PEST SURVEY CARD

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Pest survey card on *Agrilus planipennis*

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Abstract

This pest survey card was prepared in the context of the EFSA mandate on plant pest surveillance (M-2017–0137), at the request of the European Commission. Its purpose is to guide the Member States in preparing data and information for *Agrilus planipennis* surveys. These are required to design statistically sound and risk-based pest surveys, in line with current international standards. The buprestid beetle *A. planipennis* is a highly destructive Union quarantine and priority pest of ash trees (*Fraxinus* spp.) native to Asia. The pest has ravaged ash resources in North America and is currently spreading in European Russia and eastern Ukraine. The introduction of plant material of host taxa into EU Member States from areas where the pest is present is regulated. All European *Fraxinus* species are suitable hosts. Larvae feed on the phloem of infested ash causing extensive dieback and tree death. The beetle requires one year to complete one generation, or two years in colder climates. Adults are found in spring and summer. Climatic conditions in the EU territories are suitable for the establishment of *A. planipennis* and host trees are widely available across the EU in forests, parks and cities. The pest spreads by active flight and human-assisted dispersal at an average rate of 1,500 m per year. Infested trees show distinctive symptoms and signs, but due to their late appearance surveillance based on symptoms is not suited for detection at low insect densities. The use of traps baited with attractants targeting adult beetles is an effective method to detect the pest at early stages of the infestation and is the recommended approach for implementing surveillance within the EU.

Keywords: emerald ash borer, *Fraxinus* spp., invasive alien species, plant pest, priority pest, risk-based surveillance, Union quarantine pest

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Introduction

The information presented in this pest survey card was summarised from pest risk assessments of *Agrilus planipennis* for the EU territory (EPPO, 2013a; VKM, 2014) and other documents like EFSA opinions (EFSA PLH Panel, 2011; EFSA, 2019), European and Mediterranean Plant Protection Organisation (EPPO) standards on phytosanitary measures (PM9/14) (EPPO, 2013b), and International Standards for Phytosanitary Measures (ISPMs) from the International Plant Protection Convention (IPPC) and relevant literature.

The objective of this pest survey card is to provide the relevant information needed to conduct surveys for detecting *Agrilus planipennis* in EU Member States (MSs) according to the methodology described in EFSA (2018). This survey card is part of a toolkit designed to assist MSs with planning a statistically sound and risk-based pest survey approach in conformity with ISPMs (ISPM 6: FAO, 2018; ISPM 31: FAO, 2016a) and International Plant Protection Convention (IPPC) guidelines for surveillance (FAO, 2016b). The toolkit consists of pest-specific and generic documents (relevant for all pests to be surveyed):

1) Pest-specific documents:
   a) Pest survey card on *Agrilus planipennis*[1]
   b) Guidelines for statistically sound and risk-based surveys of *Agrilus planipennis* that guide the reader through the entire process of survey design including the sample size calculations (EFSA, in preparation).
2) General documents:
   a) General guidelines for statistically sound and risk-based survey of plant pests (EFSA, 2020)
   b) RiBESS+ manual[2]
   c) RiBESS+ and SAMPELATOR[3] statistical tools for survey design.

1. The pest and its biology

1.1. Taxonomy

Scientific name: *Agrilus planipennis* Fairmaire, 1888

Class: Insecta, **Order**: Coleoptera, **Family**: Buprestidae, **Genus**: *Agrilus*, **Species:** *Agrilus planipennis*

Synonym(s): *Agrilus feretrius* Obenberger, *Agrilus marcopoli* Obenberger, and *Agrilus marcopoli ulmi* Kurosawa (EPPO, 2013a)

EPPO Code: AGRLPL

Common name: emerald ash borer (EAB)

Agrilus planipennis (Figure 1) is a phytophagous beetle within family Buprestidae and is native to eastern Asia. The synonymisation of *A. marcopoli ulmi* accessions from Japan with *A. planipennis* is questioned by some authors (Cipollini and Peterson, 2018; Orlova-Bienkowskaja and Volkovitsh, 2018).

Conclusions on taxonomy

Agrilus planipennis is a clearly distinct species within the genus *Agrilus* (Coleoptera: Buprestidae).

[1] The Pest Survey Card will be updated in the form of a Story Map that will be available in the Plant Pests Story Maps Gallery available online: https://efsa.maps.arcgis.com/apps/MinimalGallery/index.html?appid=f91d6e95376f4a5da206eb1815ad1489
[2] https://zenodo.org/record/2541541/preview/ribess-manual.pdf
[3] https://shiny-efsa.openanalytics.eu/

Figure 1: Adult of *Agrilus planipennis* (Coleoptera: Buprestidae). The insect body is bright metallic green, with distinctive coppery red coloration of the abdomen visible underneath the elytra (Source: David Cappaert, Bugwood.org)

1.2. EU pest regulatory status

Agrilus planipennis is a Union quarantine pest listed in part A of Annex II of Commission Implementing Regulation (EU) 2019/2072[4] and a priority pest under Commission Delegated Regulation (EU) 2019/1702[5]. Annual surveys must be conducted by Member States.

Special requirements for importing plants, wood, wood products and bark of *Fraxinus* L., *Juglans ailantifolia* Carr., *Juglans mandshurica* Maxim., *Ulmus davidiana* Planch. and *Pterocarya rhoifolia* Siebold and Zucc from countries where the beetle is present are laid down in Annex VII of Commission Implementing Regulation (EU) 2019/2072 with the aim of preventing the introduction of *A. planipennis*. Commission Implementing Regulation (EU) 2020/1292[6] introduced additional measures to prevent the entry of *A. planipennis* into the Union territory from Ukraine.

Derogations from Commission Implementing Regulation (EU) 2019/2072 on certain provisions for the same host plants mentioned above, and requirements for introduction into the Union of ash wood (*Fraxinus* spp.) originated and processed in USA and Canada are currently in place (Commission

[4] Commission Implementing Regulation (EU) 2019/2072 of 28 November 2019 establishing uniform conditions for the implementation of Regulation (EU) 2016/2031 of the European Parliament and the Council, as regards protective measures against pests of plants, and repealing Commission Regulation (EC) No 690/2008 and amending Commission Implementing Regulation (EU) 2018/2019. OJ L 319, 10.12.2019, pp. 1–279.

[5] Commission Delegated Regulation (EU) 2019/1702 of 1 August 2019 supplementing Regulation (EU) 2016/2031 of the European Parliament and of the Council by establishing the list of priority pests. C/2019/5637 OJ L 260, 11.10.2019, pp. 8–10.

[6] Commission Implementing Regulation (EU) 2020/1292 of 15 September 2020 as regards measures to prevent the entry into the Union of Agrilus planipennis Fairmaire from Ukraine and amending Annex XI to Implementing Regulation (EU) 2019/2072.

Implementing Regulation (EU) 2020/1164[7], Commission Implementing Regulation (EU) 2020/1002[8], Commission Implementing Regulation (EU) 2020/918[9]).

Plants in the genera *Fraxinus, Juglans* and *Ulmus* are included in the list of high-risk plants under Commission Implementing Regulation (EU) 2018/2019[10].

The general requirements for survey of quarantine organisms in the EU territory are laid down in Regulation (EU) 2016/2031[11] and the format and instructions are laid down in Regulation (EU) 2020/1231[12].

Overview of EU regulatory status

Agrilus planipennis is a Union quarantine pest, also listed as a priority pest. Special requirements for the introduction of plants and plant products of host taxa from areas where the pest is present are currently in place.

1.3. Distribution

Agrilus planipennis, or emerald ash borer (EAB), is an eastern Asian species, accidentally introduced into North America and European Russia (Haack et al. 2002, Liu et al., 2003; Baranchikov et al., 2008, Evans et al., 2020) (Figure 2). Its native range includes China (Beijing, Hebei, Heilongjiang, Jilin, Liaoning, Shandong, Tianjin and Xinjiang provinces), Russian Far East (Khabarovsk and Primorsky krai), and the Korean peninsula (Orlova-Bienkowskaja and Volkovitsh, 2018). Presence in Japan (Hokkaido, Honshu, Kyushu and Shikoku provinces) is based on the assumption that *A. marcopoli ulmi* is a valid synonym of *A. planipennis* (Orlova-Bienkowskaja and Volkovitsh, 2018). Similarly, records from Mongolia and Taiwan are ambiguous (Orlova-Bienkowskaja and Volkovitsh, 2018). Detected in the USA in 2002, the EAB spread quickly throughout eastern and mid-western North America. It is currently found in 35 eastern and mid-western US states and five Canadian provinces (USDA APHIS PPQ, 2020).

[7] Commission Implementing Regulation (EU) 2020/1164 of 6 August 2020 providing for a temporary derogation from certain provisions of Implementing Regulation (EU) 2019/2072 in respect of measures to prevent the introduction into and the spread within the Union of the pest *Agrilus planipennis* Fairmaire from Canada and the United States. OJ L 258, 7.8.2020, pp. 6–8.

[8] Commission Implementing Regulation (EU) 2020/1002 of 9 July 2020 establishing a derogation from Implementing Regulation (EU) 2019/2072 as regards the requirements for introduction into the Union of ash wood originating or processed in the United States. OJ L 221, 10.7.2020, pp. 122–126.

[9] Commission Implementing Regulation (EU) 2020/918 of 1 July 2020 establishing a derogation from Implementing Regulation (EU) 2019/2072 as regards the requirements for the introduction into the Union of ash wood originating or processed in Canada. OJ L 209, 2.7.2020, pp. 14–18.

[10] Commission Implementing Regulation (EU) 2018/2019 of 18 December 2018 establishing a provisional list of high risk plants, plant products or other objects, within the meaning of Article 42 of Regulation (EU) 2016/2031 and a list of plants for which phytosanitary certificates are not required for introduction into the Union, within the meaning of Article 73 of that Regulation. C/2018/8877 OJ L 323, 19.12.2018, pp. 10–15.

[11] Regulation (EU) 2016/2031 of the European Parliament of the Council of 26 October 2016 on protective measures against pests of plants, amending Regulations (EU) No 228/2013, (EU) No 652/2014 and (EU) No 1143/2014 of the European Parliament and of the Council and repealing Council Directives 69/464/EEC, 74/647/EEC, 93/85/EEC, 98/57/EC, 2000/29/EC, 2006/91/EC and 2007/33/EC. OJ L 317 23.11.2016, pp. 4–104.

[12] COMMISSION IMPLEMENTING REGULATION (EU) 2020/1231 of 27 August 2020 on the format and instructions for the annual reports on the results of the surveys and on the format of the multiannual survey programmes and the practical arrangements, respectively provided for in Articles 22 and 23 of Regulation (EU) 2016/2031 of the European Parliament and the Council OJ L280, 28.08.2020, pp. 1–15.

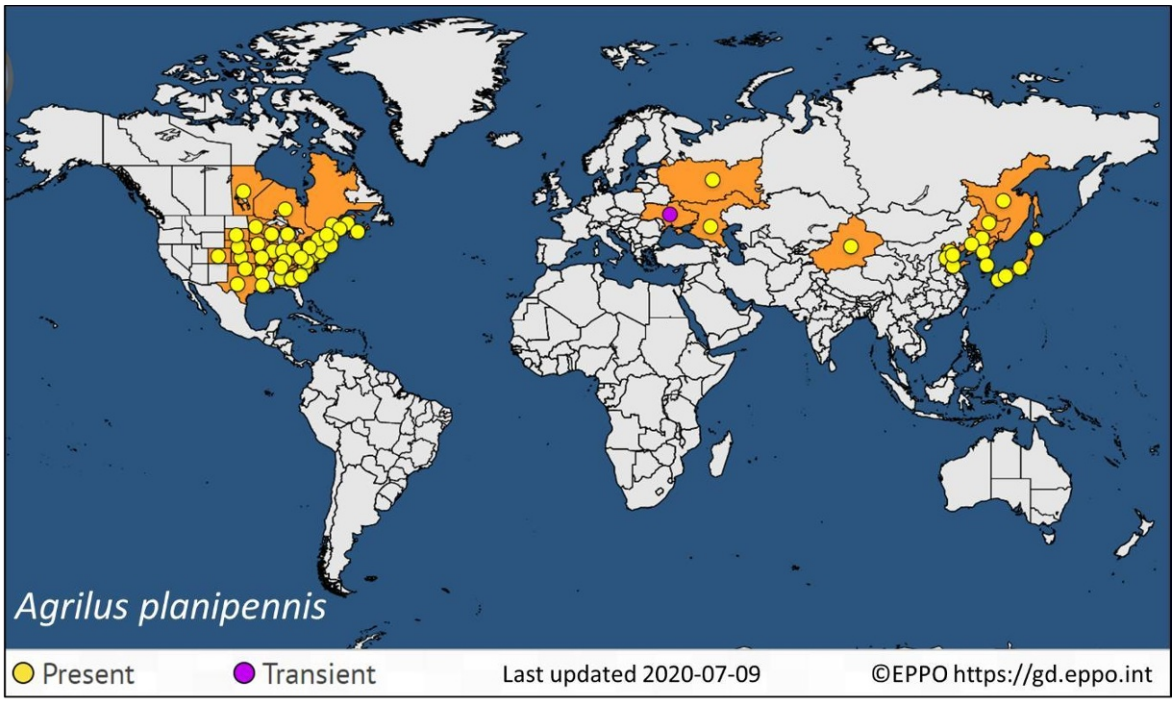

Figure 2: Global distribution of *Agrilus planipennis* (Source: EPPO Global Database, online)

The European distribution of *A. planipennis* is currently restricted to 16 regions of the Russian Federation (Belgorod, Bryansk, Kaluga, Kursk, Lipetsk, Moscow, Orel, Ryazan, Smolensk, Tambov, Tula, Tver, Vladimir, Volgograd, Voronezh and Yaroslavl) and to the Luhansk Oblast province in Ukraine, where outbreaks are under attempt of eradication (CABI, 2019a; EPPO, 2020; Orlova-Bienkowskaja et al., 2020). Further surveys in the Ukraine and bordering Belarus did not detect the pest (Orlova-Bienkowskaja et al., 2020) (Figure 3).

