose is to assist the Member States in preparing their survey activities for *Aromia bungii* using a statistically sound and risk-based pest survey approach. *A. bungii* is a quarantine species in the EU. The cerambycid beetle is native to East and South-East Asia and in the EU is currently reported as 'present under eradication' in Italy and 'transient under eradication' in Germany. *Prunus* species are its main host, in particular stone fruit trees, such as peach, apricot, plum, cherry and almond. However, other species such as pomegranate, kaki and olive trees are reported as potential hosts. Depending on local conditions the life cycle takes from two to four years (from egg laying to adult emergence). The larvae and pupae complete their development in the trunks of the host trees or in its basal branches. In the EU, the climate suitability and host plant availability are not limiting factors for the establishment of the species. Wood packaging, wood or wooden products from *Prunus* species are the main introduction pathways of *A. bungii* into the EU. Therefore, areas where *Prunus* species are grown that surround ports, packhouses and warehouses are risk areas that are particularly relevant for detection surveys. In addition, plants for planting (saplings and bonsais) can also sustain eggs or initial larval stages. Therefore, nurseries where *Prunus* species are cultivated are also prime targets for detection surveys. For early detection of *A. bungii*, the preferred method is pheromone-based trapping in combination with the visual inspection of main target host plants.

Keywords: plant pest, survey, risk-based surveillance, *Aromia bungii*, rednecked longhorn beetle, *Prunus* species.

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Correspondence: ALPHA@efsa.europa.eu

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Introduction

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 information presented in this pest survey card is summarised from several recent pest risk assessments on *Aromia bungii* (i.e. Food and Environment Research Agency in the UK, the Netherlands Food and Consumer Product Safety Authority (Anonymous, 2017), the Julius-Kühn Institute in Germany (Schrader and Schröder, 2012), the EFSA report to support EU priority pests for this species (EFSA, 2019), the European and Mediterranean Plant Protection Organization (EPPO) standards on phytosanitary treatments (PM 10), diagnostics (PM 7/41 (3)) and national regulatory control systems (PM 9/17 (1)), International Standards for Phytosanitary Measures (ISPMs), datasheets and other scientific documents.

The objective of this pest survey card is to provide the relevant biological information needed to prepare surveys for *A. bungii* in EU Member States (EFSA, 2019). It is part of a toolkit that is being developed to assist Member States with planning a statistically sound and risk-based pest survey approach in line with International Plant Protection Convention guidelines for surveillance (IPPC, 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 *Aromia bungii*
ii. General documents:
   a. The general survey guidelines
   b. The RiBESS+ manual available online[1]
   c. The statistical tools RiBESS+ and SAMPELATOR which are available online[2] with open access after registration.

1. The pest and its biology

1.1. Taxonomy

Scientific name: *Aromia bungii* Faldermann 1835

Phylum: Arthropoda **Subphylum**: Hexapoda **Class**: Insecta **Order**: Coleoptera **Family**: Cerambycidae **Subfamily**: Cerambycinae **Tribe**: Callichromatini **Genus**: Aromia Audinet-Serville 1834 **Species**: bungii Faldermann 1835

Synonym(s): *Aromia cyanicornis* Guérin-Méneville, 1844; *Aromia ruficollis* (Redtenbacher, 1868); *Cerambyx bungii*; *Aromia bungii* var. *brunnea* (Podaný, 1971); *Aromia cyanicornis* var. *ruficollis* (Redtenbacher, 1868)

Common name(s) of the pest: redneck longhorn beetle, red-necked longhorn beetle, peach red-necked longhorn, plum and peach longhorn beetle, peach borer, peach musk beetle.

Taxonomy: *Aromia bungii* is a single taxonomic entity, clearly defined and an identifiable species. The genus *Aromia* (Audinet Serville 1834) comprises four distinct species, i.e. *A. bungii*, *A. japonica* (Podaný 1971), *A. moschata* (Linnaeus, 1758) and *A. orientalis* (Plavilstshikov, 1934). Other taxa appearing in the literature within the genus *Aromia* refer to either synonyms or subspecies of these four species.

[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

1.2. EU pest regulatory status

The outbreaks of *Aromia bungii* in Germany and Italy, and the risk that this species poses to economically relevant fruit crops, resulted in implementing the emergency measures laid down in Commission Implementing Decision (EU) 2018/1503[3], which states that if there is an outbreak in EU territory, an area should be demarcated around the point of that outbreak and within that area effective eradication measures should be adopted, or under certain circumstances, Member States could decide not to establish a demarcated area and to limit the measures to the destruction of the infested plants because this would be proportionate to the phytosanitary risk for that particular area.

A. bungii is currently regulated under Council Directive 2000/29/EC[4], in Annex I A/I banning its introduction into the EU, and Annex IV A/I laying down special requirements for plants, plant products and other objects originating outside the EU to prevent introduction and movement of the pest into and within all Member States. The special requirements are summarised in Table 1.

Table 1: Summary of the special requirements laid down in Annex IV A/I of Council Directive 2000/29/EC, relevant to the survey of *Aromia bungii*

Plants, plant products and other objects	Requirements
Prunus L., other than in the form of chips, particles, sawdust, shavings, wood waste and scrap obtained in whole or part from these trees, wood packaging material, except dunnage supporting consignments of wood, which is constructed from wood of the same type and quality as the wood in the consignments and which meets the same EU phytosanitary requirements as the wood in the consignment, but including that which has not kept its natural round surface, originating in China, the Democratic People's Republic of Korea, Mongolia, Japan, the Republic of Korea and Vietnam.	Originates in an area officially free from *Aromia bungii*, or has undergone an appropriate heat treatment, or has undergone appropriate ionising radiation.
Wood in the form of chips, particles, sawdust, shavings, wood waste and scrap obtained in whole or part from *Prunus* L., originating in China, the Democratic People's Republic of Korea, Mongolia, Japan, the Republic of Korea and Vietnam.	Originates in an area officially free from *Aromia bungii* or has been processed into pieces of not more than 2.5 cm thickness and width or has undergone an appropriate heat treatment.

