PEST SURVEY CARD

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Pest survey card on *Popillia japonica*

European Food Safety Authority (EFSA),
Gritta Schrader, Melanie Camilleri, Ramona Mihaela Ciubotaru, Makrina Diakaki, Sybren Vos

Abstract

This pest survey card was prepared in the context of the mandate on plant pest surveillance (EFSA-Q-2017-00831), upon request by the European Commission. The purpose of this document is to assist the Member States in planning annual survey activities of quarantine organisms using a statistically sound and risk-based pest survey approach, in line with the current international standards. The data requirements for such activity include the pest distribution, its host range, its biology, risk factors as well as available detection and identification methods. This document is part of a toolkit that consists of pest-specific documents, such as the pest survey cards and generic documents relevant for all pests to be surveyed, including, the general survey guidelines and statistical software such as RiBESS+.

Keywords: plant pest, survey, risk-based surveillance, *Popillia japonica*, Japanese beetle

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Correspondence: ALPHA@efsa.europa.eu

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Introduction

The information presented in this pest survey card was summarised from a pest risk assessment of *Popillia japonica* for the UK territory (Korycinska et al., 2015), the EPPO Datasheet (EPPO and CABI, 1997), the EPPO diagnostic protocol (EPPO, 2006), the EPPO Standard on National Control Systems (EPPO, 2016), the EPPO Global Database (EPPO, 2018, the CABI datasheet on *P. japonica* (CABI, 2018), the *P. japonica* (Japanese beetle) Fact Sheet from CFIA (CFIA, 2017), and the EFSA pest categorisation of *P. japonica* (EFSA PLH Panel, 2018).

The objective of this pest survey card is to provide the relevant biological information that is needed to prepare surveys for *P. japonica* in EU Member States (EFSA, 2018). This document is part of a tool kit that is being developed to assist and support Member States plan a statistically sound and risk-based pest survey approach in line with International Plant Protection Convention (IPPC) guidelines for surveillance (FAO, 2016). The tool kit consists of pest-specific documents and more general documents relevant for all pests to be surveyed:

i. Pest-specific documents:
   a. The pest survey card *P. japonica.* [1]
ii. General documents:
   a. The general survey guidelines (to be finalised in 2019)
   b. The RiBESS+ manual available online[2]
   c. The statistical tools RiBESS+ and SAMPELATOR which are available online[3] with open access after registration.

1. The pest and its biology

1.1. Taxonomy

Scientific name: *Popillia japonica* Newman, 1841

Class: Insecta, **Order:** Coleoptera: Scarabaeidae: **Subfamily:** Rutelinae: **Tribe:** Anomalini (or **Family:** Rutelidae), **Genus:** *Popillia*, **Species:** *japonica*

Common name in English: Japanese beetle

Popillia japonica (Figure 1) is an insect native to Japan and a pest of a great variety of trees and shrubs. It is a clearly distinguished species among others of the same genus. It varies in size from 8 to 11 mm length and 5 to 7 mm width.

[1] The content of this EFSA Supporting Publication is reproduced as a live document available at https://efsa.maps.arcgis.com/apps/MinimalGallery/index.html?appid=f91d6e95376f4a5da206eb1815ad1489 where it will be updated whenever new relevant information becomes available.
[2] https://zenodo.org/record/2541541/preview/ribess-manual.pdf
[3] 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

Figure 1: Adult of *Popillia japonica* – a clearly distinguished species (Source: Steven Valley, Oregon Department of Agriculture, Bugwood.org)

1.2. EU pest regulatory status

Popillia japonica is listed in Annex I Part A/1 of Council Directive 2000/29/EC[4], banning its introduction into the EU. There are no specific requirements laid down in the Council Directive.

1.3. Pest distribution

The Japanese beetle originates from north-eastern Asia where it is native in northern China, and Japan. It was introduced into North America and has become a more serious pest in the USA than in its area of origin (EPPO, 2006). In the EU, the pest occurs in Portugal (Azores) and in Italy (Milan) (Korycinska et al., 2015, EPPO Global Database).