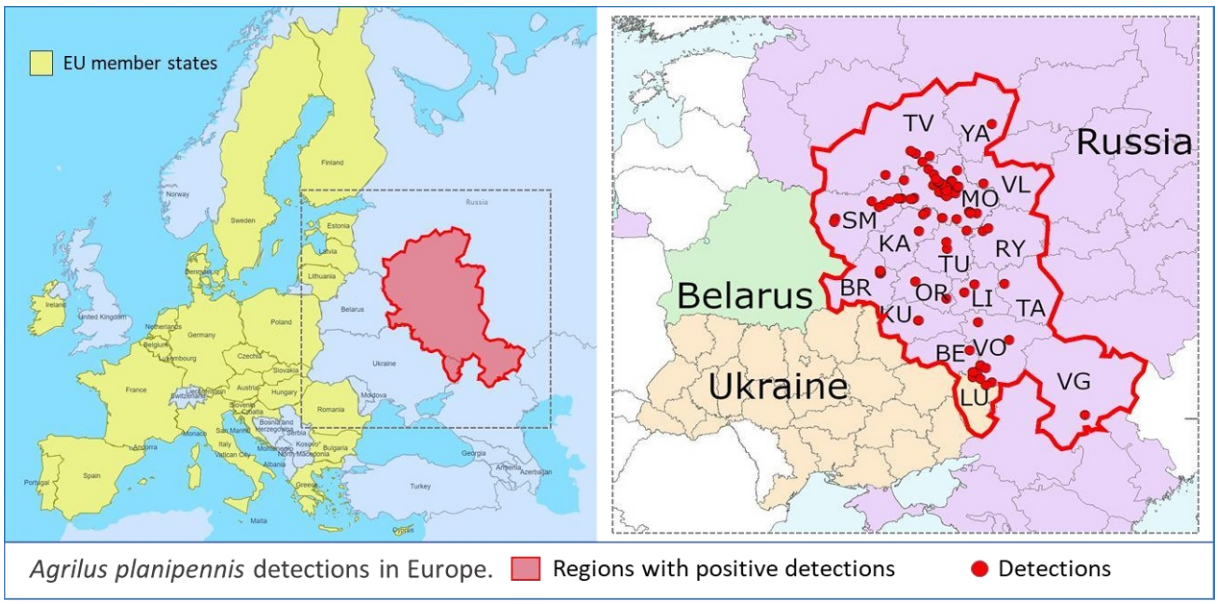

Figure 3: Distribution of *Agrilus planipennis* in Europe (Source: EU background map EC-GISCO; *A. planipennis* detection map modified from Orlova-Bienkowskaja et al., 2020, courtesy of Marina Orlova-Bienkowskaja)

Conclusion on distribution

Agrilus planipennis is currently not known to occur within the EU, but is present in European Russia and Eastern Ukraine, North America and native Asia. Surveillance is needed in all parts of the EU, where the beetle can establish. In particular, the eastern EU borders need specific attention (Finland, Estonia, Latvia, Lithuania, Poland, Slovakia, Hungary, Romania).

1.4. Life cycle

Agrilus planipennis usually completes one generation per year but individuals with a two-year life cycle are observed in colder climates, at lower insect densities on vigorous or less susceptible host trees, and when oviposition is late in the season (Cappaert et al., 2005; Wei et al., 2007; EPPO, 2013a; Herms and McCullough, 2014), including in Europe (Orlova-Bienkowskaja and Bieńkowski, 2016). Adults emerge from host trees during spring/early summer, feed on ash leaves (obligatory maturation feeding) and mate. Mated females lay eggs on and into bark cracks of host trees. Eggs hatch within 2 weeks from oviposition and first instar larvae penetrate the bark and feed on the phloem and cambial tissue through summer and autumn, moulting three times until they reach fourth instar, then dig 1–2 cm deeper in the outer wood or thicker barks of large trees finally moulting to overwinter as prepupae. In the following spring the beetles pupate and emerge from trees by chewing exit tunnels through outer wood and bark (Figure 4). Insects with a two-year life cycle overwinter as earlier larval instars at the end of the first year, and in the second year as prepupae.

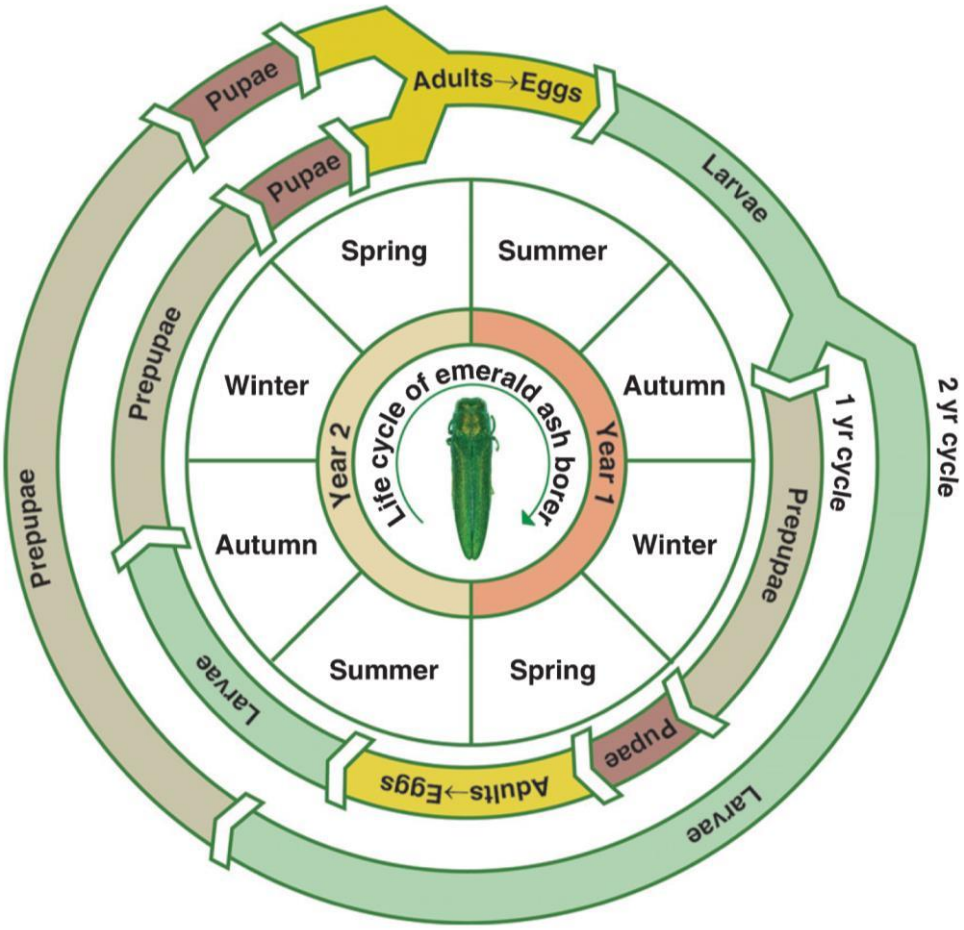

Figure 4: Life cycle of the *Agrilus planipennis*. Development can be completed in one or 2 years, depending on climatic conditions (accumulation of degrees days), oviposition time and host vigour (Source: Villari et al., 2015, courtesy of the New Phytologist Trust)

Agrilus planipennis requires at least 150 frost-free days (with minimum temperatures above 0°C) to complete one generation (Wei et al., 2007), and an accumulation of 450 degree days (base 10°C) of development (growing degree days, or GDD) to reach the emergence of adult beetles (USDA APHIS PPQ, 2018; Herms et al., 2019).

Conclusion on life cycle

Adults are present in late spring and during the summer, and this is the recommended period for trapping flying beetles. Insects undergo four larval instars, and usually overwinter as prepupae. One generation per year is common, but two years are required in certain conditions.

1.5. Host range and main hosts

Agrilus planipennis primary hosts are ash trees, *Fraxinus* species (Oleaceae) (Jendek and Poláková, 2014). All native European ash species *F. excelsior*, *F. angustifolia* (syn. *F. oxycarpa*, *F. oxyphylla*) and *F. ornus* are confirmed as susceptible hosts (EFSA PLH Panel, 2011; EPPO, 2013a; Baranchikov et al., 2014; Herms, 2015). Native hosts include Asian *F. mandshurica* and *F. chinensis*. All North American ash species including *F. americana*, *F. nigra, F. pennsylvanica* and *F. velutina*, are suitable hosts (Herms, 2015; Orlova-Bienkowskaja and Volkovitsh, 2018). *Fraxinus nigra* and *F. pennsylvanica* are the most susceptible to *A. planipennis*, while *F. quadrangulata* shows some resistance (EPPO, 2013a; Herms and McCullough, 2014; Villari et al., 2015). Asian ash species *F. chinensis*, *F. rhynchophylla* and *F. mandshurica* suffer significant damage from the EAB only when already stressed, with the latter species being the most resistant (Wei et al., 2004; EPPO, 2013a; Tanis and McCullough, 2015).

The North American white fringe tree *Chionanthus virginicus* (Oleaceae) is a secondary and sub-optimal host, and commercial olive *Olea europaea* was found to be susceptible in laboratory trials, but no reports exist of successful attack and breeding on plants in the wild (Cipollini and Peterson, 2018). *Ulmus* (Ulmaceae), *Juglans* and *Pterocarya* (Juglandaceae) species were described as potential hosts in Asia, but this has not been confirmed (Cipollini and Peterson, 2018; Orlova-Bienkowskaja and Volkovitsh, 2018), and species of those genera were found to be not suitable for larval development in field tests (Anulewicz et al., 2008). The susceptibility of other genera within the family Oleaceae was tested and none was found to be susceptible, but further host range expansion in newly invaded regions cannot be discounted (Cipollini and Peterson, 2018).

Conclusion on host range and main hosts

Agrilus planipennis utilises ash trees (*Fraxinus* spp.) as the main hosts, and the North American species *Chionanthus virginicus* as suboptimal host. All three European species *Fraxinus excelsior*, *F. ornus* and *F. angustifolia* are suitable.

1.6. Environmental suitability

1.6.1. Climatic suitability

Agrilus planipennis can tolerate high temperatures and shows remarkable thermal plasticity (Sobek et al., 2011). Climatic conditions in the EU territory are generally not a limiting factor for EAB establishment and spread (VKM, 2014), and maximum entropy modelling (MaxEnt) shows a high environmental suitability overall for the EAB in Europe (Flø et al., 2015). Both mean temperatures and precipitation across Europe are suitable for the development of all life stages of the beetle, and there is not likely to be a climatic barrier to its establishment (Figure 5). Even in northern European countries such as Sweden and Finland, minimum winter temperatures should not impede pest establishment as they do not fall below the insect's supercooling point (Orlova-Bienkowskaja and Bieńkowski, 2020), but beetles

populations in those countries are expected to require 2 years to complete development rather than one year (Orlova-Bienkowskaja and Bieńkowski, 2016).

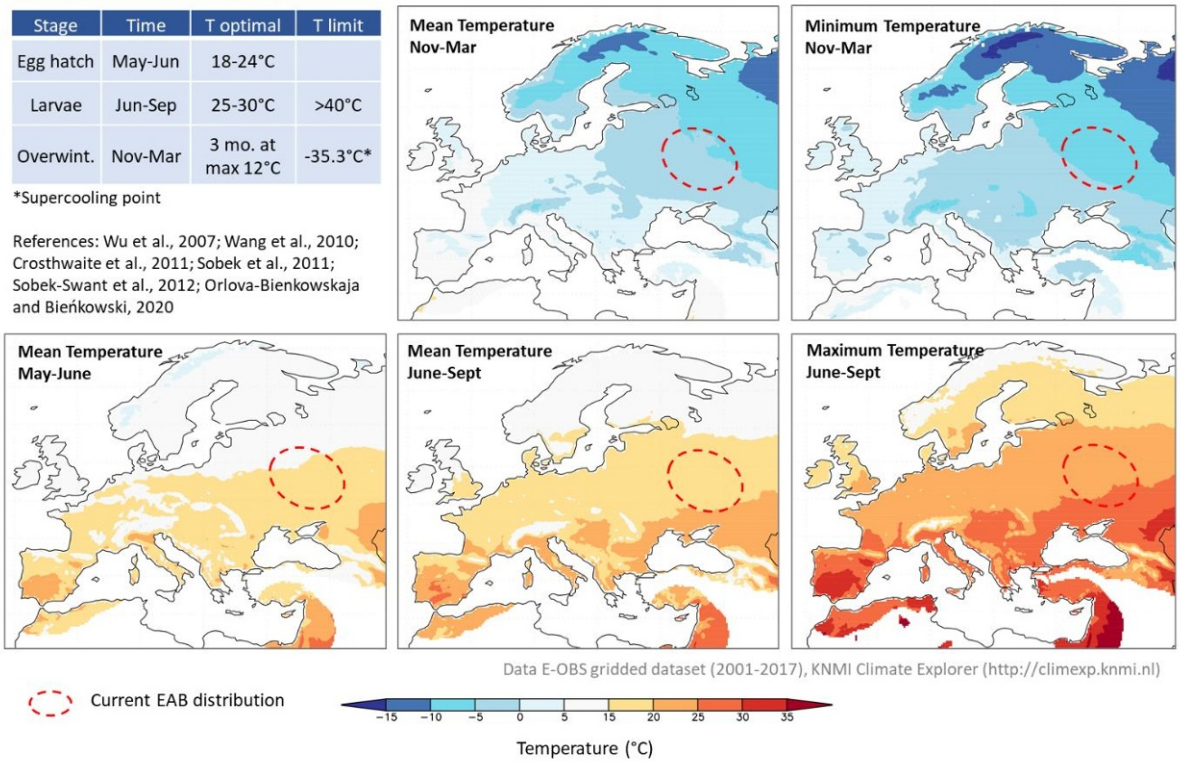

Figure 5: Thermal requirements of *Agrilus planipennis* stages and average temperatures for key months in Europe (Courtesy of Ignazio Graziosi, Giulia Villani and Lynne Rieske-Kinney)