1.3. Pest distribution

Aromia bungii is a native species in East Asia (south-eastern Palaearctic). In the native range, it is present in China (Anhui, Fujian, Gansu, Guangdong, Guangxi, Guizhou, Hainan, Hebei, Heilongjiang, Henan, Hong Kong, Hubei, Hunan, Inner Mongolia, Jiangsu, Jiangxi, Jilin, Liaoning, Shaanxi, Shandong, Shanxi, Sichuan, Yunnan and Zhejiang), North Korea, South Korea, Mongolia and Vietnam. The species has also been reported in areas of Russia close to the Mongolian border (EPPO, 2015). In Japan, the species was reported for the first time in 2013 when several cherry trees (*Prunus* spp.) and Japanese plums (*P. mume*) were found showing symptoms of infestation (larval galleries) and some adults were found on those trees. It was first detected in the Aichi prefecture and has since spread towards other areas (i.e. Saitama, Gunma, Tokyo, Osaka, Tokushima and Tochigi prefectures – Anonymous, 2013). The National Institute for Environmental Studies of Japan states that most probably the route for entry

[3] Commission implementing decision (EU) 2018/15033 of 8 October 2018 establishing measures to prevent the introduction into and the spread within the Union of *Aromia bungii* (Faldermann). OJ L 254, 10.10.2018, p. 9-18.

[4] 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. Consolidated version of 01/09/2019

in the country was infested wood and/or packaging material (NIES, online). Nursery plant material is also considered to be a pathway for further spread.

A. bungii has also been intercepted by phytosanitary authorities in the United States in a warehouse containing wood and packaging material from China (Smith, 2009) and in cargo containers in the port of Blaine (Washington) as reported in EPPO (2014).

For EU Member States, the first detection of *A. bungii* by phytosanitary inspection services was in 2008 when three adults were intercepted among wooden pallets in a warehouse in the UK. Other than that, the pest is present in Italy and transient in Germany (Duffy, 1968; Anderson et al., 2013; EPPO, 2012a; EPPO, 2012b; EPPO 2015). *A. bungii* has also been reported in Spain, where an adult male of *A. bungii* was retrieved from a drift net arranged in the Caselas river (Caldelas de Miño, Galicia, Spain) (Otero and Cobo, 2018), although the species is considered to be absent from Spain.

A. bungii was detected in Germany for the first time in 2011 in southern Bavaria (Burmeister et al., 2012). The pest species was immediately placed on the EPPO Alert List in May 2012 (EPPO, 2013). *A. bungii* appeared again near Rosenheim in July 2016. By March of 2019, adult beetles of *A. bungii* had been found in two locations in Bavaria. Official measures have been taken. A survey including traps around the locations is being carried out and two demarcated zones have been established. Suspicious plants have been felled and the presence of the beetle was confirmed. Control measures to eradicate the pest are currently being implemented and therefore the species is officially considered to be a transient species in Germany.

In September 2012, *A. bungii* was detected in Italy when several *Prunus* spp. (plum and apricot trees) showing symptoms of infestation and two adults were found in the Campania region (EPPO, 2013a and EPPO, 2013b). In July 2013, *A. bungii* was also detected in Lombardy, Italy. In 2016, a second outbreak was reported in the Campania region. In 2017, two outbreak foci for *A. bungii* were again identified. In this case, 252 plants were found to be infested, including *P. armeniaca* (apricot) and *P. domestica* (plum). Because of these large outbreaks and the successive time intervals at which the species has been observed, this pest is now considered to be established in Italy but with restricted distribution.

In Germany, *A. bungii* is under eradication and measures are being taken to monitor and control possible outbreaks (Figure 1). In Italy, *A. bungii* is under containment (official communication from Italy to the European Commission).

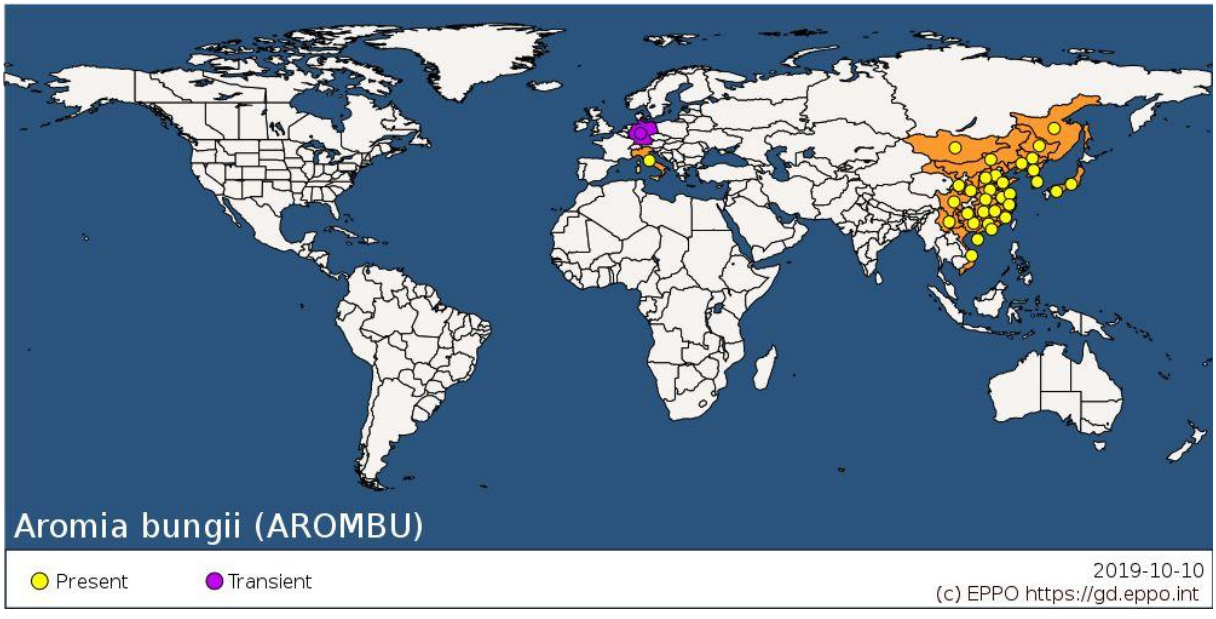

Figure 1: Global distribution of *Aromia bungii* (Source: EPPO Global Database, https://gd.eppo.int)