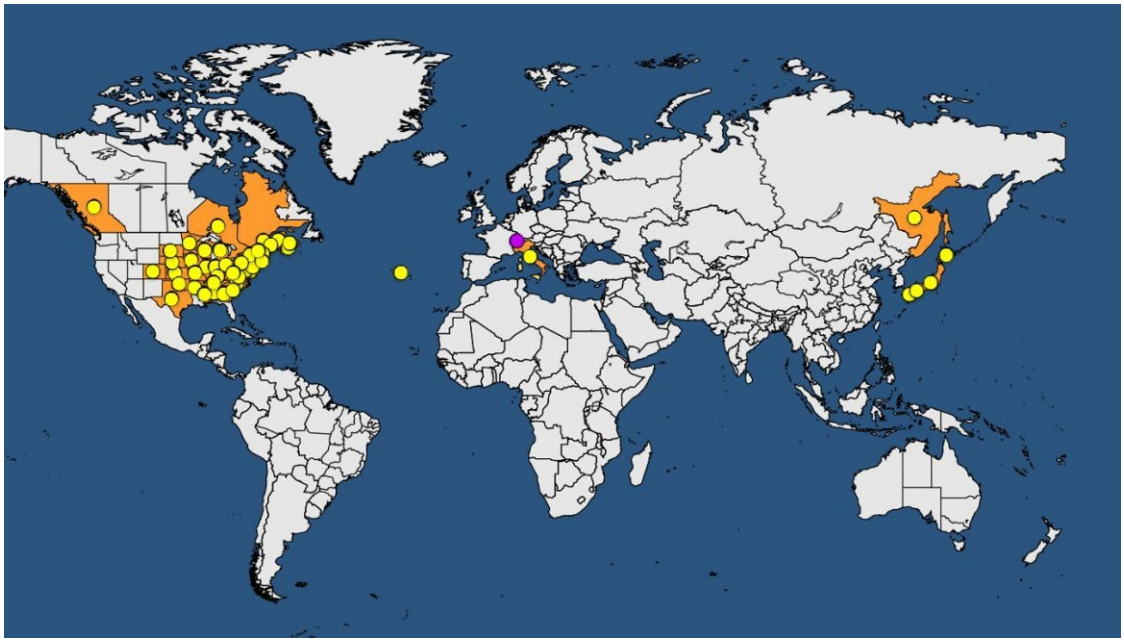

Figure 2: Distribution map of *Popillia japonica* according to EPPO Global Database. The pest status in countries or states is reported as present (yellow dots) or transient (purple dots) (Source: EPPO global database, www.eppo.int). Accessed on 22/02/2019

[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 01/04/2018

1.4. Life cycle

Normally, the Japanese beetle has one generation per year but, at the northern edge of its range, a few individuals may need 2 years to complete the life cycle (EPPO and CABI, 1997).

In 2016, EPPO highlighted the importance of the oviposition sites for the females with preference for moist grassland and turf. Thus, detection surveys need to locate larvae below ground and/or adult beetles above ground (Figure 3).

This life cycle (Figure 3) indicates seasonality of the different life stages of the pest. The timing of each stage is variable depending on the climate conditions of each area under surveillance and may occur on different months in different Member States. Females lay eggs after burrowing approximately 5–10 cm below ground. After the eggs hatch, grubs spend 10 months in the soil. Initially, they are 10–20 cm below ground and will only move towards the turf and start feeding on roots in spring, before pupation.

