Pest survey card on *Anthonomus eugenii*

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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 *Anthonomus eugenii* surveys. These are required to design statistically sound and risk-based pest surveys, in line with current international standards. *Anthonomus eugenii* is a clearly defined taxonomic entity that is subject to specific measures in the EU to prevent entry of the pest via the fruit of *Capsicum* spp. *Capsicum* spp. and *Solanum melongena* are the most relevant hosts in the EU with the former having the highest risk of infestation. The areas around packing and sorting stations where imported fruit of these crops is handled would also be at a higher risk. *Anthonomus eugenii* could become established indoors in all areas in the EU where *Capsicum* and *S. melongena* plants are grown in greenhouses, provided that a nearly continuous production cycle is in place. In the southernmost parts of the EU, it is also expected that *A. eugenii* could become established outdoors. Detection of *A. eugenii* should either be performed by visual examination to detect the pest and its signs of infestation in the growing plants, and by using traps to catch adult beetles in the absence of host plants. It is recommended that the initial morphological identification of *A. eugenii* is confirmed by DNA barcoding.

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**Keywords:** *Anthonomochaeta eugenii*, *Capsicum* spp., pepper weevil, plant pest, *Solanum melongena*, survey, risk-based surveillance

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Introduction

The information presented in this pest survey card was summarised from a recent pest risk analysis of *Anthonomus eugenii* for the EU territory (van der Gaag and Loomans, 2013), a rapid Pest Risk Analysis by Baker et al. (2012), the EPPO Global Database (EPPO, online) and other documents.

The objective of this pest survey card is to provide the relevant biological information needed to prepare surveys for *A. eugenii* in EU Member States (MSs) following the methodology described in EFSA (2018). It is part of a toolkit that is being developed to assist MSs with planning a statistically sound and risk-based pest survey approach in line with International Plant Protection Convention standards (ISPMs) and guidelines for surveillance (FAO, 2016a,b; FAO, 2018). The toolkit consists of pest-specific documents and generic documents relevant for all pests to be surveyed:

i. Pest-specific documents:
   a. The pest survey card on *Anthonomus eugenii*  
ii. General documents:
   a. The general survey guidelines
   b. The RiBESS+ manual
   c. The statistical tools RiBESS+ and SAMPELATOR.

1. The pest and its biology

1.1. Taxonomy

**Scientific name:** *Anthonomus eugenii* Cano, 1894  
**Class:** Insecta, **Order:** Coleoptera, **Family:** Curculionidae, **Genus:** Anthonomus, **Species:** Anthonomus eugenii  

**Synonym(s):** Anthonomochaeta eugenii (Cano), Anthonomus aeneotinctus Champion  

**EPPO Code:** ANTHEU  

**Common name:** pepper weevil  

**Taxonomy:** *Anthonomus eugenii* (Figure 1) is a clearly distinguished insect pest of several *Capsicum* and *Solanum* species.

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1 The Pest Survey Card will be updated in the form of 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://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
**Conclusions on taxonomy**

*Anthonomus eugenii* is a clearly defined taxonomic entity.

### 1.2. EU pest regulatory status

*Anthonomus eugenii* is a Union quarantine pest listed in Annex II of Commission Implementing Regulation (EU) 2019/2072\(^4\), and it is also listed as a priority pest under Commission Delegated Regulation (EU) 2019/1702\(^5\).

Special import requirements are laid down in Annex VII of Commission Implementing Regulation (EU) 2019/2072 regarding *Capsicum* fruit from third countries where *A. eugenii* is present.

The general requirements for surveys of quarantine organisms in the EU territory are laid down in Regulation (EU) 2016/2031\(^6\).

### Overview of the EU regulatory status

*Anthonomus eugenii* is a regulated priority Union quarantine pest and there are specific import requirements aiming to prevent the entry of the pest via *Capsicum* fruit.

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\(^4\) 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, p. 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. OJ L 260, 11.10.2019, p. 8–10.

\(^6\) 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, p. 4.
1.3. Pest distribution

*Anthonomus eugenii* probably originates from Mexico or surrounding regions in Central America (Goff and Wilson, 1937) from where it has spread to the Caribbean and southern states of the USA (Figure 2). Outbreaks have been reported from greenhouses in British Columbia (Canada) in 1992, but the pest was eradicated. The pest was found again in greenhouses in Canada (Ontario) in 2009 and 2010; no statutory action was taken to eradicate the pest (CFIA, 2011). In Canada, repeated annual invasions may result in localised, transient populations that cause significant damage. Such an event, for example, occurred during the pepper-growing season in 2016 in southern Ontario (Labbé et al., 2018). In the EU, the pest was reported in greenhouses in the Netherlands in 2012 and was eradicated with the application of official measures. In 2013, the pest was found in Italy (province of Latina) in greenhouses and in an open field (Speranza et al., 2014). Statutory action was taken and the pest was eradicated (EPPO, 2020).