1.4. Life cycle

The biology of the species is typical for cerambycids. However, the duration of the life cycle given the environmental/climatic variation in Europe can vary significantly according to prevailing local climatic conditions. The beetle lives in forests, urban and peri-urban green areas and orchards, and is mainly associated with *Prunus* species. *Aromia bungii* overwinters as larvae of different ages. Adult beetles emerge from infested trees in the late spring and summer months (usually from May to August), although the peak moments occur at the end of May and beginning of July. Adult females lay eggs in bark crevices on the trunk and main branches (e.g. eggs have been found up to 2 m off the ground). Originally, it was thought that old, senescent trees were preferred by this pest, though in areas where the pest has been recently introduced (as the outbreaks in Italy demonstrate), females prefer young and healthy trees for oviposition. The presence of the species in senescent trees is probably the result of recolonisation events after establishment. Each female lays 350–730 eggs during its lifetime. First-instar larvae hatch in approximately 10 days and then bore a gallery in the phloem. Intermediate larval instars develop, damaging the sapwood. Larvae may overwinter two or three times and usually mature after 21–36 months (Liu et al., 1999; Ostojá-Starzewski and Baker, 2017) (Figure 2). Larvae can survive for relatively long periods of time without feeding before pupation. The pupation of 4th-instar larvae takes place in chambers excavated in the trunk of the tree and/or at crown height, in the main branches. The pupal period lasts ca. 20 days, and pupation generally occurs in spring or early summer. Based on all this, the lifecycle from egg hatching to adult emergence ranges from 2-4 years depending of local conditions, quality of the host, latitude and climatic conditions, being mean temperature a good predictor of the duration of the life cycle.

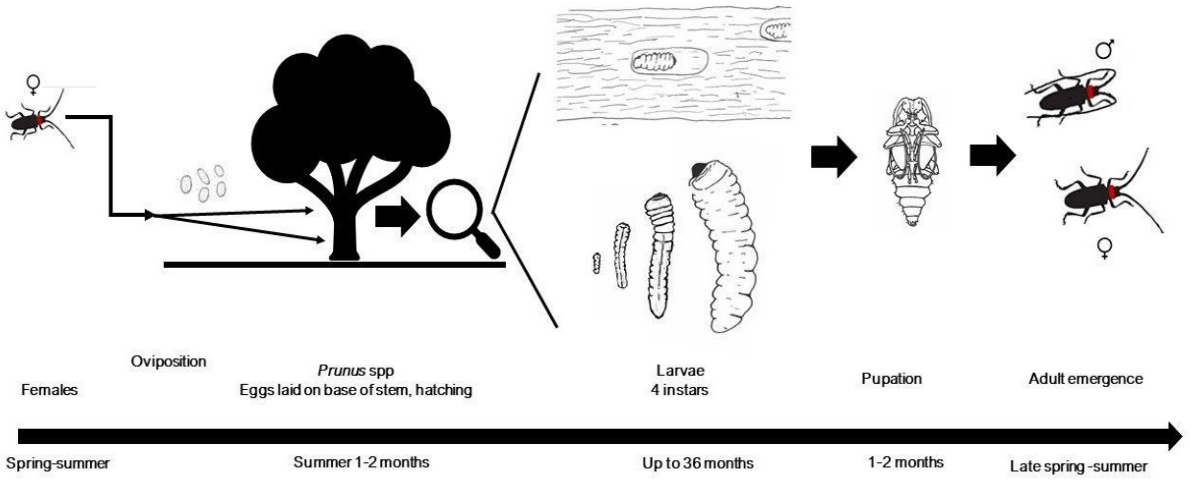

Figure 2: Schematic representation of the life cycle of *Aromia bungii*

1.5. Host range and main hosts

Aromia bungii is an oligophagous species. In the native area of distribution, the main hosts are *Prunus armeniaca* (apricot), *P. persica* (peach), *P. domestica* (plum) and *P. avium* (cherry) (Wu and Li, 2005; Ma et al., 2007; Wang et al., 2007). Other confirmed hosts are *P. americana* (American plum), *P. grayana*, *P. japonica* (Japanese bush cherry), *P. mume* (Chinese plum), *P. pseudocerasus* (Chinese sour cherry), *P. salicina* (Japanese plum) and *P. yedoensis* (Yoshino cherry). In China, other tree species have been reported as potential hosts for *A. bungii*, i.e. *Diospyros kaki* (persimmon), *D. lotus* (Caucasian persimmon), *D. virginiana* (American persimmon) and *Punica granatum* (pomegranate) but these records are unconfirmed. In Europe the species has been reported in *P. domestica* (plum) and *P. cerasifera* (cherry plum), a common rootstock of stone fruit, *P. armeniaca* (apricot), *P. avium* (cherry), *P. persica* (peach) and on *P. dulcis* (almond). Additional hosts mentioned in literature sources are *Populus* spp. (poplar), and *Olea europaea* (olive) (EPPO, 2015, citing others). The EPPO Global Database also lists *Prunus padus*, *P. pseudoceraus*, *P. salicina* and *Prunus* x *yedoensis* as major host species, and *Azadirachta indica*, *Bambusa textilis*, *Castanea mollissima*, *Juglans regia*, *Populus alba*, *Populus*

tomentosa, *Pterocarya stenoptera*, *Pyrus bretschneideri*, *Schima superba*, *Zanthoxylum bungeanum* as minor hosts. The genus *Quercus* is also reported to be a potential host by EPPO. Because of the host range of the species and its preference for *Prunus* spp., *A. bungii* is considered to be an important pest of stone fruit trees such as *P. persica* (peach), *P. armeniaca* (apricot), *P. domestica* (plum), *P. avium* (sweet cherry) and *P. dulcis* (almond).

In Europe, cultivated areas of *P. armeniaca* (apricot) and *P. persica* (peach) are mainly located in the Mediterranean Member States (Spain, Portugal, southern France, Italy, Slovenia, Croatia, Greece, Malta and Cyprus) but also occur in Austria, Bulgaria, Czechia, Germany (north up to the Baltic Sea), Hungary, Romania and Slovakia. *Prunus domestica* (plum) and *P. avium* (sweet cherry) also occur in these countries but also further north (the UK, Belgium, the Netherlands, Denmark, Poland, Latvia, Estonia, southern parts of Norway and Sweden) (EPPO, 2014; EFSA, 2019). Therefore, the genus *Prunus* and several species of *Prunus* susceptible to being hosts of *A. bungii,* in particular *P. avium* (sweet cherry), are well represented and spread throughout western Europe (Figure 3). From a detection survey perspective it is important to bear in mind that although *Prunus* species are the main hosts, trees of other genera need to be considered in detection and delimiting surveys of (potential) infested areas. Thus, *Prunus* spp. should be prioritised for detection surveys but once the pest has been detected, other hosts should be included in in delimiting surveys.