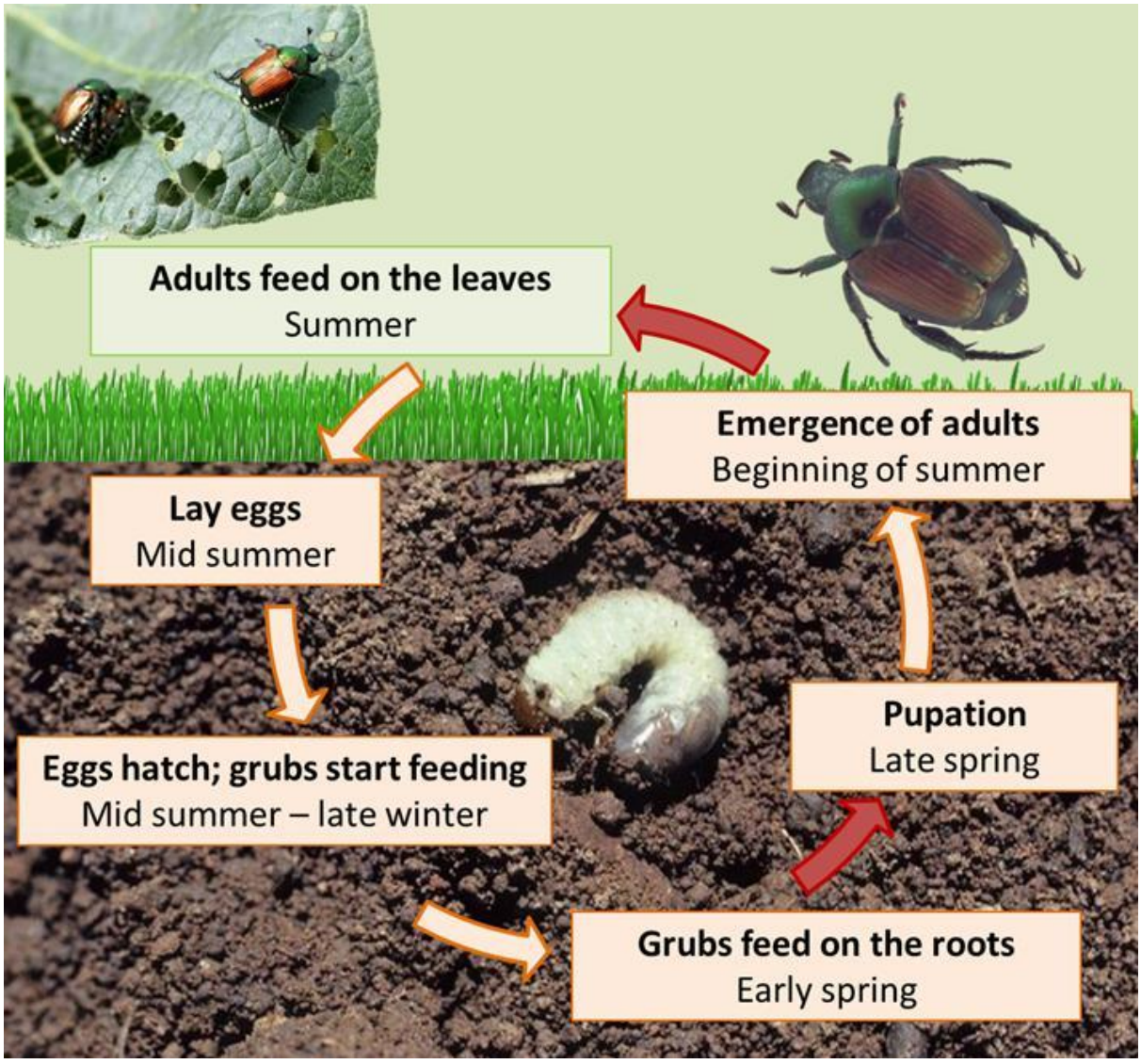

Figure 3: Life cycle of *Popillia japonica*. The most appropriate times to do the survey are marked in red. This diagram includes three images: (top left, adults on leaf) (Source: Roger Schmidt, University of Wisconsin-Madison, Bugwood.org); (top right, adult beetle) (Source: Emmy Engasser, Hawaiian Scarab ID, United States Department of Agriculture (USDA) APHIS ITP, Bugwood.org); (bottom, larva in the soil) (Source: Jim Baker, North Carolina State University, Bugwood.org)

1.5. Host range and main hosts

Popillia japonica is a highly polyphagous species and the adults can be found feeding on a wide range of trees, shrubs, wild plants and crops (EPPO, 2016). Very important factors in the selection of host plants by the beetle are the odour and the location in direct sun. Usually, the beetles feed in groups, starting at the top of a plant and working downward (Vieira, 2008).

According to USDA (2016) the pest has a host range of more than 300 plants in 79 plant families. Among the preferred hosts are: *Abutilon hybridum*, *Acacia baileyana*, *Acer palmatum*, *Acer plantoides*, *Aesculus hippocastanum*, *Alcea rosea*, *Althaea* sp., *Arbutus unedo*, *Bauhinia variegata*, *Betula populifolia*, *Castanea dentata*, *Ceanothus griseus*, *Citrus sinensis*, *Cydonia oblongas*, *Eucalyptus sideroxylon*, *Fremontodendron californicum*, *Glycine max*, *Grewia caffra*, *Hibiscus syriacus*, *Juglans nigra*, *Lagerstroemia indica*, *Larix occidentalis*, *Malus domestica*, *Nandina domestica*, *Parthenocissus quinquefolia*, *Platanus acerifolia*, *Podocarpus macrophyllus*, *Polygonum* spp., *Populus nigra*, *Prunus* spp., *P. Domestica*, *P. Persica*, *Punica granatum*, *Quercus palustris*, *Rosa* spp., *Rubus* spp., *Sassafras albidum*, *Sorbus americana*, *Tilia* spp., *Ulmus americana*, *U. Procera*, *Vitis* spp., *Zea mays*, *Zinnia elegans*.

In 2006, EPPO stated that *Vitis* and *Zea mays* are the main hosts of concern in Europe.

In the Azores (Portugal), the adult beetles were reported to feed on a wide range of hosts: *Acer* spp. (maples), *Asparagus officinalis* (asparagus), *Glycine max* (soybean), *Malus* spp. (apples), *Medicago sativa* (alfalfa), *Phaseolus vulgaris* (pea), *Populus* spp. (poplar), *Prunus* spp. (stone fruit including plums, peaches, etc.), *Quercus* spp. (oaks), *Rosa* spp. (roses), *Rubus* spp. (blackberry, raspberry), *Tilia* spp. (lime trees), *Ulmus procera* (English elm), *Vitis* spp. (grapes) and *Zea mays* (maize) (Vieira, 2008).