1.6.2. Host availability

Considering the widespread climatic suitability of the EU territory for establishment of *A. planipennis*, the relevant indicator of environmentally suitable areas is the availability of ash trees. *Fraxinus* species native to Europe are European ash *F. excelsior*, south European flowering ash *F. ornus* and narrow-leafed ash *F. angustifolia* (syn. *F. oxycarpa*, *F. oxyphylla*) (Wallander, 2008). Their combined native range covers the entire territory of the EU (Figure 6). *Fraxinus excelsior* is the most widespread species and can be found in natural mixed and planted broadleaved forests and as a common urban tree across Europe and is widely used as a hedgerow tree in Great Britain (EFSA PLH Panel, 2011; EPPO, 2013a; Beck et al., 2016). *Fraxinus excelsior* is a very large component of floodplain forests in northern European countries, where it can be locally found at high densities (Valenta et al., 2915), and is the most abundant European ash species in eastern EU countries bordering Ukraine and Russia. *Fraxinus ornus* has a more southern distribution, while *F. angustifolia* presence coincides with the southern portion of *F. excelsior* range (Caudullo and de Rigo, 2016; Caudullo and Houston Durrant, 2016; Caudullo et al., 2017).

Several non-native *Fraxinus* species are cultivated in the EU as ornamental trees in cities and parks (especially in Eastern Europe), including North American *F. americana, F. latifolia*, *F. pennsylvanica*, *F. nigra*, *F. velutina*, and Asian species *F. mandshurica* (EFSA PLH Panel, 2011; EPPO, 2013a; CABI, 2019b). *Fraxinus pennsylvanica* in particular is commonly found in north-eastern European cities and widely planted in floodplains as a shelter belt and timber tree (Schmiedel et al., 2013).

Initial outbreaks of *A. planipennis* in the Moscow region of Russia were detected on *F. pennsylvanica*, but the pest then spread to *F. excelsior* in rural forest locations (Straw et al., 2013). In the Ukraine, the

pest is impacting shelter belts of *F. pennsylvanica*, and its occurrence in *F. excelsior* stands is under evaluation (EPPO, 2020; Orlova-Bienkowskaja et al., 2020).

The health of *F. excelsior* in many EU Member States is currently under threat by the fungal pathogen *Hymenoscyphus fraxineus,* which is causing ash dieback, thus increasingly impacting ash resources (McMullan et al., 2018). The interactions between the pathogen and *A. planipennis* are unknown and raise serious concerns. The spread of ash dieback could undermine the resilience of ash resources in respect to an upcoming invasion by *A. planipennis*, and canopy-level dieback caused by the pathogen might mask symptoms of insect attack and make visual detection of *A. planipennis* in areas impacted by the disease unfeasible. Furthermore, potential resistance of *F. excelsior* to *H. fraxineus* was associated with low levels of metabolites known to trigger insect deterrence in plants (iridoid glycosides), thus raising concerns that ash trees selected for resistance to the fungus might be highly susceptible to *A. planipennis* (Sollars et al., 2017).

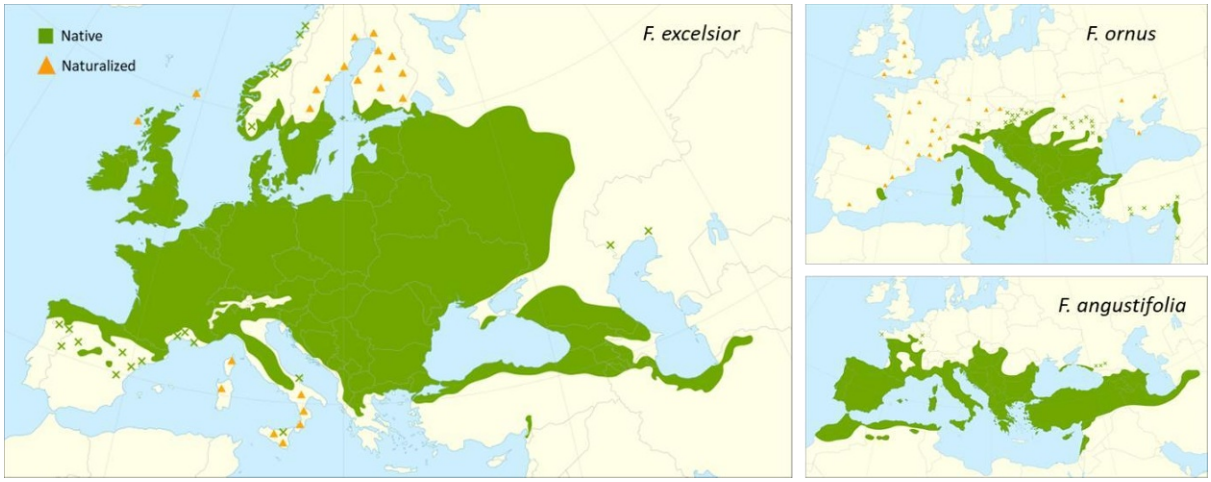

Figure 6: Range of native *Fraxinus* species in Europe (Source: modified from Caudullo et al., 2017)

Conclusion on environmental suitability

Climatic conditions in the EU territory are suitable for *Agrilus planipennis* establishment, but in colder regions the insect is expected to require two years to complete one life cycle. Host trees are widely available across the EU as native *Fraxinus* species in the forest, and both native and exotic trees in cities and parks.

1.7. Spread capacity

Main pathways for the potential introduction of *Agrilus planipennis* to the EU territory are identified as timber trade, solid wood packing material, wood chips, and live ash for planting material from countries where the pest is present (EPPO, 2013a; Flø et al., 2015; Evans et al., 2020).

Once populations are established, the pest disperses by natural (active flight), and human-assisted (passive transportation) means, resulting in a stratified (both local and long-distance) spread (Muirhead et al., 2006).

Assessing natural spread potential at a local level is crucial for delimiting areas of surveillance around points of potential introduction. EAB adults do not often fly great distances over open areas but they are good fliers (EPPO, 2005; Taylor et al., 2006; Lyons et al., 2007). The spread tends to be slower during the initial phases of population build-up and then accelerates. Monitoring efforts using systematic grids of girdled ash trees in two sites located 25 km apart in Michigan (USA) over a three-year period revealed that the rate of spread was slower in the newly infested site (0.4–0.7 km per year) compared with the older infested site (1.2–1.7 km per year) (Mercader et al., 2016). Females can lay eggs on

trees as far away as 2,000 m from the epicentre of an area with high population density, but tend to lay 90% of their eggs within 100 m of the source when within infestations from a single introduction point (Mercader et al., 2009, 2016). Pest control treatments such as insecticides can have a substantial influence on spread rates (McCullough et al., 2015).

Additional dispersal of *A. planipennis* is generated by human-assisted spread, involving the movement and transportation of infested ash material such as firewood, timber, wood packaging material and potted plants. *Agrilus planipennis* is thought to have been introduced accidentally to the USA through infested solid wood packing material from China (Poland and McCullough, 2006; EPPO, 2013b), and its devastating spread within North America was greatly facilitated by the unchecked movement of infested firewood (Muirhead et al., 2006).

After establishment, the spread of *A. planipennis* in invaded areas continues through a combination of natural and human-assisted mechanisms. Moving infested firewood or timber from the infested area accelerates the spread initially through jump spread beyond normal flight ranges followed by natural beetle spread at local scales. This combined mechanism can cause long jumps responsible for the high variability of invasion speed. *Agrilus planipennis* invasion in North America progressed at rates between 2.5 and 80 km per year, while in European Russia a progression of 13 to 41 km per year has been observed, likely facilitated by the ability of the pest to hitchhike (Prasad et al., 2010; Baranchikov and Kurteev, 2012; Valenta et al., 2017).

Based on an expert knowledge elicitation (EKE) about the spread rate, EFSA (2019) estimated that the maximum distance expected to be covered in one year by *A. planipennis* due to natural spread combined with human-assisted spread at local level (long jumps being excluded) is below 1,500 m in 50% of the cases and below 3,000 m per year in 75% of the cases and ranges from 100 to 10,000 m in 98% of the cases.

Conclusion on spread capacity

Agrilus planipennis exhibits stratified spread through a combination of local dispersal by active flight and long-range dispersal due to the movement of infested plant material. Females tend to lay 90% of their eggs within 100 m of the source when within infestations from a single introduction point, but the pest is expected to spread below 1,500 m per year in 50% of the cases (with a 98% uncertainty range of 100 to 10,000 m) resulting from combined natural and human assisted movement. However, in 75% of the cases, the spread rate would be below 3,000 m per year. These distances (i.e. 100, 1,500 and 3,000 m) are the key spread values recommended for designing a survey.

1.8. Risk factor identification

Identification of risk factors and their relative risk estimation are essential for performing risk-based surveys. A risk factor is a biotic or abiotic factor that increases the probability of infestation by the pest in the area of interest. The risk factors that are relevant for surveillance need to be characterised by their relative risk (should have more than one level of risk for the target population) and the proportion of the overall target population to which they apply. The identification of risk factors needs to be tailored to the situation of each Member State. This section presents examples of risk factors for *Agrilus planipennis* and is not necessarily exhaustive (Table 1).

For the identification of risk areas, it is first necessary to identify the activities that could contribute to introduction or spread of *A. planipennis*. These activities should then be connected to specific locations. Around these locations, risk areas can be defined, knowing that their size depends on the spread capacity of the target pest and the availability of host plants around these locations.

Example 1: Trade from non-EU countries where the pest occurs

The trade of host plant commodities from non-EU countries where the pest occurs is subject to special requirements laid down in Commission Implementing Regulation (EU) 2019/2072 (see Section 1.2). However, the risk of introduction of individuals hitchhiking into the EU from areas where the pest is present through main trade routes (timber, wood material and commodities of host taxa) cannot be

excluded. The pest is already present in European Russia and Ukraine, and it is likely that it would enter the EU from those eastern borders by hitchhiking from infested forested areas along the land transport network. The pest would then be able to further spread in the EU by natural dispersal. As an example, it is around 600 km on the highway (M9/E22) from Tver in Russia to Rēzekne in Latvia or 750 km to Tartu in Estonia (M9 and E95).

Therefore, risk activities are linked to the transport and trade of host plant commodities from areas where the pest occurs (Flø et al., 2015; Valenta et al., 2015). The corresponding risk locations (Lyons et al., 2007) include international airports and harbours, stops along main roads and railways, truck parking lots, wood storage and trade facilities, hardwood sawmills, trade centres such as nurseries and garden centres.

Example 2: Declining trees within healthy forests

Another risk factor is the presence of declining ash trees within a generally healthy forest. *Agrilus planipennis* is strongly attracted by stressed trees (Tluczek et al., 2011; Mercader et al., 2013). The occurrence of ash hosts impacted by biotic or abiotic stresses with patchy distributions within an area, is likely to increase the chance of infestation and the probability of detecting beetles through surveillance. This is particularly relevant in areas affected by the agent of ash dieback *Hymenoscyphus fraxineus* (Vasaitis and Enderle, 2017; Coker et al., 2019), although there is little information on the interaction. This risk factor could be considered when planning trap deployment during surveillance operations, for example for areas with patchy distribution of declining trees affected by ash dieback.

Table 1: Examples of risk activities and corresponding risk locations relevant for surveillance of *Agrilus planipennis*

Risk activity/source	Risk locations
Transport and trade of host plant commodities from areas where the pest occurs	Locations related to the transport, processing, distribution and trade of host plant commodities (e.g. international airports and harbours, stops along main roads and railways, truck parking lots, wood storage and trade facilities, hardwood sawmills, nurseries and garden centres)
Biotic or abiotic stress (for example infection from the agent of ash dieback *Hymenoscyphus fraxineus*)	Forests and areas with a patchy distribution of declining trees

1.8.1. Risk factors and survey design

Risk areas can be defined as specific areas in a set of epidemiological units (see Glossary) adjacent to the risk locations defined above. The definition of a risk area around a certain risk location takes into consideration the spread capacity of the pest and the availability of host plants.

For detection surveys, where the aim is to substantiate pest freedom (FAO, 2017), based on the estimated spread capacity of *A. planipennis* (see Section 1.7), three risk areas to be surveyed can be defined around the risk locations as the first 100 m (i.e. high-risk area), up to 1,500 m (i.e. medium risk area) and beyond 1,500 m (i.e. baseline risk area) (Figure 7).