1.6. Environmental suitability

Aromia bungii is mainly found in natural, reforested, urban and peri-urban forests, and orchards. Temperature is an important factor determining the length of the *A. bungii* life cycle; the lowest threshold for survival is unknown but in laboratory conditions adults of *A. bungii* can survive for weeks at temperatures as low as 8 °C. As larvae of this insect complete their development in the trunk of the tree, the larval stages are well protected from harsh climatic conditions and therefore the species can complete its life cycle in a wide variety of climatic areas. *A. bungii* is present in the Lioning province (China) where the annual temperature is 6–9 °C with only 140–160 frost-free days per year. The pest distribution in China and Mongolia indicates that the northern limit for occurrence is determined by the number of accumulated degree-days (base 10 °C) above 500, corresponding to hardiness zones ranging from 4 to 13, even though under this scenario, the completion of the life cycle may take several years. Considering the hardiness zones where the species occurs in East Asia, and that these zones are also present in the EU, climate does not seem to pose a limitation for the establishment of the species in the EU. Because of the climatic characteristics and the fact that *Prunus* spp. are widespread in the area, the potential area of occurrence in the EU is very extensive (Magarey et al., 2008; Figure 4).

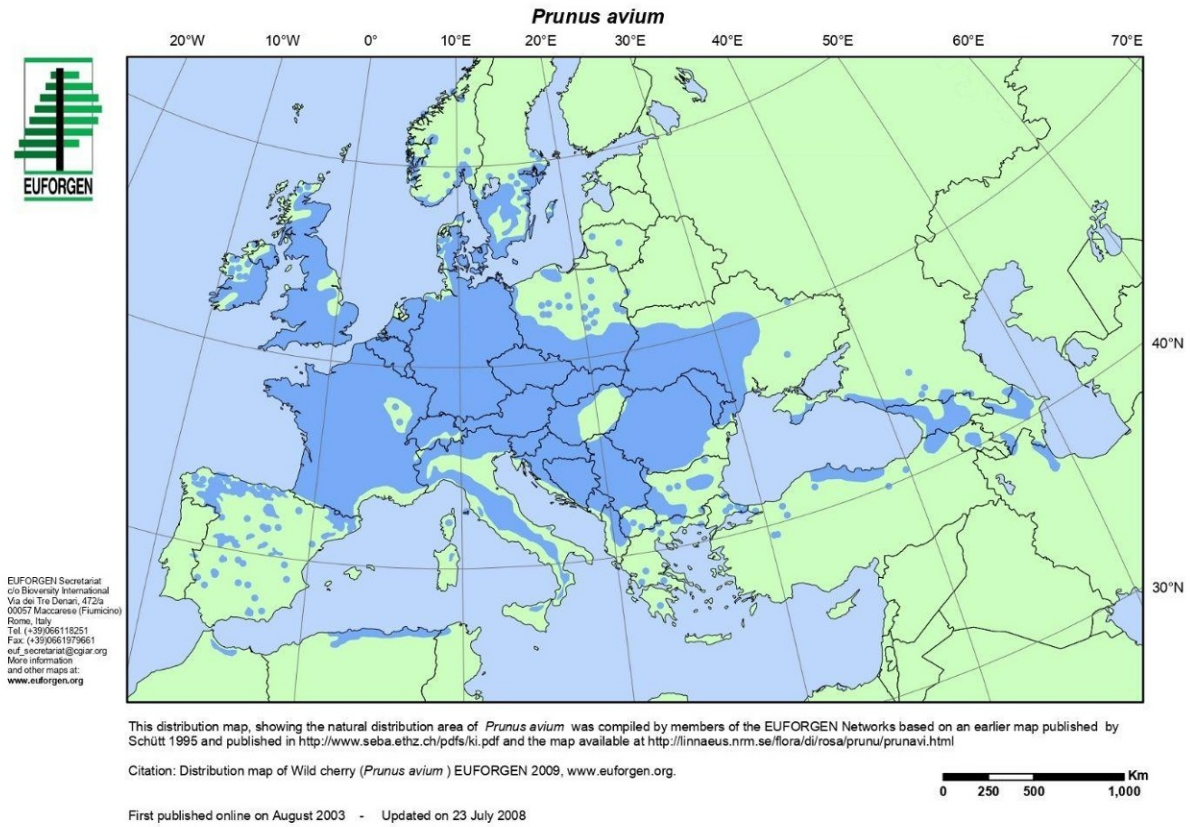

Figure 3: Distribution map of wild cherry (*Prunus avium*) EUFORGEN 2009. www.euforgen.org