In Italy, at the Ticino Valley outbreak site, *P. japonica* was observed on wild plants (*Rubus*, *Ulmus*, *Urtica*, *Rosa*, *Populus* and *Parthenocissus*) and crops of soybean (*Glycine max*) (EPPO, 2014).

According to EPPO (2016) *Popillia japonica* can cause significant damage to nurseries, seedbeds, orchards, field crops, landscape plants, turf and garden plants due to the larval feeding. The main species attacked within the grassland belong to the genera *Festuca*, *Poa* and *Lolium*.

1.6. Environmental suitability

A review of the thermal requirements of *P. japonica* for climate mapping was summarised from rearing experiments by Korycinska et al. (2015; Table 1).

Table 1: Thermal requirements for the development of *Popillia japonica* (rearing experiments) (Source: Korycinska et al., 2015)

Minimum threshold for development	Degree days	Details	Reference
Between 13 and 15°C (depending on life stage)	1317.1	At a temperature of 20°C, egg-adult	Ludwig (1928)
	1596.5	At 22.5°C, egg-adult	
	1970.9	At 25°C, egg-adult	
10°C	1305	Egg-adult	Régnière et al. (1981)
10°C	1422	Egg-egg	Régnière et al. (1981)
50°F (= 10°C)	1030	From 1 January, cumulative degree days before adult emergence in Iowa	Hodgson and Kuntz (2013)
50°F (= 10°C)	970	From 1 January, cumulative degree days before adult emergence in Ohio	Herms (2004)
Not stated	Min: 1029; Max: 2154	'Growing degree days' but no details of what is being measured, or the threshold temperature. Location: Long Island, New York, USA using a 20-year dataset	Johnson (2000)

Temperature and particularly soil moisture are the main factors that may limit the potential spread of the beetle into new areas. *P. japonica* is adapted to regions were the mean soil temperature is between 17.5 and 27.5°C during the summer, and above −9.4°C in the winter (CABI, 2018).

Popillia japonica feeds less on cloudy and windy days and does not feed on rainy days. When the temperature is between 21°C and 35°C, and the Relative Humidity is above 60% on clear summer days beetles feed actively (CFIA, 2017).

Based on Régnière et al. (1981), the sum of the degree days the beetle might need one or two years to complete its development into an adult. In places with degree days above 1422 and a threshold of 10°C the insect can finish the life cycle in one year, whereas in places with degree days above 711 and the same threshold the life cycle is completed in two years (Figure 4).