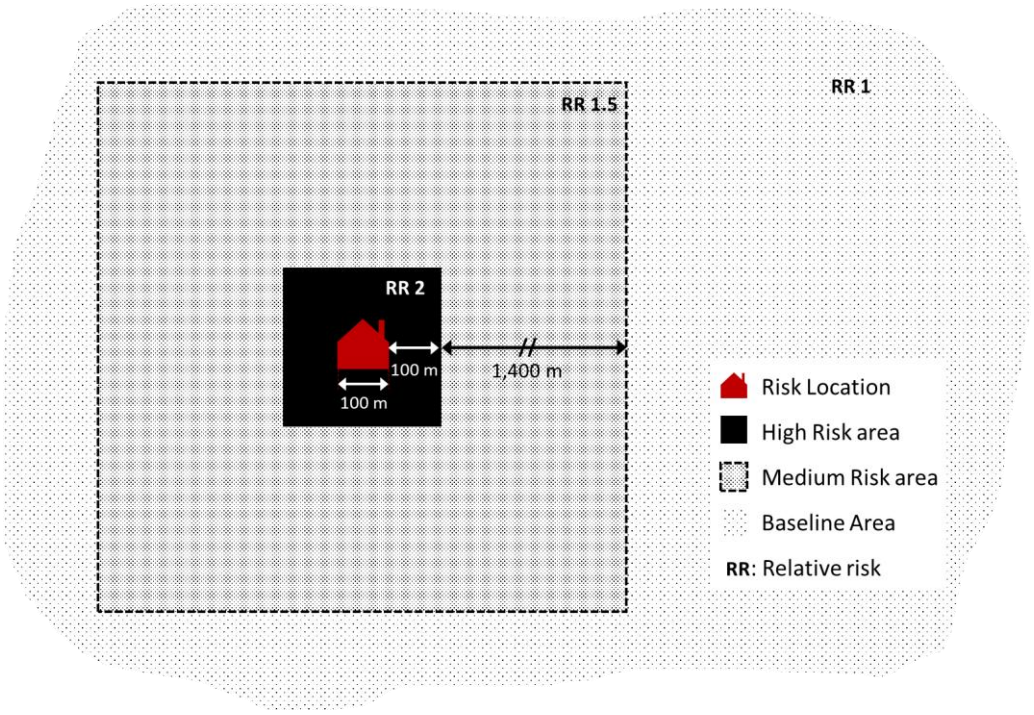

Figure 7: Risk areas and relative risk should be defined around risk locations when designing detection surveys. High risk area corresponds to risk location of 1 hectare (100 m side) surrounded by additional 100 m on each side. Medium risk area corresponds to the next 1,400 m (i.e. 1,500 m from the risk location) and baseline area corresponds to the area beyond this distance

The relative risk should also be taken into account in the survey design (EFSA, in preparation) in order to allocate survey efforts in the three different risk areas. Table 2 shows an example of the relative risk for the three risk areas (see also Figure 7). These areas might vary in size depending on the host plant availability (location and density) and other landscape features.

Table 2: Definition of three risk areas and their corresponding relative risk. Different risk areas (high risk, medium risk and baseline) around risk locations are connected to different surveillance efforts

Risk areas	Relative risk
High-risk areas are defined within 100 m around the risk locations where according to Mercader et al. (2009) about 90% of the larvae of a newly established EAB population are found	2
Medium risk areas are defined between 100 m and 1,500 m around the risk location as this corresponds to the median of the maximum yearly spread rate of the adult insects (EFSA, 2019)	1.5
Baseline area is the survey area beyond 1,500 m from the risk location	1

For delimiting surveys, i.e. once the pest is detected, in order to delimit the boundaries of the infested zone, the survey should start in a 1,500 m peripheral band (first survey band) around a provisional infested zone 3,000 m wide (based on spread capacity, see Section 1.7), for each year since the last detection survey was conducted, around the source of the infestation (e.g. the risk location where the pest was introduced). For example, in sites were detection surveys are conducted every year the provisional infested zone is set at 3,000 m, for sites surveyed every two year 6,000 m, and so on. Two situations can be distinguished (Figure 8):

- A positive detection is carried out in the first survey band: the provisional infested zone is enlarged by including the infested peripheral survey band. An additional peripheral band of 1,500 m should be surveyed. The procedure should be repeated until a survey band is found to be negative. The infested zone is then delimited, and a buffer zone of 10,000 m should be established around it, where intensive monitoring and detection surveys should be conducted.
- No detection is carried out in the first survey band: the provisional infested zone is narrowed down and an inner peripheral survey band of 1,500 m should be surveyed. The procedure should be repeated until the first positive detection in a survey band. The infested zone is then delimited, and a buffer zone of 10,000 m should be established where intensive monitoring and detection surveys should be conducted.

This procedure is described in detail in EFSA (in preparation) "Guidelines for statistically sound and risk-based surveys of *Agrilus planipennis*".

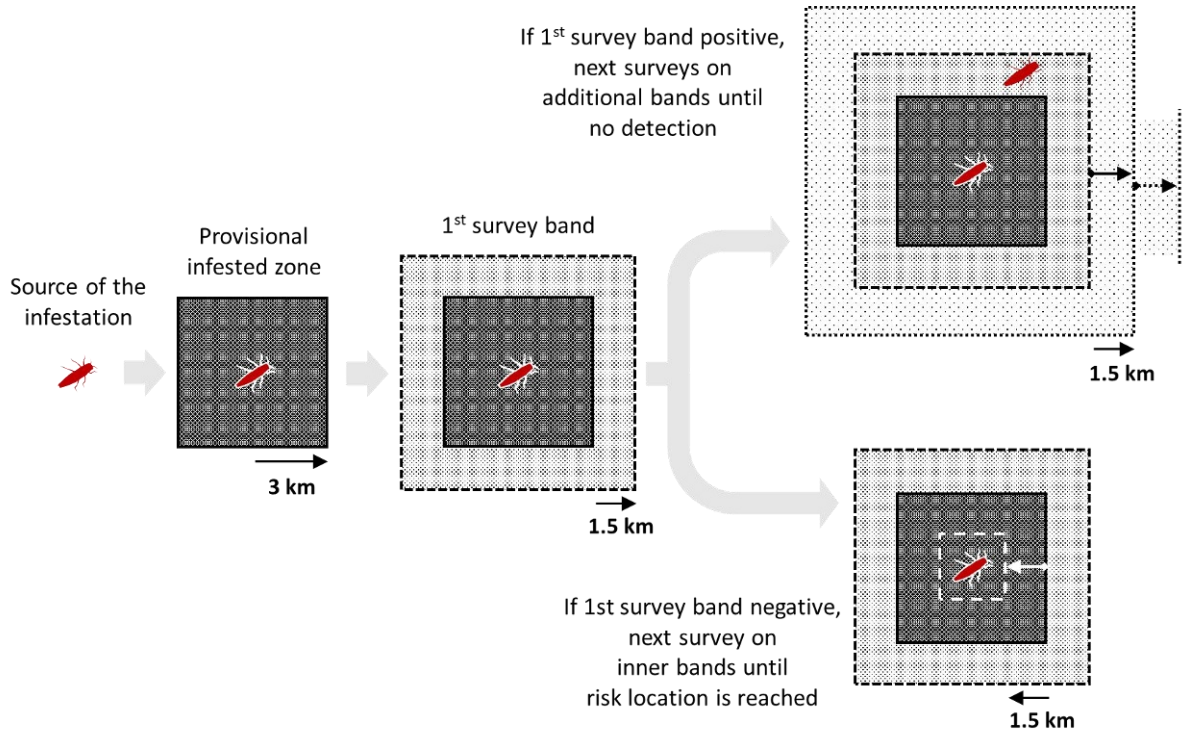

Figure 8: Once the pest is detected, the source of the infestation (e.g. risk location) is determined, the delimiting survey aims to define the boundaries of the infested zone. The illustration describes a site where the detection survey is conducted every year (i.e. 3,000 m provisional infested zone)

2. Detection

Agrilus planipennis infestations can be detected by visual examination of symptoms on attacked trees, by trapping adult beetles with traps, and by sampling potentially infested branches or stems. Symptoms are only noticeable at a landscape level years after insect populations have increased locally. This makes surveillance of *A. planipennis* based on visual examination and branch sampling unsuited for detecting new infestations. Proactive surveillance based on trapping adult beetles is the most appropriate tool for detection at early stages of the infestation. However, the observation of symptoms and branch sampling activities can aid the delimiting of infestations and can be used for evaluating population densities. A comprehensive description of sampling techniques for the detection of *A. planipennis*, including pros and cons for each approach, is provided by Ryall (2015).

2.1. Visual examination

Visual examination of tree symptoms and signs of *A. planipennis* infestation is only effective after the trees have been infested for several years (EPPO, 2013b: 3–4 years; DeSantis et al., 2013: up to 10 years). In North America the pest started spreading approximately 10 years before it was detected through visual signs (Siegert et al., 2014). By the time symptoms appear and signs are noticeable, trees are typically heavily infested, and there might be numerous additional infested trees with no clear signs in the same vicinity (Ryall et al., 2011).

Declining trees due to *A. planipennis* attack show distinctive symptoms at canopy level. Infestations on larger trees usually start from the upper canopy, on branches, then progress downwards, while on smaller trees the main stem could be attacked earlier. Larval feeding disrupts mostly the tree's phloem, thus impeding translocation of nutrients, and triggering canopy dieback and tree decline. Depending on larval density and tree size, ash can die within 5–7 years from initial infestation, but smaller trees can die within 1–2 years (Knight et al., 2013). Several other signs of *A. planipennis* can be noticed on stems and branches. Main symptoms of *A. planipennis* infestation and signs of insect activity can be summarised as follows (Wilson and Rebek, 2005; de Groot et al., 2006).

Symptoms:

- Canopy dieback, which increases with the progression of the infestation and the larval density in infested ash, usually starts from the top of the tree (EPPO, 2013b) (Figure 9A, B, D).
- Epicormic shoots produced on branches and stems below the infested area (Figure 9B, C).
- Smaller and sometimes chlorotic leaves. Leaves do not abscise well in autumn and petioles may stay on the tree in winter.

Figure 9: Dieback of sections of the canopy triggered by *Agrilus planipennis* on *Fraxinus pennsylvanica* (A); complete canopy dieback and growth of basal shoots on *Fraxinus* spp. (B); epicormic growth (water sprouts) and bark removed to show larval galleries on *F. pennsylvanica* (C); tree mortality on *Fraxinus* spp. in the forest (D) (Sources: (A, C, D) courtesy of Ignazio Graziosi; (B) Daniel Herms, Bugwood.org)

Signs:

- Serpentine larval galleries on the phloem under the bark (de Groot et al., 2006; EPPO, 2013b) (Figure 10A, B, C, D).
- Characteristic D-shaped exit holes produced by emerging adults, 6–9 mm wide, on the bark of stems and branches (EPPO, 2013b) (Figure 11A, B, C).
- Splitting of the bark of infested branches and small stems, occasionally with larval galleries exposed underneath (Figure 10B, D) (Lyons et al., 2007).
- Stripping damage and holes in the bark of stems and branches by woodpeckers feeding on *A. planipennis* larvae and pupae (de Groot et al., 2006). In heavily infested forests the noise produced by woodpecker activity is an additional sign (Jennings et al., 2013).
- Small notches on leaf laminae, produced by adult feeding (de Groot et al., 2006) (Figure 10D).

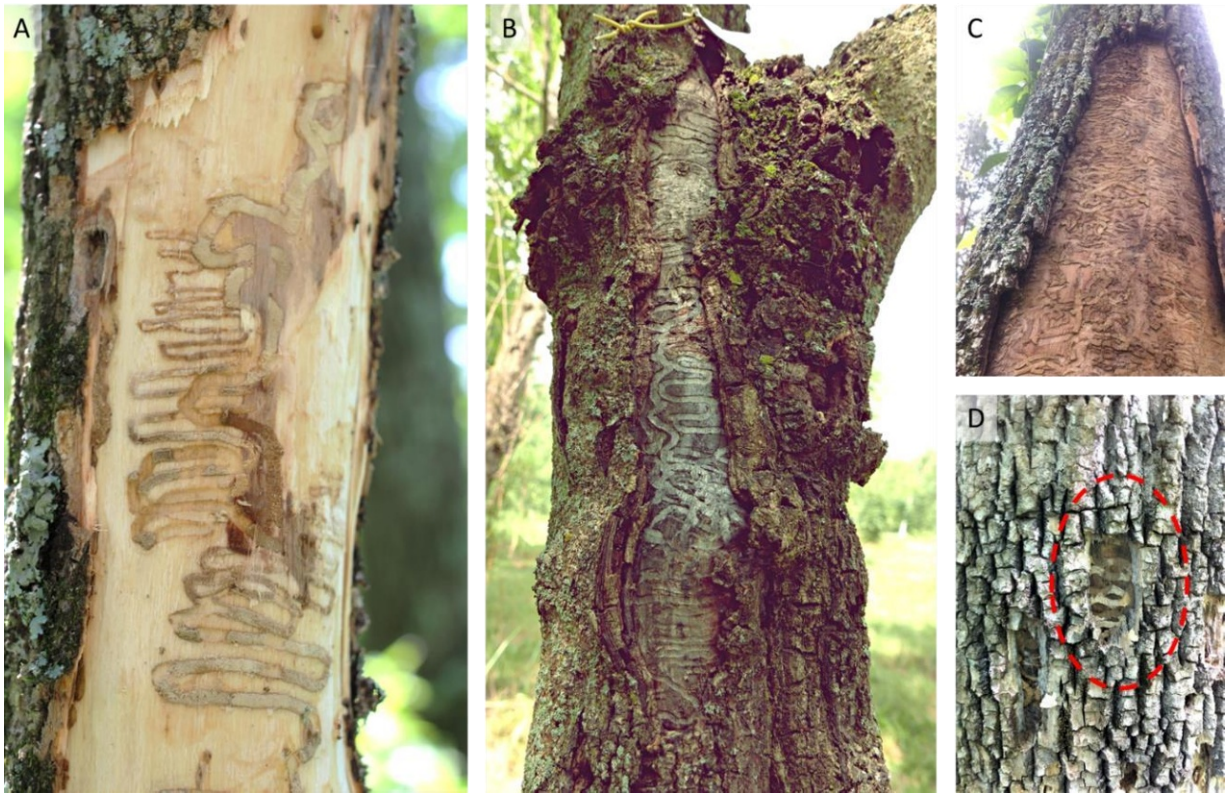

Figure 10: Typical serpentine larval galleries of *Agrilus planipennis* on a debarked stem of *Fraxinus pennsylvanica* (A); bark splitting and *A. planipennis* larval galleries on *F. angustifolia* in a common garden at the USDA Forest Service Northern Research Station, Ohio, USA (B); disrupted bark and old *A. planipennis* larval galleries on dead *F. pennsylvanica* (C); bark splitting and larval galleries on *Fraxinus* spp. (D) (Courtesy of Ignazio Graziosi)