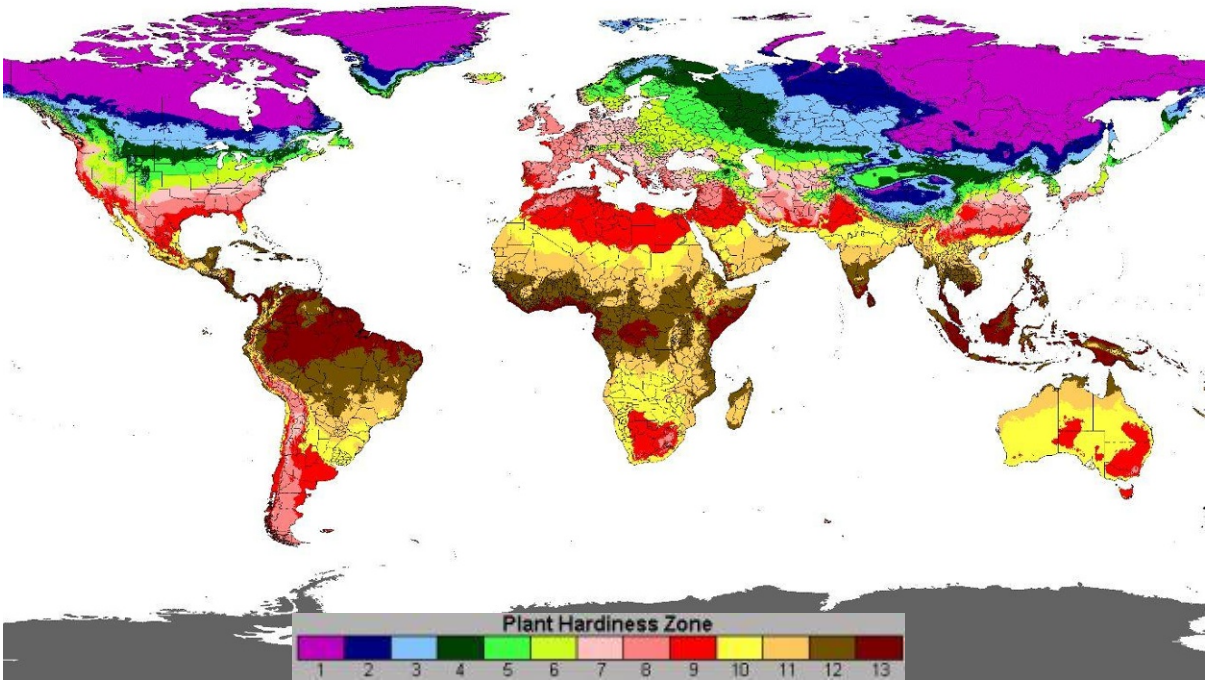

Figure 4: Global plant hardiness zones as reported in Magarey et al., 2008. *Aromia bungii* is known to occur in areas of East Asia corresponding to plant hardiness zones from 4 to 13 which are also well represented in western Europe (Source: Roger D. Magarey, North Carolina State University)

1.7. Spread capacity

Two types of movements that are relevant for the spread of this pest species can be distinguished: first, the capacity of the species to move at local level, this is once it has been introduced or become established in an area; and second, the capacity of the species for long-distance dispersal or assisted dispersal through other pathways.

Regarding local movement or spread of the species at local level, adult beetles of *Aromia bungii* can fly. However, there are very limited experimental or observational data on its active dispersal. The flight pattern of *A. bungii* is not only dependent on the abundance and presence of suitable host plants, but is probably also influenced by pheromonal cues (although these have still not been completely characterised) released by the male (Xu et al., 2017). Experimental assessment of flight behaviour in males and females implies the existence of an airborne factor released by *A. bungii* males which attracts the females (Yasui et al., 2019). In field conditions, adults of *A. bungii* were observed to fly to heights of more than 10 m. Moreover, adult individuals respond rapidly to an approaching object. Therefore, it is assumed that they possess good eyesight and can use vision in addition to olfactory senses for their orientation, movement and avoidance behaviour.

Although there are no detailed studies available on the rate of *A. bungii* spread by natural means, flight behaviour is estimated as being comparable to that of *Anoplophora* spp. (i.e. *A. glabripennis* or *A. chinensis*) which is estimated to have a spread range of 2–3 km per season (Smith et al., 2009. However, this distance is influenced by the abundance of and degree of proximity to suitable host plants (Smith et al., 2009). In situations where no host plants are available, these species can fly longer distances. In Lombardy, Italy, the pattern and spread of new infestations of *A. chinensis* indicate that new infestations are mainly found within a radius of 500–670 m from previously infested sites (Cavagna et al., 2013) and the chance of finding a new infestation is close to 99% within a radius of 400 m from a previous infestation focus. This means that adults will tend to fly and infest neighbouring trees and spread over short distances (less than 400 m). So, assuming that *A. bungii* shows a similar behaviour, these scales of magnitude should be considered. This view is supported by analyses of infestation foci in Italy (Lombardy and Campania) which indicate a spread rate of approximately 2 km in 6 years in Lombardy, while in Campania the species reached 5 km after 6 years.

At local scales (within or near infestation sites), human-assisted short distance dispersal of eggs and larvae is considered unlikely. Eggs, larvae and pupae may be present on and in cut branches. However, cut branches will probably be too small for the larvae to complete their development (EPPO, 2014). Although the risk of survival in small branches and saplings is regarded as unlikely, in infested areas the following activities should be considered as potentially relevant regarding the spread of the pest, i.e. local displacement of infested logs, wood and plants for planting.

There are indications that adults of *A. bungii* may hitch-hike in wood commodities as some beetles were found on premises where packaged goods have been imported (EPPO, 2014). Long-distance and international dispersal is likely to happen by means of assisted dispersal of eggs, larvae or pupae hidden in packaging material. This has been the pathway/commodity in some of the phytosanitary interventions of *A. bungii* (in the UK, USA and Japan), confirming that this packaging material could be a significant pathway for the introduction of specimens.

1.8. Risk factor identification

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 *Aromia bungii*. These activities should then be connected to specific locations also called 'risk locations'. Risk areas can be defined by taking into consideration the spread capacity of the pest and the availability of host plants around these locations. For *A. bungii* there are two relevant pathways that should be considered when considering pest surveys.

Wood packaging material

The main introduction pathway of *A. bungii* in the EU Member States is probably wood packaging material (pallets, crates, dunnage, etc.). Other potential pathways are wood or wooden products of *Prunus* species which are large enough to sustain live larvae until adult emergence. Living adults may hitch-hike in imported goods but this is probably only occasional. Therefore, areas with *Prunus* species present that surround ports and warehouses are particularly exposed to the pest and should be carefully considered for detection purposes.

Infested plant material

Plants for planting (saplings), and bonsais can also sustain eggs or initial larval stages. Therefore, nurseries where *Prunus* species are cultivated are also prime targets for detection surveys. Moreover, although the risk of survival in small branches and saplings is regarded as unlikely, in infested areas the local displacement of infested logs, wood and plants for planting should be considered as potentially relevant regarding the spread of the pest.

Figure 5: Signs of *Aromia bungii* infestation: (A) frass deposition on the base of the trunk; (B) frass on the base of a *Prunus* branch in the crown; (C) detail of larval galleries on a senescent tree; (D) detail of larval galleries in a transversal section of an infested branch. (Source: Bayerische Landesanstalt für Landwirtschaft (LfL))

2. Detection and identification

2.1. Visual examination

The life cycle, behaviour and feeding habits of *Aromia bungii* is similar to those of wood-boring cerambycids. Beetle larvae bore tunnels in the vascular tissues of the trunk and the tree's basal branches and at later infestation stages, they can reach the heartwood. At the end of these tunnels, they form pupal chambers where pupae overwinter. As is the case with most subcortical insects and other cerambycids detection based only on symptoms can be difficult. Surveys based on the superficial inspection of trees and/or wood for packaging is inefficient because larvae and pupae are usually found deep in the wood (EPPO 2014). During later developmental stages (i.e. development of mature larvae and during pupation) the frass produced in the excavation of larval galleries may accumulate around the base of the trunk or on the crown giving therefore indication of an infestation. Detection of infested trees is possible by detection of frass, mainly at the base of the trunk (although some may be seen on the bark or near the crown in upper branches) (Xu et al., 2017, Zou et al., 2019). If infestation is suspected, it is recommended to remove the bark of the tree to reveal larval galleries and holes (Figure 5).