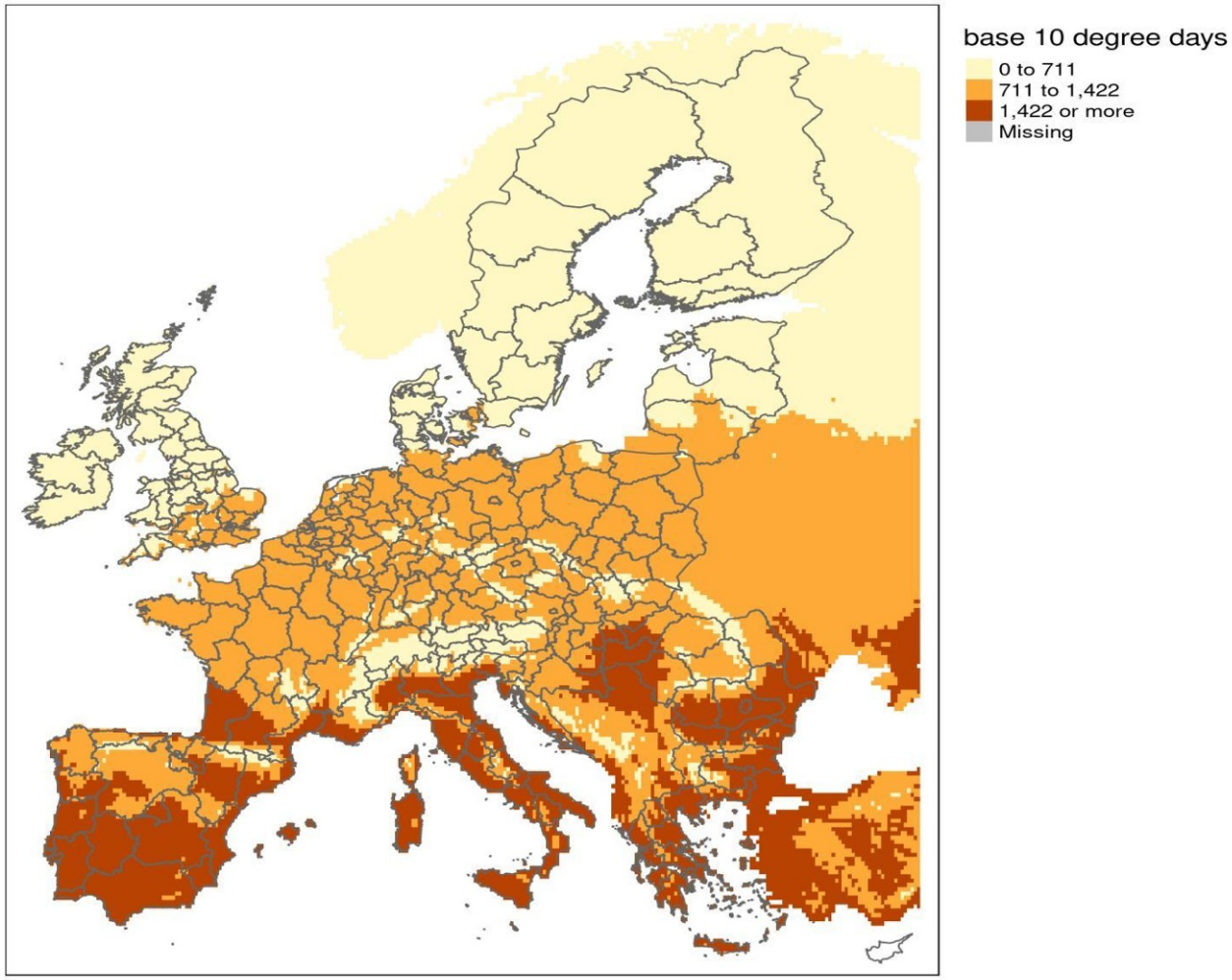

Figure 4: Thermal suitability area for *Popillia japonica* based on degree days above 711 (for two-year life cycle) and 1422 (for one-year life cycle) with threshold of 10°C (Source: Régnière et al., 1981). Note that soil moisture – corresponding with rainfall – is not considered here but is important, since precipitation and soil moisture should be taken into account when considering establishment of *P. japonica*

As the Japanese beetle has a broad host range, host plants are not the limiting factor for its establishment. It is expected to be able to establish in all Member States where climatic conditions are suitable. The beetle has established in the Azores (Portugal) and in Milan (Italy), therefore, a high risk of spread to other countries with favourable conditions is assumed. However, as mentioned above, the temperature and the soil moisture are key parameters to limit the potential spread of the Japanese beetle into new areas. According to Bourke (1961; in Fleming, 1972) the Mediterranean region is not suitable for the establishment of the beetle due to the lack of summer rainfall while in northern Europe establishment was predicted to be less likely because summer temperatures are lower. In central France, southern Germany and parts of Switzerland, Austria, Czech Republic, Hungary, Poland, Romania and Slovakia, climatic conditions for establishment were assumed to be most suitable, since summer rainfall is abundant and temperature is favourable. Furthermore, extensive irrigation could increase suitability in some areas in southern Europe (EFSA PLH Panel, 2018).

1.7. Spread capacity

Although beetles can fly up to 8 km, they rarely do (Fleming, 1972). Lacey et al. (1995) recaptured 70% of beetles within 50 m of the release point in a mark–release–recapture study in the Azores. Only less than 1% were recaptured at 1 km. According to Sara et al. (2013), adult density significantly decreased with higher distance from a field edge. A much higher spread rate (16–24 km per year) was found in the decade after *P. japonica's* establishment in the USA (EPPO, 2006). After that period, Fox (1932) found spread rates varying between 3 and 24 km per year. Allsopp (1996) estimated *P. japonica* spread at 7.7 km $year^{-1}$ between the years 1927 and 1938 followed by 11.9 km $year^{-1}$ between the years 1939 and 1951. This could have been both due to natural dispersal and human-assisted spread, e.g. with plants for planting.

The greatest flight activity is reported to be on clear days and when the temperature is between 29°C and 35°C, relative humidity >60% and wind is <20 km h^{-1} (CABI, 2018).

The adult's flight period extends from late May throughout early November, with peak numbers caught during the last half of July and the first half of August, obtaining in this period 82% of the total number of beetles captured (Vieira, 2008). Odour and location in direct sun seem to be very important factors in plant selection. The beetles usually feed in groups, starting at the top of a plant and working downwards (Vieira, 2008).

1.8. Risk factor identification

A risk factor is a biotic or abiotic factor that increases the probability of infestation in the epidemiological unit by the pest. The risk factors that are relevant for the surveillance are those that have more than one level of risk for the target population.