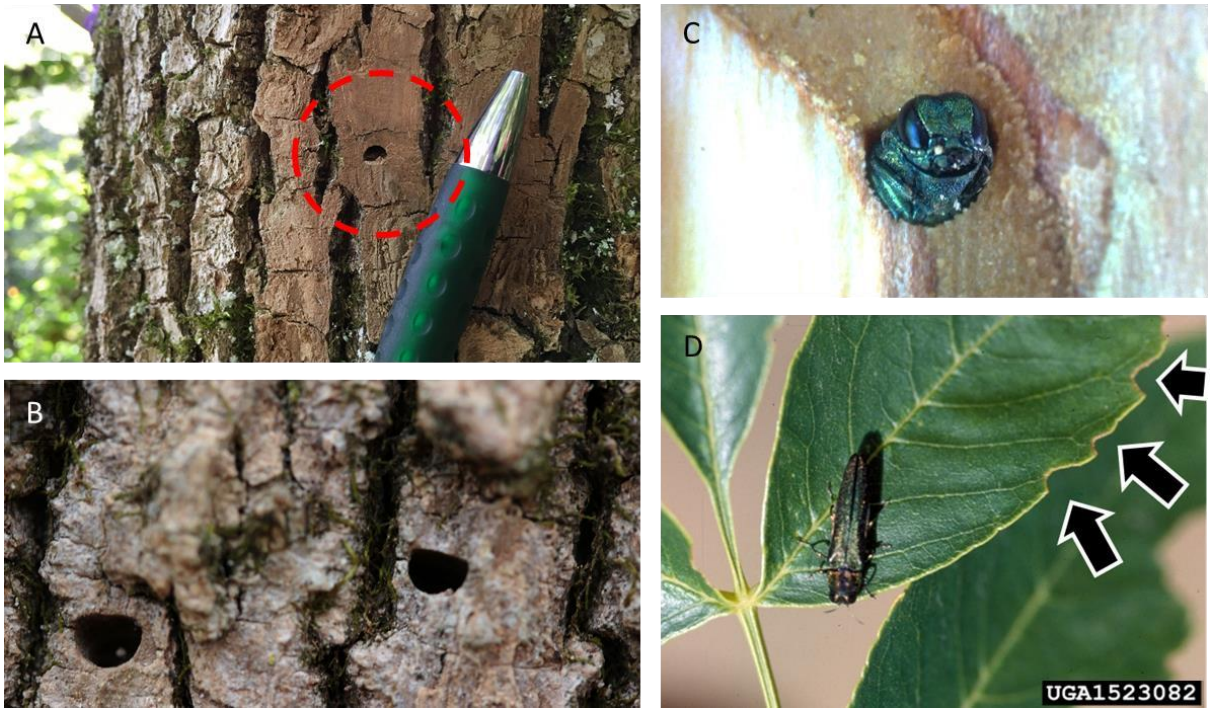

Figure 11: *Agrilus planipennis* adults on ash bark. Exit holes (6–9 mm diameter) of emerged adults visible on ash bark (A, B); adult beetle emerging from the host plant (C); adult beetle on foliage and leaf notches on leaves caused by the insect' feeding (D) (Sources: (A, B, C) courtesy of Ignazio Graziosi; (D) Daniel Herms, Bugwood.org)

Risk of misidentification of symptoms

D-shaped exit holes are common for *Agrilus* spp. and other Buprestidae (Lyons et al., 2007) and, in Europe, a possible confusion with the native species *A. convexicollis*, which also feeds on *Fraxinus*, is possible. Similar non-pest-specific symptoms could be caused by adverse weather conditions such as frost damage or by other pests and diseases, e.g. ash yellows, anthracnose, ash dieback in general (Lyons et al., 2007) and ash dieback caused by the fungus *Hymenoscyphus fraxineus* (NAPPO, 2009), which is already widely distributed in northern European countries. The situation in Estonia, Lithuania and Sweden is severe (Vasaitis and Enderle, 2017). Other bark beetles (*Hylesinus varius* and *H. crenatus*) may as well cause symptoms on *Fraxinus* (Orlova-Bienkowskaja, 2015), but the appearance of larval galleries is clearly distinct and exit holes are round, not D-shaped. There are several cerambycid beetles that utilise ash trees, but emergence holes made by these species are typically round and usually larger than those of *A. planipennis* (Lyons et al., 2007). If, during inspection, suspicious symptoms are identified, it is recommended that plant samples are sent to a specialist laboratory for further identification, and detection in the suspected area of infestation should be carried out by trapping as soon as possible.

Conclusions on visual examination

Trees infested by *Agrilus planipennis* show distinctive symptoms and signs, including canopy dieback, epicormic growth, larval tunnelling under the bark and D-shaped adult emergence holes. Due to the gap between initial infestation and the appearance of clear signs of *A. planipennis* attack, surveillance based on symptoms is not suited for detection of early stages of infestations.

2.2. Trapping

Trapping targets *Agrilus planipennis* flying adults. Several different trap designs have been developed, and extensive research has been conducted on trap type, trap colour, trap placement and attractant compounds (Poland et al., 2019). In addition, the use of girdled ash trap trees has been shown to be effective for detecting the beetle at low prevalence, and a detection method employing sentinel trees in high-risk areas is currently being developed.

The goal of trapping is to detect the initial presence of the pest in an area, and the effectiveness of trapping systems relies on the ability to capture at least one individual of the pest species as soon it starts spreading in an area (not on the capacity to capture a high number of individuals). Trapping systems used to detect *A. planipennis* exploit the insect's visual and olfactory attraction to host trees. Traps are placed on *Fraxinus* trees (or trees can be modified to function as trap), thus providing visual and olfactory cues to beetles. Subsequently trap colour and odour lures amplify host attractiveness and direct the insects to the trap.

2.2.1. Trap types, colour, attractant and placement

The trap types validated in North America by official use and extensive research for detecting *A. planipennis* are the modified Lindgren multifunnel traps, the triangular sticky prism traps and the double-decker traps (consisting of two sticky prisms) (Figures 12 and 13).

Trap type

Multifunnel traps designed for detecting *A. planipennis* consist of 12 connected funnels made of green UV plastic, with a collecting cup at the bottom, and with lure vials attached on the inside of a funnel (Figure 12A). Traps are placed within the canopy of ash trees (Figure 12B, C). Coating funnels with the slippery fluoropolymer fluon is necessary for trap efficacy as it prevents insects from climbing out of the traps and escaping (Francese et al., 2013; USDA APHIS PPQ, 2018).

Prism traps are 60 × 40 cm triangular prisms made of corrugated plastic coated with clear insect trapping glue, loaded with a lure, and hung on branches in the canopy of ash trees (Abell et al., 2015) (Figure 13A, B, D).

Double-decker traps consist of two sticky prism traps mounted at 1.8 and 3 m on a 3 m tall PVC pipe supported by a T-post (McCullough and Poland, 2017) (Figure 13C, D). Prisms can be both purple, or with green at the top of the pipe and purple at the bottom and baited with an attractant lure.

Other trap designs originally used for trapping native buprestids, consisting of elongated multifunnels, were recently evaluated, and validation for detecting *A. planipennis* is ongoing (Imrei et al., 2020).

Trap colour

Colour is as a significant factor affecting trap captures, as it depends on the response of EAB to lights of different wavelengths (Silk et al., 2011). Females are generally attracted to purple because it mimics bark colour and oviposition sites, while males respond strongly to green, as emerging males tend to fly towards host foliage (Poland et al., 2019). As a result, purple traps typically catch more females than males (Francese et al., 2008), while green traps capture mostly males especially when deployed high in the canopy (Crook et al., 2009; Silk et al., 2011). Because of the complex response to colour, experimental comparisons of traps with different colours are challenging (Marshall et al., 2010a; Francese et al., 2013; Crook et al., 2014; Poland and McCullough, 2014; McCullough and Poland, 2017). However, recent assessments found that dark green (530–540 nm wavelengths and 49% reflectance), and Sabic purple (420 nm 21.7% reflectance and 670 nm 13.6% reflectance) are the most attractive (Poland et al., 2019).

Attractant lures

Attractants most commonly used within surveillance programmes in North America are manuka or phoebe oil and (3Z)-hexenol (synonym cis-3-Hexenol), which function as kairomones providing host cues and attracting insects to the tree (Ryall et al., 2011; Silk et al., 2011; Ryall et al., 2013). Green sticky prism traps baited with the pheromone (3Z)-lactone in combination with (3Z)-hexenol have been

proposed (Parker et al., 2020). The attractive range of lures baited traps is not fully clear and it might vary depending on site-specific factors (Poland et al., 2019; Parker et al., 2020).

Trap placement

Multifunnel and sticky prism traps are always hung high in the canopy of ash trees and are ideal for surveillance within forests. Double-decker traps are to be placed in close proximity to ash trees but in open areas exposed to sun, such as forest margins or parks preferentially facing south. Multifunnel traps can be reused for several years, while prism sticky traps are lighter, but are discarded at the end of the trapping season and more complicated to process due to the glue coating and risks of saturation by a wide range of trapped insects in addition to the target species. The height of trap positioning in the canopy has a strong effect on trapping effectiveness. Traps can be hung in the mid canopy between 3 to 8 m but hanging traps high in the upper canopy is found to improve the effectiveness (Crook et al., 2008; Poland et al., 2019).

Figure 12: Green multifunnel traps placed within ash tree upper canopy (Courtesy of Hugh Evans, Forest Research, UK)

Figure 13: Different types of traps for *Agrilus planipennis*. Placement of green sticky prism trap on a tree (A); green prism trap within ash tree canopy (B); double- decker trap with two purple sticky prisms (C); *A. planipennis* specimen on the surface of a purple sticky prism trap (D, left) (Sources: (A, B) courtesy of Hugh Evans, Forest Research, UK; (C, D) courtesy of Ignazio Graziosi)

2.2.2. Trap trees and sentinel trees

Girdled trees produce plant stress volatiles highly attractive to *A. planipennis*. Trap trees are prepared by girdling the trunk (removing a strip of bark around the trunk) and adding sticky bands for capturing adults or checking larval establishment by removing the tree bark (McCullough et al., 2009a, 2009b). This method has proven to be extremely effective in detecting beetles at low population densities such as early stages of the infestations, as volatiles released by girdled trees are found to be more attractive for the insect compared with volatiles in artificial lures used in traps (Mercader et al. 2013). Using three trap trees within an 800 m radius has an effectiveness of 90% at very low insect densities (Mercader et al., 2013). Ideal detection trees are open-growth trees and those located along roadways, forest edges and in canopy gaps. Trees should measure 10–15 cm at breast height to reduce the debarking operations for detecting larvae. On selected trees a 20–30 cm wide band of bark is removed (on the main stem at a height of 110–130 cm). Trees are felled in autumn and winter and every branch and stem >5 cm in diameter is carefully debarked with a drawknife to assess the presence and density of larvae (Figure 14). A detailed protocol for deploying trap trees is described by Mercader et al. (2013). Using trap trees is very labour intensive and trees weakened due to girdling could be a safety hazard when of large size and located in public areas.

An additional detection method might be using potted sentinel trees as a valuable aid for the detection of early infestations in high-risk areas. These could be potted with highly susceptible *Fraxinus* species (e.g. *F. pennsylvanica*) (BFW, 2018). For unavailability of traps in emergency situations (outbreak and no traps), trap trees and sentinel trees could be used for detections.