Adults can also be observed in field conditions because of their diurnal activity, their large size (23–37 mm), and often shiny colouration (Figure 6).

2.2. Description of the pest

In infested areas, different stages in the development of *Aromia bungii* can be detected during visual inspections:

- **Eggs:** elongated, subcylindrical approx. 2 mm long. Average size of the eggs found in infested areas in Italy is 2 mm long and 1 mm wide, and light green in colour (CABI, 2019). Eggs are laid in bark crevices or under lichens growing on bark surfaces of older trees. Due to their small size and hidden oviposition sites, eggs are not easily detected.
- **Larvae:** hatched larvae are 2-2.5 mm long; mature larvae are 42- 52 mm (Figure 7). There are two distinct morphological types (Garonna, 2012) similar to what occurs with *Aromia moschata* (Duffy, 1968), designated as larval types 'a' and 'b'. The 'a' forms are usually *ca.* 50 mm long and ca. 10 mm wide and show the broadest body area across the prothorax, with body segments narrowing towards the abdominal apex. The 'b' forms are cylindrical and compact (Garonna, 2012). Both larval types present very pale colours (yellowish-white) and show 4-segmented legs. Form 'a' larvae show strong and very prominent mandibles in which the basal part is as dark as the apical part. The 'b' forms show shorter mandibles with basal parts whitish and separated from a darker apical part by a deep transverse incision.
- **Pupae:** the pupae show a light yellow colour and are 22-38 mm long showing clearly defined legs, and long coiled antennae. As reviewed by CABI (2019), the pupae become gradually darker resembling the coloration of immature adults.
- **Adults:** the adult form of *A. bungii* is recognizable by its brightly black elytrae and the red dorsal region of the prothorax which renders its common name i.e. red neck longhorn beetle. However, *A. bungii* ssp. *cyanicornis* Guérin-Méneville, 1845 is entirely black (Figure 8). In Europe, the only native species of the genus *Aromia* is *A. moschata* which is considerably smaller (13-35 mm) than *A. bungii,* presents a different habitus, metallic green/blue elytrae and in consequence is easily differed from *A. bungii* (Figure 9).

Figure 6: (A–B) General appearance (habitus) of *Aromia bungii* male under field conditions; (C) detail of red dorsal region of the prothorax (Sources: Photo A: Bruno Espinosa; Photos B: and C: shutterstock.com)