The first risk factor retained is related to the entry points of the pest in the EU, in particular airports, ferry docks, bus stations and railway stations, nurseries, garden centres.

Another risk factor considered for this pest is the host species. The areas with abundant moist grassland and turf are suggested in EPPO (2016) as the most attractive oviposition sites for the females. Therefore, the epidemiological units that include this type of environment could be considered with a higher relative risk than the others.

2. Detection and identification

2.1. Visual examination

2.1.1. Pest

Larvae and adults are the life stages that can be detected by visual examination in a distinguishable way. The larvae (Figure 5) live in the fibrous root zone of the plants and, they can, therefore, be detected by examination of the soil and roots.

Figure 5: A typical C-shaped creamy white grub of *Popillia japonica* in the soil (Source: David Cappaert, Bugwood.org)

A distinctive morphological characteristic of P. japonica larvae is a V-shaped arrangement of the last two rows of spines (raster) on the last body segment, 6–7 in number, ventral to the anal opening (Figure 6). They may be seen with a hand lens and if they are not present, the larva belongs to a species other than *P. japonica* (CFIA, 2017).

Figure 6: The raster pattern on the last abdominal segment of *Popillia japonica* (Source: Mike Reding and Betsy Anderson, USDA Agricultural Research Service, Bugwood.org)

Microscopic identification might be needed to distinguish *P. japonica* larvae from closely related species (EPPO, 2016). Further details on the visual identification and a dichotomous key for Scarabaeoidea families and the *Popillia* genus are provided in Appendix 1 of EPPO (2006).

The adult beetle is brightly coloured metallic green and coppery bronze, oval in shape, and varies in size from 8 to 11 mm in length and 5 to 7 mm wide (Figure 7). The female is typically larger than the male. Along each lateral side of the elytra, there are five tufts of white hair present and two dorsal spots of white hair on the last abdominal segment. Male and female beetles can be differentiated from each other by the shape of the tibia and tarsus on the foreleg. The male tibial spur is more sharply pointed and the tarsi are shorter and stouter than those of the female (EPPO, 2006).

Figure 7: The adult Japanese beetle *Popillia japonica* (Source: Emmy Engasser, Hawaiian Scarab ID, USDA APHIS ITP, Bugwood.org)

According to USDA (2016) a larval survey should be conducted if the turf damage indicates a large number of grubs in the soil. Based on the European situation in Milan (Italy), ERSAF (2016) recommends that the larval monitoring should be carried out in grassy meadows, especially irrigated ones, located in the infested area.

The most used method for finding *P. japonica* larvae is coring or extraction of cubic portions of soil, 20 cm in depth, width and height. It is recommended to take four core samples from the surface area under 0.5 hectares and six samples from fields with surface areas between 0.5 ha and 1 ha. For a surface area greater than 1 ha, two additional core samples need to be collected for each extra hectare, over and above the basic six samples (ERSAF, 2016).

The starting point for extraction should consider that *P. japonica* tends to prefer the cooler and shadier portions. In America the insect appeared to prefer downwind locations, near bushes, more to the south and east than north and west. Thus, it is recommended at least half the core samples to be collected near the edge where conditions are most favourable for egg deposition, and the others two-three dozen meters towards the middle. The distance between one core and the next should not be less than 20 m, unless the reduced dimensions of the field make this impossible (ERSAF, 2016).

After extraction, the soil is searched to identify the larvae in it, by tipping it into a white tray and breaking soil apart with hand tools. All larvae suspected of being beetle larvae are collected in sample tubes containing 70% alcohol and labelled with a sample code (ERSAF, 2016).

According to CABI (2018), larvae can be found by cutting sections of turf with a spade or golf cup cutter in late summer, autumn or early spring. The soil and roots are examined about 8 cm in depth. For larvae infesting nursery trees, examining soil down to 30 cm may be needed to get a reliable sample (CABI, 2018). Adults can be detected by visual examination of green parts of plants. Further details on how to carry out the visual examinations for the adult beetles can be found in Appendix 2 of EPPO (2016).

It needs to be considered, that if a suspected specimen is collected in North America or Italy, there is high confidence that a correct morphological identification will be made. However, the genus is large and other species in Asia could be confused with *P. japonica* morphologically. Therefore, if found in the vicinity of locations importing plants from Asia, there is a chance that another species of *Popillia* could be present. Nevertheless, gene sequence data are available and many labs in Europe could confirm identification.

2.1.2. Symptoms

Symptoms caused by adults of *P. japonica* are easily recognised in particular defoliation (Figure 8). On leaves the adult beetle chews out the tissue between the veins (EPPO, 2006).