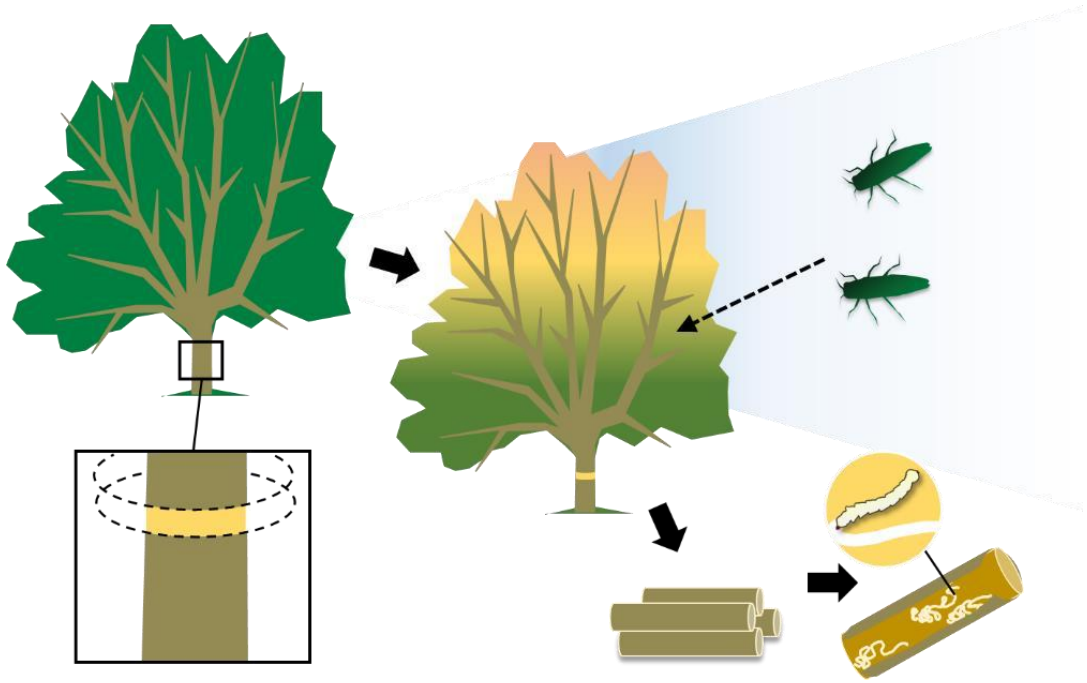

Figure 14: Preparation and evaluation of girdled trap trees for detecting *Agrilus planipennis* (Courtesy of Ignazio Graziosi)

2.2.3. Trapping effectiveness

Trap effectiveness (ability to capture at least one *A. planipennis*) of different combinations of trap type, colour, placement and attractants can vary significantly depending on the methods by different authors used for assessing low population densities (Table 3), and we indicate a range of effectiveness (minimum and maximum values) for each trap. Moreover, effectiveness values presented here refer to trapping *A. planipennis* in North America, where native host species in the forest are known to be attractive to the beetle. Attractiveness of European ash and the effect of composition of European forests on trapping is unknown. The attractive range of traps is not fully known (Poland et al., 2019; Parker et al., 2020).

In light of the body of experimental work and new advances on trap design, surveillance should be conducted using the traps validated for *A. planipennis*, which are green multifunnel, sticky prism, and double-decker traps loaded with attractant lures, and trap trees.

Dark green multifunnel traps (wavelength = 530–540 nm, reflectance 49%) coated with fluon and hung high in the upper part of the canopy of ash trees (Francese et al., 2013; USDA APHIS PPQ, 2018; Poland et al., 2019) strike a good balance between effectiveness and convenience due to improved handling (no glue) and reusability, and they can be used in all landscape settings. Effectiveness associated with dark green funnel traps loaded with (3Z)-hexenol lures ranges between 82.5 and 100% and effectiveness at low insect densities 87.5 ± 12.5% (Poland et al., 2019). Coating with fluon is necessary to ensure effectiveness. Traps can be reused for several years. In open field locations and at 5–10 m around edges, double-decker traps with green prism at the top and purple at the bottom both baited with (3Z)-hexanol can be used (Poland et al., 2019).

Light green sticky prism traps might not be as effective in detecting beetles at low densities (Ryall et al., 2013; Poland and McCullough, 2014; Poland et al., 2019).

Purple and light green sticky traps loaded with (3Z)-hexenol and or manuka oil have an effectiveness at low insect densities of between 72–82% and 55–75%, respectively (Ryall et al., 2013; Poland and McCullough, 2014; Poland et al., 2019). While trap trees are a highly effective (90% detection rate), they are a labour-intensive technique.

Double-decker traps with green (top) and purple (bottom) prisms loaded with (3Z)-hexenol are the most effective trap design at low insect densities, but better suited to being placed in open areas and at 5–10 m from forest edges (Poland and McCullough, 2014; Poland et al., 2019).

Table 3: For designing *Agrilus planipennis* surveillance in the EU (see EFSA, in preparation) we consider the dark green multifunnel traps coated wityn fluon and baited with (3Z)-hexenol. In light of the uncertainty around its attractive range, we consider a trapping effectiveness of 75%, which correspond to the lower range of the selected trap type (Poland et al., 2019). *Agrilus planipennis* disperses at least within 100 m from the emergence site on 99% of the cases (EFSA, 2019; and Section 1.7), therefore we propose a density of one trap per hectare (100 m distance between traps) to be sufficient to detect populations within that hectare.Effectiveness for different trapping methodologies at sites with low *A. planipennis* densities (see also Poland et al., 2019)

Trapping method	Effectiveness at low EAB densities	References
Dark green multifunnel traps with (3Z)-hexenol	87.5±12.5%	Francese et al., 2013; USDA APHIS PPQ, 2018; Poland et al., 2019
Double-decker traps with (3Z)-hexenol and manuka oil	100%	Poland and McCullough, 2014; McCullough and Poland, 2017
Green prism traps with (3Z)-hexenol and (3Z)-lactone	75–98%	Ryall et al., 2013; McCullough and Poland, 2017; Parker et al., 2020
Double-decker traps with manuka oil	56–95%	Marshall et al., 2010a, 2010b; McCullough et al., 2011
Green or purple prism traps with (3Z)-hexenol	37–82%	Ryall et al., 2013; Crook et al., 2014; Poland and McCullough, 2014
Girdled trap trees	57 to >100% (if 3 trees within an 800 m radius: 90%)	Marshall et al., 2010a; McCullough et al., 2011; Mercader et al., 2013
Green or purple prism traps with Manuka oil	25–80%	Marshall et al., 2010a, 2010b; McCullough et al., 2011

2.2.4. Trap deployment and insect collection

Different detection methods could be integrated into survey programmes tailored to local conditions (McCullough and Poland, 2017). Low value or declining ash trees along fence lines, roads or in forested settings can be girdled or debarked to function as trap trees. Prism traps or reusable funnel traps can be deployed at larger scales. Free-standing double-decker traps may be especially appropriate for high-risk areas, as long as the traps are near ash trees and in the open, so prisms are exposed to sunlight. Examples are highway medians, along railroads, in powerlines running through forested areas and around the perimeters of sawmills and waste wood disposal yards. Multifunnel traps should be hung on branches in the upper canopy of ash trees using ropes. Collection cups of multifunnel traps are filled with 2 inches of propylene glycol as killing agent and preservative (USDA APHIS PPQ, 2018).

For trapping operations refer to the EAB guidelines from USDA APHIS PPQ (2018) and the EAB programme manual (USDA APHIS PPQ, 2015). Traps should be deployed immediately before adult emergence (i.e. 450 GDD, see Section 1.4), inspected every two weeks and lures should be replaced according to the product's instructions (usually four to six weeks). The accumulation of degree days depends on local climatic conditions at the surveyed areas. In the USA, traps are recommended to be used continuously from 1 May to 30 September (USDA APHIS PPQ, 2018). The exact timing of the trap deployment for different EU MSs should be determined appropriately based on the beginning of adult emergence, 450 DD base 10°C (in the USA this is mid-May to June; EPPO, 2013b).

As soon as traps are checked, beetles, if alive, must be immediately killed by placing them in vials filled with 70% ethanol and tightly sealed. For multifunnel traps, insects are usually collected by pouring the content of each collection cup (killing agent plus insects) through a coffee filter into a waste bottle, then insect specimens are put into wire closed bags or zipper bags (USDA APHIS PPQ, 2018). Insect specimens should never be transported alive, to avoid accidental escape into new areas.

Any collected larvae, prepupae and pupae should be heat treated in boiling water for about 1 minute and stored in vials filled with 70% ethanol (USDA APHIS PPQ, 2015). This will preserve specimens for further examination and morphological identification. If this operation cannot be performed in the field, samples should be kept wet and cool before being preserved. They can be placed in vials or specimen jars with moist wipes or paper towels, securely sealed and transported in a cooled container. As soon as possible, the larvae, prepupae and pupae must be processed (Lyons et al., 2007).

Trapping protocols detailing procedures and materials for implementing surveillance tasks are available (Lyons et al., 2007; EPPO, 2013b; USDA APHIS PPQ, 2018).

Conclusions on trapping

The use of traps baited with attractants, particularly dark green multifunnel traps coated with fluon and baited with (3Z)-hexenol, and the deployment of trap trees are appropriate methods for *Agrilus planipennis* detection at the early stages of an infestation and should be used for implementing surveillance within the EU.

2.3. Branch sampling

Branch sampling involves cutting branches of ash trees, debarking and inspection for the presence of larval galleries and live larvae. This approach is best suitable for verifying the origin of a new detection, for delimiting infested areas, or for evaluating population density within trees and assessing the effectiveness of eradication programmes as described in ISPM 9 (FAO, 1998). Branch sampling is best effective for estimating the prevalence of *A. planipennis*, before canopy dieback and other symptoms are evident (Ryall et al., 2011).

The method requires the removal of two >6 cm diameter branches (50 cm long) selected within the middle canopy of each tree and has been applied to open-grown urban ash trees of 20–50 cm diameter at breast height (Ryall et al., 2011). Such branch sampling had an effectiveness of 75% to detect the EAB in asymptomatically infested trees at low pest density, but when density is extremely low (less than one gallery per branch) effectiveness decreases significantly (Ryall et al., 2011; Ryall, 2015; Turgeon et al., 2016). Branch sampling is a destructive method, is very labour intensive, and is not advised for detecting the pest at early stages (Ryall et al., 2011), although it is the only method for quantifying beetle density in a host tree.

Conclusions on branch sampling

Sampling tree branches (50 cm long, >6 cm diameter, sections removed from the middle canopy) is a destructive method that assesses the presence of larvae within the tree before symptoms and signs of the pest appear. It can be used as complementary detection methodology for confirming the origin of a new detection or determining the extent of an infestation and allows assessment of beetle density within trees.

2.4. Other detection methods

In North America, trained sniffer dogs have been used with promising results for detecting *Fraxinus* plants infested with *A. planipennis*, and feasibility studies are currently being conducted in Europe (Evans et al., 2020). Results for detecting *A. planipennis* in nurseries and forests, and at very low larval densities within trees, are very promising (Hoyer-Tomiczek and Hoch, 2020).

A biosurveillance methodology using the beetle-hunting wasp *Cerceris fumipennis* (Hymenoptera: Crabronidae) has been developed in North America. This highly specialised wasp predates on *Agrilus* species and carries paralysed prey (adult *A. planipennis*) into underground nests for provisioning larvae. Monitoring consists of locating *Cerceris* nests for *A. planipennis* presence and capturing with nets adult wasps returning to the nests carrying adult *A. planipennis* (Rutledge et al., 2013). This approach is time consuming and requires numerous trained staff to cover a significant area, although volunteers can be trained to supplement trained staff, and it would be highly suited as a citizen science project. At this time, there are no studies available on European *Cerceris* species as biomonitoring candidates.

Remote sensing by aerial photography, high spatial resolution and multispectral imagery has been used for detecting EAB infestations, mapping outbreaks and evaluating canopy-level mortality in natural and urban settings (Souci et al., 2009; Pontius, 2014; Murfitt et al., 2016). These tools are very valuable for developing management programmes of EAB infestations.

Conclusions on detection

Agrilus planipennis should be detected preferentially by using traps (in particular fluon-coated green multifunnel traps) targeting adults during the flight season. Other detection methods can be employed as complementary to trapping in particular around risk locations, and for delimiting the infested zone following an outbreak. Trap trees and sentinel trees can be deployed in high risk areas prior the insect flying season and could then be inspected during the winter. Branch sampling can be used year-round as a complementary method for delimiting infestations following a positive detection and for assessing population density. Visual examination of insect signs and canopy dieback can be conducted for selecting trees for branch sampling for both detection and delimiting surveys. The use of sniffer dogs can be considered during detection surveys in high-risk areas, and also to support delimiting surveys. Remote sensing can be utilised as an additional complementary tool for prioritising the survey sites, for determining the extension of an infested zone and to locate ash resources during surveillance planning.

2.5. Identification

2.5.1. Morphology

The following illustrated guides provide assistance for the identification of *A. planipennis* adult and immature stages:

- Chamorro et al. (2015)[13]
- Parsons (2008)[14]
- Lompe (2017)[15]

Adults

Agrilus planipennis is a metallic green beetle with typically elongated body, 7.5–13.5 mm long (Figures 15B, E and 16H) (Parsons, 2008; EFSA PLH Panel, 2011). Beetles have a very distinctive coppery red coloration of the abdomen visible underneath the elytra (Figure 1). Females are typically larger and have wider bodies compared with males. Coloration of adult beetles is mostly green, but it can vary, with specimen showing brass, copper or red shades. Specimens entirely coppery red, entirely bluish-green, or green with bluish elytra are rare (Parsons, 2008).

[13] https://www.fs.fed.us/nrs/pubs/jrnl/2015/nrs_2015_chamorro_001.pdf
[14] http://www.emeraldashborer.info/documents/eab_id_guide.pdf
[15] http://www.coleo-net.de/coleo/texte/agrilus.htm#anKer5

Immature stages

Eggs are light brown, elliptical in shape and 1 mm long, and are laid by females into bark cracks (Figures 15A and 16F). Larvae are yellowish white with characteristic trapezoidal abdominal segments and a pronotal groove that is posteriorly bifurcated (Figure 15C, D and 16B–E). Fourth instar larvae reach 26–32 mm in length (16B), while prepupae are shorter and fold in a J-shape when overwintering (Chamorro et al., 2015) (Figure 16A, I). Pupae are of similar size to adults, yellowish white to light brown and get darker closer to eclosion (Figure 16G).