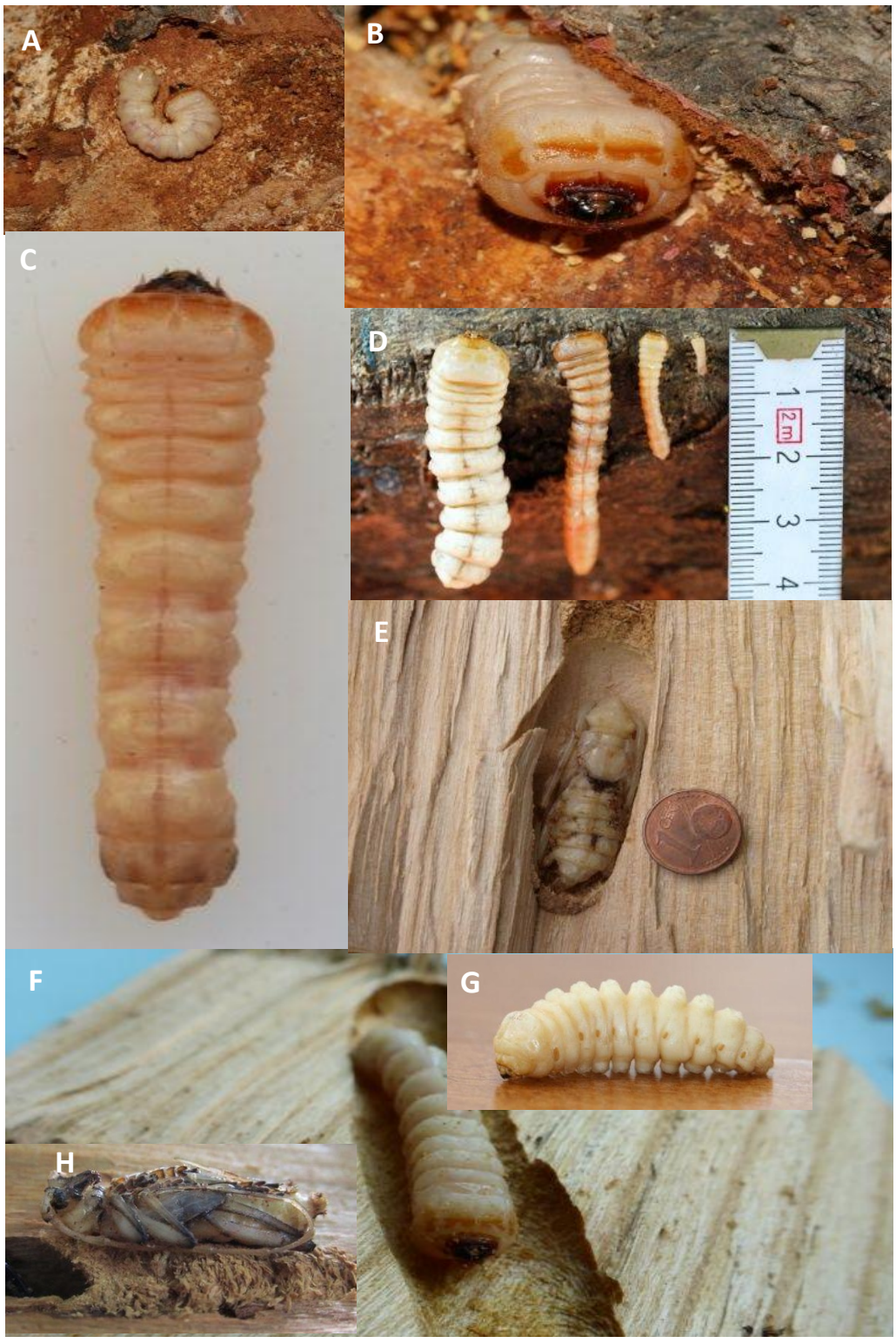

Figure 7: General views of *Aromia bungii* larvae and pupae: (A) lateral view of fourth instar larvae; (B) front view of fourth instar larvae with detail of mouth parts; (C) full dorsal view of fourth instar larva (Photos A–C, Source: Bruno Espinosa); (D) comparison of four instar larvae (Source: Bruno Espinosa); (E) pupa of *A. bungii*; (F) larval gallery and larva of *A. bungii*; (Photos D–F Source: Bayerische Landesanstalt für Landwirtschaft (LfL)); (G) lateral view of larva (Source: Antonio Garonna); (H) pupa laid on frass plug produced by last instar larva (Source: Antonio Garonna)

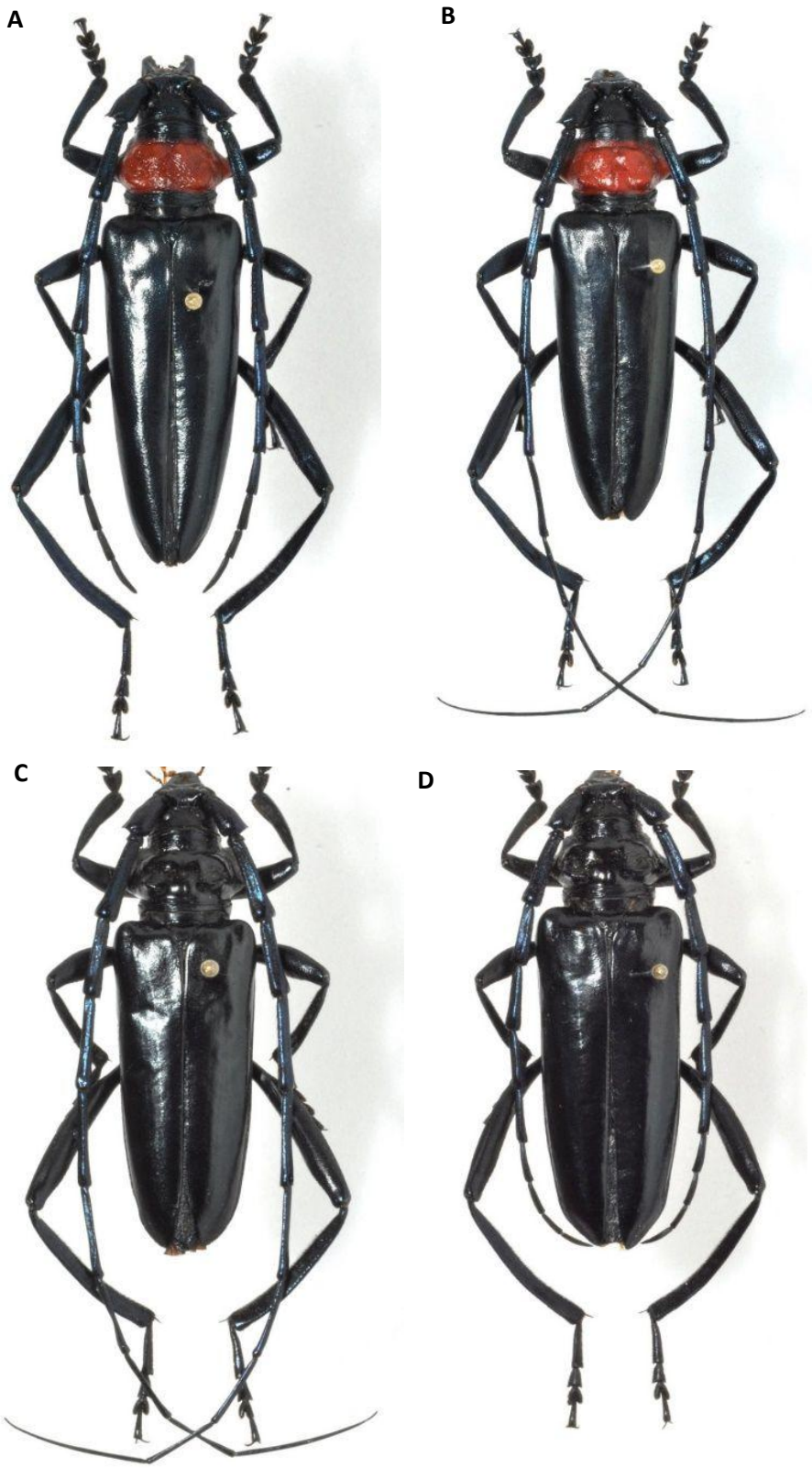

Figure 8: General morphology of (A) male and (B) female of *Aromia bungii*; in the lower part of the panel, *A. bungii* ssp. *cyanicornis* male (C) and female (D) (Source: Pierre Haller)

Figure 9: General morphology of the only congeneric species of *Aromia bungii* in Europe: *A. moschata* (Source: Wikimedia image registered under Creative Commons (CC BY-SA 2.5))

2.3. Symptoms

Aromia bungii larvae bore into both the sapwood and heartwood of main trunks and large branches, weakening trees and occasionally killing them (Gressitt, 1942; Duffy, 1968). More recent literature reports that the larvae mainly tunnel the subcortical area beneath the bark and the sapwood and less commonly in the heartwood, leading to loss of fruit production and weakening of the trees (EPPO, 2013a). So visual inspection during surveys may focus on senescent or decaying-looking trees to detect advanced stages of infestation. However, the presence of the pest on younger trees as demonstrated in the outbreaks in Italy means that inspection should not neglect younger individuals (Yu and Gao, 2005; Li et al., 2018).