Figure 8: Skeletonised leaves of grapevine (*Vitis vinifera*) (Source: David Cappaert, Bugwood.org)

The beetles can also feed on flower petals (Figure 9).

Figure 9: Japanese adult beetle feeding on a rose (*Rosa* sp.) flower (Source: M.G. Klein, USDA Agricultural Research Service, Bugwood.org)

The larvae cause feeding damage to the roots of host plants, and the symptoms caused are not all specific (EPPO, 2006). The pest prefers areas with moist, loamy soil covered with turf or pasture grasses (Figure 10). They feed just below the surface, cutting and consuming the grass roots. Early symptoms include thinning, yellowing, and wilting of grass (CABI, 2018).

Figure 10: Grass turf damaged by larvae of *Popillia japonica* (Source: M.G. Klein, USDA Agricultural Research Service, Bugwood.org)

On corn, the beetles feed on the maturing silk, preventing pollination; this results in malformed kernels and reduced yield (Figure 11) (CABI, 2018).

Figure 11: Damage on corn silk caused by *Popillia japonica* (Source: Daren Mueller, Iowa State University, Bugwood.org)

Risk of misidentification:

P. japonica larvae and adults are very similar to the pest of European cultivated grasslands *Phyllopertha horticola* (Figure 12), which has a similar life cycle and biology (Korycinska et al., 2015).

P. japonica can be distinguished from the latter by its shiny golden green thorax, lateral tufts of white hair on the abdomen, and two patches of white hair on the pygidium (EPPO and CABI, 1997).

Figure 12: Garden chafer *Phyllopertha horticola* (Source: Malcolm Storey, www.bioimages.org.uk)

2.1.3. Traps

Potter and Held (2002) consider traps most useful for the detection of new infestations and for monitoring populations. Traps containing a PEG food-type lure (phenethyl propionate + eugenol + geraniol) and a sex attractant (Japonilure) (Ladd et al., 1981; cited in CABI 2018) are widely used in the USA and the Azores for monitoring and survey, and for delimiting infestations (CABI, 2018). According to EPPO (2006), these could also be useful in warehouses with imported commodities. EPPO (2016) recommends that the traps should be put up at the end of May, checked once a month during the summer (frequency can be reduced at low-risk sites) and collected in September. However, Alm et al. (1996; cited in Potter and Held, 2002) stated that trap yields increased when traps were emptied daily, possibly because the odour of decaying beetles repels the beetles or masks the lure activity.

Hamilton et al. (2007) supposed that the region of the plume where concentrations of the lure are sufficient to provoke a response from the beetles, could extend at most 500 m downwind of the traps, and therefore beetles could be attracted from distances of several hundred metres. They found high trap catches near agronomic land with the two preferred host plants corn or soybeans. Catches were generally low in areas with no preferred host plants. Since putting pheromone traps too close together may reduce their effectiveness for monitoring purposes, as shown at least for other insects, it is recommended that traps should not be placed closer than 200 m apart (EPPO, 2016).

Traps with both food and pheromone lures are most effective in attracting adults when placed at approximatively 56 cm above ground level (USDA, 2016), but the height depends also from the presence of host plants in the survey area – if there is only turf or turf and high-growing hosts (trees) present, the trap height should be 28–56 cm from the funnel rim of the trap to the ground, but if turf and low-growing hosts are available, then the trap height should be at host level (EPPO, 2016). Usually, traps are yellow, but white and green traps are equally effective (Figure 13).

Figure 13: Types of traps used for *Popillia japonica* (left: Source; EPPO 2016, right: Trap used in Dutch surveys, Source; NVWA, 2018)

It is recommended to place the traps:

- In direct sunlight (all-day sun or at least midday sun) because they are twice more effective as those placed in the shade.
- In close but not immediate proximity to host plants, at 3–6.4 m from favoured trees, shrubs, and vines (see Section 1.5). Traps placed immediately adjacent to tall, bushy plants or other objects could be of lower efficacy since dissemination of the lure may be hindered (USDA, 2016).

Trapping in areas where *P. japonica* is not known to occur should be conducted at the rate of one trap per 5 km^2 in areas suitable for *P. japonica* establishment (Government of Canada, 2015). The California Department of Food and Agriculture (2013) recommends 0.7 traps per km^2 urban and rural residential areas of 300 or more homes per 2.5 km^2. Furthermore, when a beetle is trapped, the number of traps should increase at 450 traps in 12 km^2 surrounding the finding. In high-risk areas, e.g. airports receiving significant travel from areas infested with Japanese beetle, the California Department of Food and Agriculture (2013) suggests that 25 traps per 2.5 km^2 should be placed in a 1.6 km buffer zone. Traps should be evenly spaced.