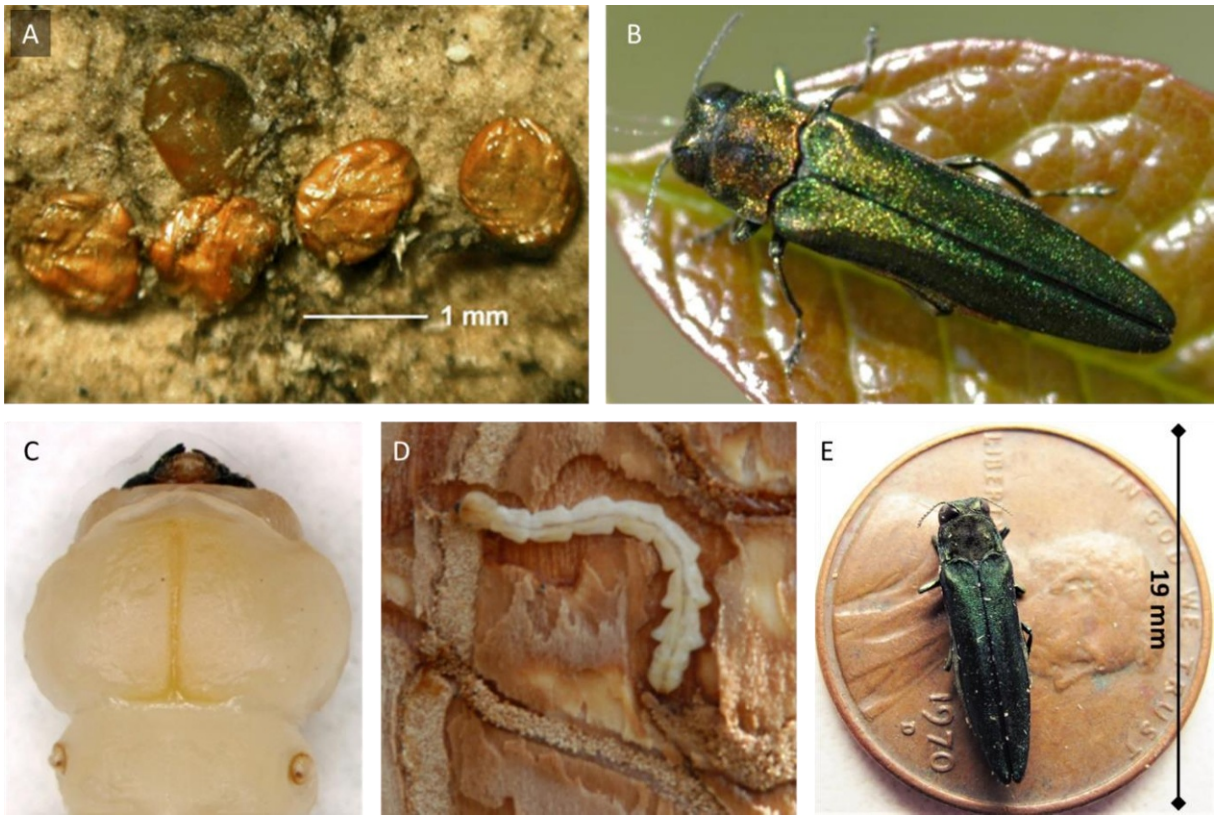

Figure 15: Appearance of *Agrilus planipennis* egg, larvae and adult. Egg cluster on ash bark (A); adult on foliage (B); head capsule of larva (C); IV instar larva in gallery (D); adult beetle on coin (19 mm diameter) (E) (Sources: (A) David Cappaert, Bugwood.org; (B, D) Daniel Herms, Bugwood.org; (C) Pennsylvania Department of Conservation and Natural Resources, Bugwood.org; (E) Ignazio Graziosi)

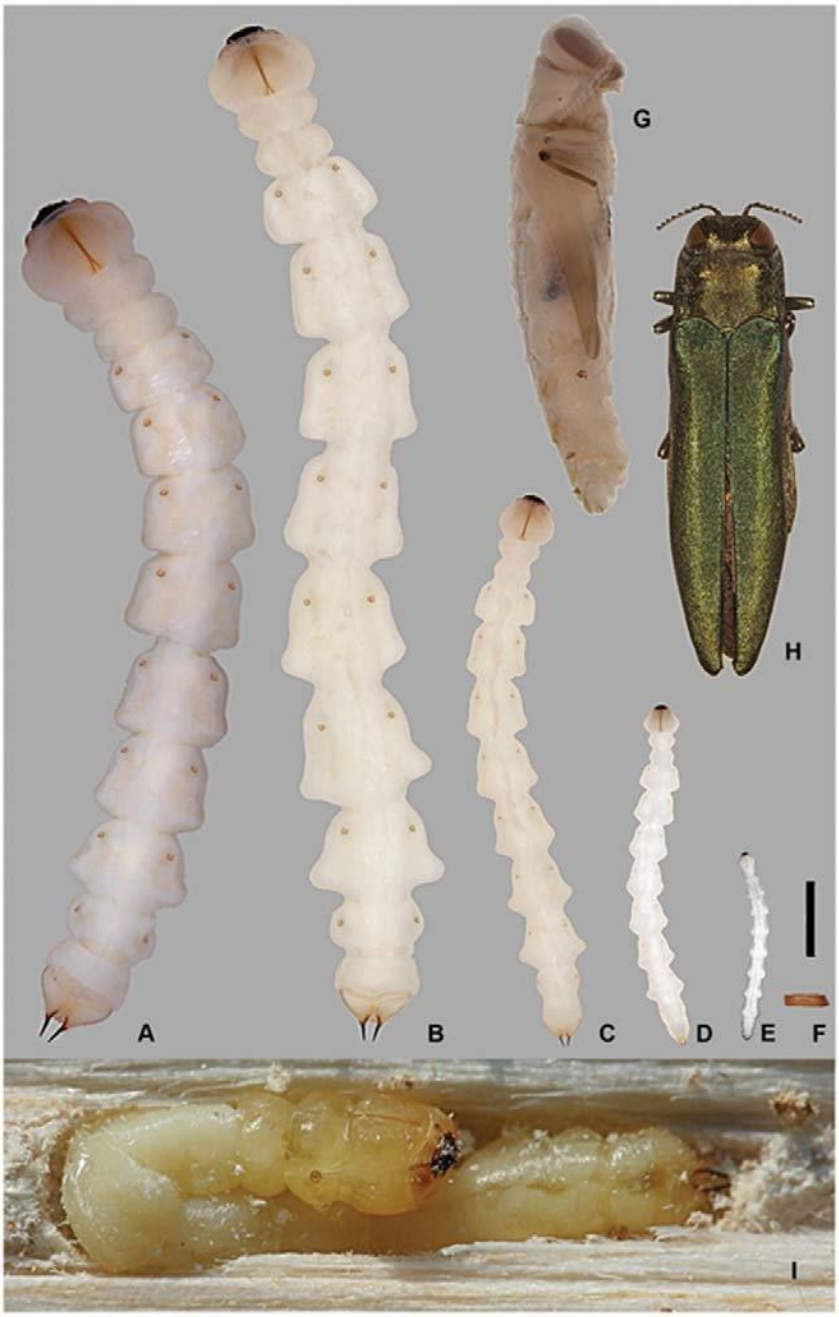

Figure 16: Immature and adult stages of *Agrilus planipennis*. Egg (F), instar I (E), instar II (D), instar III (C), instar IV (B), prepupa (A, I), pupa (G), and adult (H). Scale bar 2 mm (Sources: (A-G) Chamorro et al., 2012, courtesy of Maria Lourdes Chamorro; (I) courtesy of Eduard Jendek)

Risk of misidentification of the pest

The adult stage of *A. planipennis* is fairly distinctive and usually easy to identify by a trained taxonomist. Nevertheless, non-specialists might confuse similarly shaped or coloured *Agrilus* with *A. planipennis*. Colour variants might be more easily confused, but the coppery red coloration of the abdomen underneath the elytra is very distinctive to *A. planipennis* (Figure 1).

Seventy-two European *Agrilus* species are known, and several of these could be misidentified as *A. planipennis* (Figure 17) but are not found on *Fraxinus* (Jendek and Poláková, 2014). Of these,

A. biguttatus is about the same size as *A. planipennis* (Figure 17C) and special care must be taken when undertaking surveillance within the area of distribution of *A. biguttatus*[16].

The only *Agrilus* species native to the EU that is also relevant for ash trees is *A. convexicollis*, which is much smaller (up to 5 mm long) compared with the EAB (Figure 17J), and is a secondary pest targeting declining and dying trees. This beetle can also be found on *Cornus*, *Euonymus*, *Rosa*, *Quercus*, *Rosa*, and *Ulmus* (Davis et al., 2006; Jendek and Poláková, 2014).

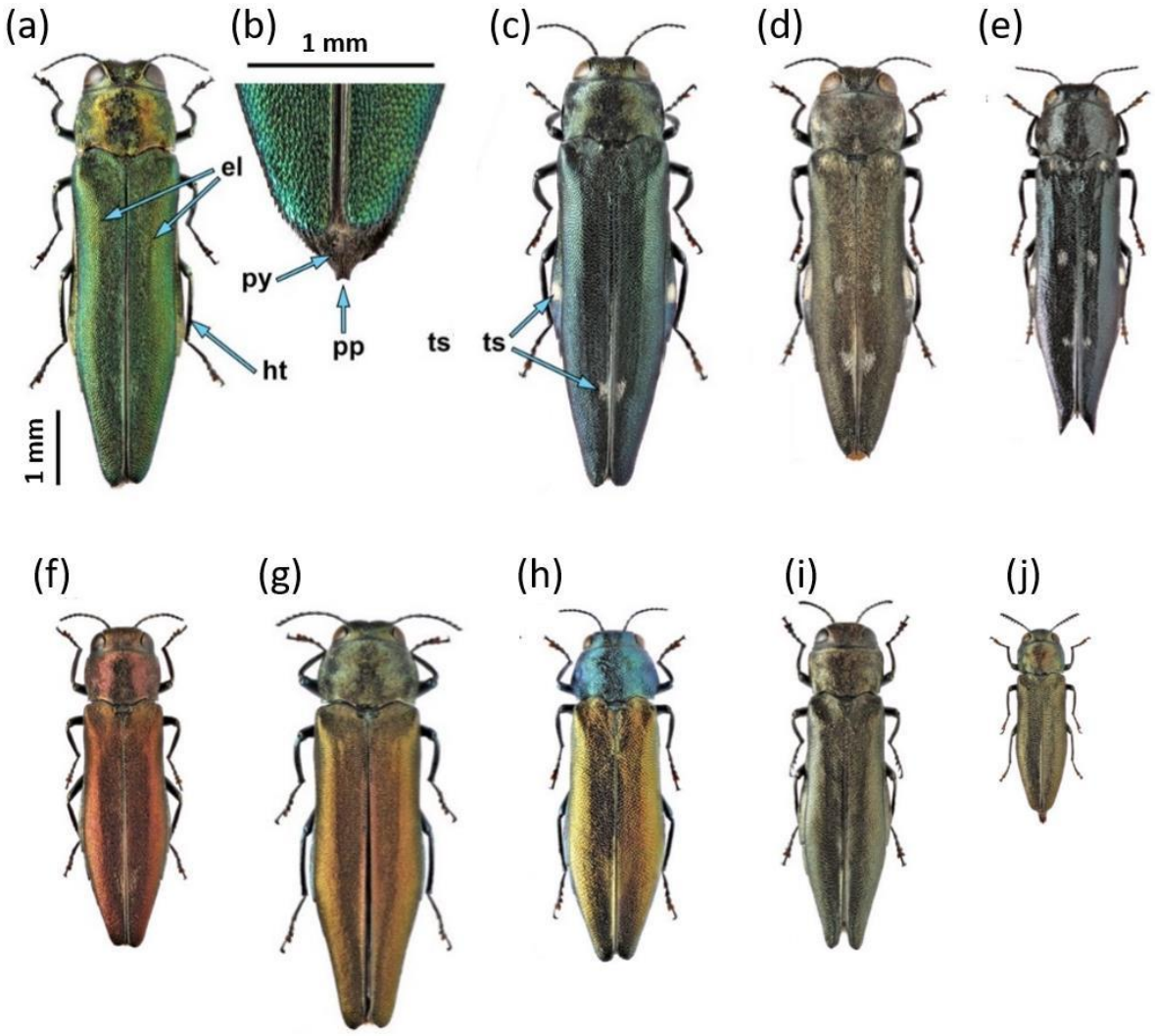

Figure 17: *Agrilus planipennis* and other European *Agrilus* spp. *A. planipennis* (A, B), *A. biguttatus* (C) *A. ater* (D), *A. guerini* (E), *A. sinuatus* (F), *A. mendax* (G), *A. subauratus* (H), *A. suvorovi* (I), *A. convexicollis* (J). el – elytra, ht – hind tibia, pp – pygidial process, py – pygidium, ts – tomentose spots. Scale bars: 2 mm (Source: modified from Volkovitsh et al., 2020; courtesy of A.V. Kovalev)

[16] https://fauna-eu.org/cdm_dataportal/taxon/39872b33-94e9-48b6-8063-56bffcbc153d#distribution

2.5.2. Molecular identification

Molecular identification of *A. planipennis* might be required to confirm the identity of dubious specimens. Mitochondrial markers [cox1– 5′ (DNA barcode fragment), cox1–3′, and rrnL] for 100 *Agrilus* species from the Northern Hemisphere including *A. planipennis* are available on the Barcode of Life Database[17] (Kelnarova et al., 2019).