The first symptoms that can be detected in field conditions are accumulations of larval frass at the base of tree trunks and the emergence holes that have an oval shape (with a diameter of *ca.* 12 mm) (Figure 5). Detection of infested trees is possible due to the accumulation of frass that is noticeable mainly at the base of the trunk (although in some cases may also be seen on the bark or near the crown in the upper branches). Frass has a reddish appearance (Figure 5A) and it is produced by larvae, the volume of frass produced increases with the age and size of the larvae and therefore it may be correlated with the degree of infestation. This sign is difficult to detect at the beginning of infestation but becomes easier at later stages. This symptom is not only restricted to the infestations produced by *A. bungii* but it is also produced by other species such as *Cossus cossus* (Linnaeus 1758) (Lepidoptera: Cossidae) or *Capnodis tenebrionis* (Linnaeus 1758) (Coleoptera: Buprestidae) that are common pests of *Prunus* spp. in Europe. Exit holes at the base of the trunk or main branches show that a first generation has completed its development, but younger living larvae can still be present in the wood, which will emerge

o or more years later. Therefore, if infestation is suspected it is recommended to remove the bark of the tree to reveal larval galleries and holes (Figure 5). Destructive sampling is therefore required to verify that a tree is infested, i.e. by removing the bark we may found larvae feeding in the phloem and cutting the trunk transversally will reveal excavated galleries of mature larvae in the heart wood. In late stadia of larval development, frass accumulates abundantly at the base of infested trees (Xu et al., 2017; Zou et al., 2019).

2.4. Traps

Current surveillance methods for *Aromia bungii* are limited to visual inspections of primary host plants. In the field, infestations can be detected by the accumulation of larval frass and the presence on the trunk of exit holes, and by the presence, at late infestation stages, of the bright coloured adults. Other than visual inspection, the use of bottle traps lured with fermenting liquids, fruit juice and vinegar has been reported, but the efficacy of these baits was very poor.

Fukaya et al. (2017) reported that females of *A. bungii* were attracted to the odour of males in laboratory bioassays, and Xu et al. (2017) identified the structure of the major male-produced aggregation-sex pheromone which was identified as (*E*)-2-*cis*-6,7-epoxynonenal. The synthesis of this compound is relatively costly but the racemic (*E*)-2-*cis*-6,7-epoxynonenal obtained from a commercially available precursor of the natural pheromone is relatively affordable. In field trials in China and Japan, *A. bungii* has been efficiently trapped using lures of racemic (*E*)-2-*cis*-6,7-epoxynonenal (Zou et al., 2019). Both sexes are attracted but female attraction is significantly higher (Yasui et al., 2019). These trials show that the racemate could be an appropriate method for large-scale monitoring.

Based on the results of these studies and further trials with pheromone lures (Zou et al., 2019) pheromone traps for *A. bungii* are now available on the Chinese market. The pheromone is very potent and based on trials by the manufacturing company (Nanjing Xinan SinoGreen Biological Technology Co. Ltd), yields reliable results useful for monitoring. The commercial lures predominantly attract females which also makes it potentially useful for local eradication programmes.

Given the presented advance in the use of pheromone-based methodologies, the use of these methods in pest surveys should be implemented together with the visual inspection of the main target species. Pheromone traps should be placed at the beginning of the emergence period (May–August). Although the details of pheromone use are still under evaluation in Italy (detailed trapping density, timing, etc.), it seems clear that pheromone trapping will become an efficient and accurate monitoring tool in the future.

2.5. Laboratory testing and pest identification

Molecular identification is possible by PCR amplification of the ribosomal 28S and the mitochondrial COI genes. COI sequences of *A. bungii* are available in Genbank (Zhang and Ren., unpublished data, 2016). In the Campania region, one of the outbreak regions in Italy, only one haplotype has been detected so far in the analyses of several field samples (CABI, 2019). As for other beetle species (e.g. *Anoplophora chinensis*) there are non-invasive identification methods based on PCR amplification of DNA obtained from faecal material. These methods allow confirming the identity of specimens found on infested trees (Strangi et al., 2013). These types of protocols could be used to extract DNA from frass collected under infested trees in *A. bungii* outbreak areas and be used as diagnostic method.

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 *Aromia bungii*

	Definition	**Unit**
Target population	All hectares in a Member State where *Prunus* species are present	Total number of hectares
Epidemiological unit	A single hectare that contains at least one individual of *Prunus* species or alternative host plants for *Aromia bungii*	A single hectare
Inspection units	Individual *Prunus* trees or alternative host plants or traps for *Aromia bungii*	Individual trees or traps

The general guidelines for the risk-based statistically sound surveillance are presented in a separate document and describe the process of the survey design step by step and include:

- the choice of the type of survey to develop depending on the objectives of the survey
- a manual for guiding the user through the statistical tools for sample size calculations
- essential considerations when:
  - choosing the sampling sites and taking the samples
  - collecting the data
  - reporting the data and the survey results.

To design a survey on *A. bungii* the following steps will generally be necessary:

1/ Determine the type of survey based on its objectives. For *A. bungii* the type of survey will depend on the pest status (according to 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. bungii*, it is necessary to select the host plants that are relevant for the survey area. For example, the target population could be all hectares in a Member State where *Prunus* hosts are grown or occur. The epidemiological unit would then be a single hectare where at least one individual of the *Prunus* is grown. 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. This would be the number of single hectares in a Member State where the host of interest is grown.

4/ Determine the inspection unit. In the case of *A. bungii* the inspection units are *Prunus* trees or non-*Prunus* trees reported as potential hosts.

5/ Determine the number of inspection units per epidemiological unit. In the case of an almond orchard, this is the average number of almond trees per epidemiological unit.

6/ Develop a sampling procedure within the epidemiological units and determine the method sensitivity. For example, when examining *Prunus* species, a representative number of plants should be examined by taking a representative number of samples. 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 being present or detected in a specific portion of the target population. It may not always be possible to identify or include a risk factor into 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 one needs to survey in a Member State in order to state with 95% confidence that the prevalence of *A. bungii* in *Prunus* will be at 1% or below.

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 should be adjusted, or the survey design should be adjusted, necessitating to go 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 inspection.

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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	Pest freedom can be defined, for a given target population, in a statistical framework, as the confidence of freedom from a certain pest against a preset design prevalence (threshold of concern).
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 to determine the presence or absence of pests, or the boundaries or characteristics of a pest population in an area, place of production or production site (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 examination other than visual, of plants, plant products or other regulated articles to determine whether pests are present, identify pests or determine compliance with specific phytosanitary requirements (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	Examination using the unaided eye, lens, stereoscope or other optical microscope (ISPM 5: FAO, 2019).

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