Further information and details on how to use traps for adult beetles are provided in Appendix 4 of EPPO (2016).

2.2. Laboratory testing

The samples of larvae collected need to be examined in the laboratory, under the microscope to identify the distinctive morphological characteristic of *P. japonica* larvae (see Section 2.1.1).

2.2.1. Identification of methods

Molecular identification methods are available for distinguishing larvae from native species, including Polymerase chain reaction (PCR) testing.

2.2.2. Diagnostic protocols

ERSAF (2016) recommends the morphological identification with a binocular microscope as a diagnostic method (see also EPPO, 2006). If in doubt, the morphological identification can be coupled with or replaced by bio-molecular analysis, consisting of the isolation of a fragment of DNA using PCR, with universal barcode primers and then sequencing. The sequences obtained are then compared with standard *P. japonica* sequences, deposited in international databanks.

A diagnostic protocol for PCR test (LCO1490/HCO2198) has been prepared by Folmer et al. (1994).

Airports are usually surrounded by abundant grassland and, thus, the combination of both risk factors increases the probability to find the beetle in these areas, if present. This is supported by the finding of the beetle around an international airport in Italy.

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 of 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 several pests have to be surveyed in the same crop, it is recommended to use the same epidemiological and inspection units for each pest in order to optimise the survey programme as much as possible.

Table 2 shows an example of these definitions.

Table 2: Examples of definitions of the target population, epidemiological unit and inspection unit

	Definition	**Unit**
Target population	Total number of hectares with host plants and suitable climatic conditions and soil temperature in each Member State	Total number of hectares
Epidemiological units	Number of hectares of host plants (e.g. moist grasslands)	Hectare
Inspection units	host plants above ground, soil and roots in the risk areas	Individual host plants, soil and root samples

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

1/ the choice of the type of survey to develop depending on the objectives of the survey

2/ a description of the different surveillance components required to determine statistically sound sample sizes

3/ a manual for guiding the user through the tools

4/ calculation of the sample size

5/ essential considerations when:

- choosing the sampling sites and taking the samples
- collecting the data
- reporting the data and the survey results

References

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Glossary

TERM	DEFINITION*
Component (of a survey)	In the general framework of surveillance, with the goal of demonstrating pest freedom, a component is an activity characterised by a given sensitivity of the method of detection and identification. The overall confidence of the survey for pest freedom will result from the combination of the different components. Two components of the same survey could have different target populations. E.g. Survey on an insect performed by trapping of the pest (component 1) and sampling the host plants for visual examination of signs or symptoms (component 2).
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)
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, 2018).
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, 2018).
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, 2018).
Pest freedom	An area in which a specific pest is absent as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained (ISPM 5: FAO, 2018).
Population size	The estimation of the number of the 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 on 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, 2018).
Risk factor	A factor that may be involved in causing the disease (Cameron et al., 2014). It is defined as a biotic or abiotic factor that increases the probability of infestation of the epidemiological unit by the pest. The risk factors relevant for the surveillance should have more than one level of risk for the target population. For each level, the relative risk needs to be estimated as the relative probability of infestation compared to a baseline with a level 1. Consideration of risk factors in the survey design allows the survey efforts to be enforced in those areas where the highest probabilities exist to find the pest should the pest be present.
Risk-based survey	A survey design that considers the risk factors and enforces the survey efforts in the corresponding proportion of the target population.
Sample size	The number of sites that need to be surveyed in order to detect a specified proportion of pest infestation with a specific level of confidence, at the design prevalence (McMaugh, 2005).
Survey	An official procedure conducted over a defined period of time to determine the characteristics of a pest population or to determine which species are present in an area (ISPM 5: FAO, 2018).
Target population	The set of individual plants or commodities or vectors in which the pest under scrutiny can be detected directly (e.g. looking for the pest) or indirectly (e.g. looking for symptoms suggesting the presence of the pest) in a given habitat or area of interest. The different components pertaining to the target population that need to be specified are: • Definition of the target population – the target population has to be clearly identified • Target population size and geographic boundary. (EFSA, 2018)
Test	Official examinations, other than visual, to determine whether pests are present or to identify pests (ISPM 5: FAO, 2018).
Test specificity	The conditional probability of testing negative given that the individual does not have the disease of interest (Dohoo et al., 2010). The test diagnostic specificity (DSp) is the probability that a truly negative epidemiological unit will give a negative result and is related to the analytical specificity. In freedom from disease it is assumed to be 100%.

Visual examination	The physical examination of plants, plant products or other regulated articles using the unaided eye, lens, stereoscope or microscope to detect pests or contaminants without testing or processing (ISPM 5: FAO, 2018).

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