Conclusions on identification

Morphology of insect specimens is the preferred method for identification. Adult and larval specimens of *Agrilus planipennis* can be identified morphologically by a trained entomologist, to avoid confusion with other *Agrilus* species found in the EU. Sequences for molecular identification are available and can be used for confirming identification when morphology is dubious.

3. Key elements for survey design

Based on the analyses of the information on the pest–host plant system, the different units that are needed to design the survey have to be defined and tailored to the situation in each Member State. The size of the defined target population and its structure in terms of number of epidemiological units need to be known.

When defining the target population for surveying *Agrilus planipennis*, consider that its host trees within the EU are generally found in: (i) forests (natural forests with ash trees in mixed forests and planted forests of ash trees); (ii) urban areas (streets, parks and gardens where ash trees are grown for ornamental purposes); (iii) agricultural areas (ash shelter belts and landscape trees).

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 as they are organised per crop visit and not by pest. Table 4 shows an example of these definitions.

Table 4: Example of definitions of the target population, epidemiological unit and inspection unit for *Agrilus planipennis*

	Definition
Target population	Area with ash trees growing in each Member State or region for which the survey is designed
Epidemiological unit	A single homogeneous area that contains at least one individual ash tree (e.g. hectare, forest, park and garden, NUTS region)
Inspection unit	A single trap (or ash tree)

The general guidelines (EFSA, 2020) for the risk-based statistically sound surveillance are presented in a separate document and describe the use of the toolkit for survey design, including the reasoning for choosing the type of survey to design depending on its objectives, the 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 and reporting the data and the survey results.

The specific guidelines for the survey of *Agrilus planipennis* are also presented in a separate document (EFSA, in preparation) and describe step-by-step the process of sample size calculation for risk-based surveillance. Two cases are addressed, a detection survey for pest-freedom demonstration and a

[17] https://www.boldsystems.org/

delimiting survey in the event of a first positive detection. For both cases, the description of the different surveillance components required to determine statistically sound sample size is provided.

The steps that will generally be necessary are the following:

1) Determine the type of survey based on its objectives. For *A. planipennis*, the type of survey will depend on the pest status (according to ISPM No 8; FAO, 2017) in the area of interest. The objective could be to substantiate pest freedom, to delimit an outbreak area following an infestation or to determine the pest prevalence. The next steps deal with the example of substantiating pest freedom. The overall confidence level and design prevalence of the survey has to be decided by the risk managers before designing the surveys as they reflect the acceptable level of risk of infestation of the host plants by *A. planipennis*. General guidelines for pest surveillance provide further details on the choice of these values and the related consequences in terms of pest surveys.
2) Define the target population and its size. When determining the target population for surveillance of *A. planipennis*, the host plants that are relevant for the survey area have to be selected. The size of the target population should be determined. For example, the target population could be all host trees in a Member State.
3) Define the epidemiological units. The epidemiological units should be single homogeneous areas that contain at least one individual host plant in each one.
4) Determine the inspection unit. For an ash forest, for example, the inspection unit is a single trap (or an ash tree).
5) Determine the number of inspection units per epidemiological unit. For an ash forest, this is the average number of traps (or ash trees) that have been inspected and/or sampled per epidemiological unit.
6) Implement the inspections and, if appropriate, the sampling, following the procedures suggested by the competent authorities, within the epidemiological units and estimate the method effectiveness in order to determine the overall method sensitivity (sampling effectiveness × diagnostic sensitivity). A representative number of plants should be examined and if there are suspicious symptoms they should be sampled. RiBESS+ can be used to calculate how many inspection units need to be examined or sampled for a predefined prevalence level (e.g. 1%) to obtain a particular confidence level. This confidence level is in turn needed to calculate the number of sites to be inspected (Step 8). Note that the more units that are inspected, the higher will be the confidence. The competent authorities need to align the survey efforts with the resources available.
7) Define the risk factors. A risk factor affects the probability that a pest will be present or detected in a specific portion of the target population. It may not always be possible to identify or include a risk factor in the survey design. Risk factors can only be included when both the relative risk and the proportion of the overall plant population to which they apply are known or can be reliably estimated.
8) Determine the number of epidemiological units to survey. RiBESS+ can be used to determine the number of epidemiological units to survey in order to achieve the objectives of the survey set at Step 1 in terms of confidence level (e.g. 95%) and design prevalence (e.g. 2%), while also including the method sensitivity from Step 6 and the risk factors identified in Step 7. As a result, considering, for example, fields where host plants are present, the number of fields that need to be surveyed are estimated for an MS in order to state, with 95% confidence, that the prevalence of *A. planipennis* will be at 2% or below.
9) Summarise and evaluate the survey design. At this stage, it is necessary to evaluate whether the above steps have resulted in a survey design that matches the available resources, meaning that a feasible number of inspections can be performed within an acceptable time frame per inspection, and resulting in a feasible number of samples. If not, available resources should be adjusted. This adjustment would result in a modified survey design using different input parameters of the statistical tool RiBESS+ (e.g. varying the number of components, method sensitivity).

10) Integrate the pest-based survey into a crop-based survey (optional).
11) Allocate the calculated survey effort. In the survey area, the output of RiBESS+ should be allocated proportionally to the host plant population or to the number of epidemiological units. In addition, the survey size should be selected from the list of available locations.
12) Data collection and survey reporting. Consider which data are needed and how these data will be reported together with the related assumptions.
13) Plan, develop or update the specific instructions for the inspectors. These activities are not addressed by EFSA and fall within the remit of the competent national authorities.

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General glossary for pest survey

Term	Definition*
Buffer zone	An area surrounding or adjacent to an area officially delimited for phytosanitary purposes in order to minimise the probability of spread of the target pest into or out of the delimited area, and subject to phytosanitary or other control measures, if appropriate (ISPM 5: FAO, 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, fruit). A pest survey comprises various components. The overall confidence of the survey will result from the combination of the different components.
Confidence	The sensitivity of the survey is a measure of reliability of the survey procedure (Montgomery and Runger, 2010). The term confidence level is used in 'Methodologies for sampling of consignments' (ISPM 31: FAO, 2016b).
Delimiting survey	Survey conducted to establish the boundaries of an area considered to be infested by, or free from, a pest (ISPM 5: FAO, 2019).
Design prevalence *analogous to the term **level of detection** used in 'Methodologies for sampling of consignments' (ISPM 31: FAO, 2016b)*	It is based on a pre-survey estimate of the likely actual prevalence of the pest in the field (McMaugh, 2005). The survey will be designed in order to obtain at least a positive test result when the prevalence of the disease will be above the defined value of the design prevalence. In 'freedom from pest' approaches, it is not statistically possible to say that a pest is truly absent from a population (except in the rare case that a census of a population can be completed with 100% detection efficiency). Instead, the maximum prevalence that a pest could have reached can be estimated, this is called the 'design prevalence'. That is, if no pest is found in a survey, the true prevalence is estimated to be somewhere between zero and the design prevalence (EFSA, 2018).
Detection survey	Survey conducted in an area to determine whether pests are present (ISPM 5: FAO, 2019).
Diagnostic protocols	Procedures and methods for the detection and identification of regulated pests that are relevant to international trade (ISPM 27: FAO, 2016a).
Epidemiological unit *analogous to the term **lot** used in 'Methodologies for sampling of consignments' (ISPM 31: FAO, 2016b)*	A homogeneous area where the interactions between the pest, the host plants and the abiotic and biotic factors would result in a similar 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 given geographical area. They are the units of interest for which the sample size is estimated (e.g. a tree, orchard, field, glasshouse, nursery).
Expected prevalence	In prevalence estimation approaches, it is the proportion of epidemiological units expected to be infected or infested.
Expert knowledge elicitation	A systematic, documented and reviewable process to retrieve expert judgements from a group of experts in the form of a probability distribution (EFSA, 2014).
Host plant	A host plant is a plant species belonging to the host range on which the pest could find shelter, feed or subsist at least for a period of time.

Host range	Species capable, under natural conditions, of sustaining a specific pest or other organism (ISPM 5: FAO, 2019). This definition is limited to an array of host plants species and does not include commodities other than plants or plant parts.
Identification	Information and guidance on methods that either used alone or in combination lead to the identification of the pest (ISPM 27: FAO, 2016a).
Infected versus infested	Infected is used when a pathogen is referred to in relation to its hosts (e.g. the trees are infected by the bacterium). Infested is used when an arthropod pest is referred to in relation to its hosts (e.g. the trees are infested by beetles). Infested is used when the pest is mentioned in relation to an area (e.g. an infested zone).
Inspection	Official visual examination of plants, plant products or other regulated articles to determine whether pests are present or to determine compliance with phytosanitary regulations (ISPM 5: FAO, 2019).
Inspection unit *analogous to **sample unit** used in 'Methodologies for sampling of consignments' (ISPM 31: FAO, 2016b)*	The inspection units are the plants, plant parts, commodities or pest vectors that will be scrutinised to identify and detect the pests. They are the units within the epidemiological units that could potentially host the pests and on which the pest diagnosis takes place (EFSA, 2018).
Inspector	Person authorised by a national plant protection organisation to discharge its functions (ISPM 5: FAO, 2019).
Method sensitivity *analogous to the term **efficacy of detection** used in 'Methodologies for sampling of consignments' (ISPM 31: FAO, 2016b)*	The conditional probability of testing positive given that the individual is infected (Dohoo et al., 2010). The method sensitivity (MeSe) is defined as the probability that a truly positive host tests positive. It has two components: the sampling effectiveness (i.e. probability of selecting infested plant parts from an infested host plant) and the diagnostic sensitivity (characterised by the visual inspection and/or laboratory test used in the identification process). The diagnostic sensitivity is the probability that a truly positive sample will result positive and is related to the analytical sensitivity. It corresponds to the probability that a truly positive inspection unit or sample will be detected and confirmed as positive. The sampling effectiveness depends on the ability of the inspector to successfully choose the infested plant parts in a host plant. It is directly linked to the sampling procedure itself and on the training and expertise of the inspectors to recognise the symptomatology of the pest. Furthermore, symptom expressions are dependent, among other factors, on the weather conditions as well as on the physiological stage of the host plant when the sample is taken.
Pest	Any species, strain or biotype of plant, animal or pathogenic agent injurious to plants or plant products (ISPM 5: FAO, 2019).
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 pre-set design prevalence (threshold of concern).

Population size	The estimation of the number of the plants in the region to be surveyed (EFSA, 2018).
Prevalence *analogous to the term* **incidence (of a pest)** *defined in the 'Glossary of phytosanitary terms' (ISPM 5: FAO, 2019)*	Pest prevalence is the fraction of infested units in the total population of host plants. Pest incidence is the proportion or number of units in which a pest is present in a sample, consignment, field or other defined population (ISPM 5: FAO, 2019).
Relative risk	The ratio of the risk of infestation in the exposed group to the risk of infestation in the non-exposed group (Dohoo et al., 2010).
Representative sample	A sample that describes very well the characteristics of the target population (FAO, 2014).
RiBESS+	Risk-based surveillance systems. This is an online application that implements statistical methods for estimating the sample size, global (and group) sensitivity and probability of pest freedom. 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 (FAO, 2014). It is defined as a biotic or abiotic factor that increases the probability of infestation of the epidemiological unit by the pest. The risk factors relevant for the surveillance should have more than one level of risk for the target population. For each level, the relative risk needs to be estimated as the relative probability of infestation compared with a baseline with a level 1. Consideration of risk factors in the survey design allows the survey efforts to be enforced in those areas, where the highest probabilities exist to find the pest.
Risk-based survey	A survey design that considers the risk factors and enforces the survey efforts in the corresponding proportion of the target population.
SAMPELATOR	Sample size calculator. This is an online application that implements statistical methods to estimate the sample size for pest prevalence estimation surveys. Free access to the software with prior user registration is available at https://shiny-efsa.openanalytics.eu/
Sample size	The sample size refers to the output of the statistical tools for survey design (RiBESS+ and SAMPELATOR). 'A well-chosen sample will contain most of the information about a particular population parameter but the relation between the sample and the population must be such as to allow true inferences to be made about a population from that sample.' (BMJ, online). The survey sample consists of the required number of 'inspection units' or samples thereof to be examined and/or tested in the survey to retrieve sufficient information on the pest presence or prevalence in the total population. For risk-based surveys, the sample size is calculated on the basis of statistical principles that integrate risk factors.

	If the examination for pest presence is performed by laboratory testing, at least one sample is taken from each inspection unit. These samples will undergo relevant laboratory testing.
Sampling effectiveness	For plants, it is the probability of selecting infested plant parts from an infested plant. For vectors, it is the effectiveness of the method to capture a positive vector when it is present in the survey area. For soil, it is the effectiveness of selecting a soil sample containing the pest when the pest is present in the survey area.
Specified plant	The plant species known to be susceptible to the pest. For example, for *Xylella fastidiosa*, the list of specified plants can be found in Annex II of Commission Implementing Regulation (EU) 2020/1201.
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 *analogous to* ***consignment*** *used in 'Methodologies for sampling of consignments' (ISPM 31: FAO, 2016b*)	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 of plants, plant products or other regulated articles, other than visual, to determine whether pests are present, identify pests or determine compliance with specific phytosanitary requirements (ISPM 5: FAO, 2019).
Test specificity	The conditional probability of testing negative given that the individual does not have the pest of interest (Dohoo et al., 2010). The test diagnostic specificity is the probability that a truly negative epidemiological unit will give a negative result and is related to the analytical specificity. In pest freedom 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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