![Figure 2: Global distribution of *Anthonomus eugenii*](https://gd.eppo.int)

**Conclusion on pest distribution**

*Anthonomus eugenii* is not known to be currently present in the EU. Therefore, surveys in Member States would be conducted to substantiate pest freedom. If there is a new outbreak, delimiting surveys should be conducted in that area to define the boundaries of the infested zone. Most of the outbreaks have occurred in greenhouses, so they should be taken into account in the survey design.

1.4. Life cycle

*Anthonomus eugenii* is mainly a pest of cultivated *Capsicum* species. Males and females are attracted to volatiles from flowering and fruiting pepper plants, to pepper weevil-damaged plants, and to the presence of the male aggregation pheromones (Eller et al., 1994; Addesso and McAuslane, 2009; Addesso et al., 2011). Adult weevils feed on buds, flowers, fruit and leaves (Patrock and Schuster, 1992; Rodríguez-Leyva, 2006).

Females prefer young fruit for feeding and egg laying, but they can also use flower buds, open flowers and mature fruit to lay eggs (Patrock and Schuster, 1992). A single egg is laid in feeding punctures (Addesso et al., 2007). The larvae feed on seeds and other tissue inside the developing fruit, where they also pupate. The adults develop and may remain protected for several days, feeding inside the fruit before chewing a small exit hole (Figure 3). As a consequence, fruit may become deformed and discoloured, ripen prematurely and suffer from premature abscission (Riley and Sparks, 1995).
Anthonomus eugenii develops in several generations per year, depending on thermal conditions. The pepper weevil has a lower threshold and optimum temperature for development of about 10°C and 30°C, respectively (Toapanta et al., 2005). It takes the pepper weevil about two weeks to complete its life cycle in warm conditions (27°C), three weeks at ambient conditions (21°C) and six weeks in cool conditions (15°C).

Figure 3: Life cycle of Anthonomus eugenii at 21°C (Source: Riley and Sparks Jr, 1995; courtesy of D. Riley)

### Conclusion on life cycle
Surveys can either target the adult beetles themselves or the signs of an infestation on the buds, flowers, fruit and leaves of host plants. Anthonomus eugenii detection should be carried out all year round, as long as buds and fruit are present, and the temperature is above 10°C.

1.5. Host range and main hosts
Anthonomus eugenii can reproduce on various species within the genera Capsicum and Solanum (Table 1). In addition, adults can feed on several Solanum spp., but do not reproduce on them. Capsicum species are the preferred hosts and the primary hosts that are grown on a commercial scale are Capsicum annuum and C. frutescens. The pest has the potential to cause major economic damage in cultivated pepper crops. Among the Solanum species, Solanum melongena is the most relevant host in terms of economic impact. Solanum lycopersicum and S. tuberosum are used by adults for feeding, but pepper weevils do not reproduce on them (Table 1).
Table 1: Host plants of *Anthonomus eugenii* and their presence in Europe. The table is adapted from Van der Gaag and Loomans (2013)

| Latin name | Presence in the European Union | Host plants: reproduction possible | Host plants: food source for adults, no reproduction known |
|------------|-------------------------------|----------------------------------|----------------------------------------------------------|
| *Capsicum anuum* | Yes, commercial and hobby (sweet pepper and chilli pepper) | X | |
| *C. baccatum* | Hobby (chilli pepper) | X | |
| *C. chinense* | Hobby (chilli pepper) | X | |
| *C. frutescens* | Hobby (chilli pepper) | X | |
| *C. pubescens* | Hobby (chilli pepper) | | |
| *Solanum americanum* | Cultivated, rare casual. Western and southern central Europe | X | |
| *S. carolinense* | Locally naturalised. Italy | X | |
| *S. dimidiatum* | No | X | |
| *S. elaeagnifolium* | Locally naturalised. Southern Europe | X | |
| *S. melongena* | Commercial and hobby (eggplant) | X | |
| *S. nodiflorum* | No | X | |
| *S. pseudocapsicum* | Pot plant, locally naturalised. South-western Europe | X | |
| *S. pseudogracile* | No | X | |
| *S. ptychanthum* | No | X | |
| *S. rantonettii* | Yes (greenhouses, gardens) | X | |
| *S. rostratum* | Locally naturalised. Italy | X | |
| *S. triquetrum* | No | X | |
| *S. axilfolium* | No | X | |
| *S. madrense* | No | X | |
| *S. nigrum* | Common weed (black nightshade) | X | |
| *S. trydynamum* | No | X | |
| *S. tuberosum* | Commercial and hobby (potato) | X | |
| *Datura stramonium* | Weed (jimsonweed) | X | |
| *S. lycopersicum* | Commercial and hobby (tomato) | X | |
| *Nicotiana alata* | Garden plant (sweet-scented tobacco) | X | |
| *Petunia parviflora* | Garden plant (petunia) | X | |
| *Physalis pubescens* | Hobby (husk tomato) | X | |

* Fruit not tested; **No feeding observed.

The preferred hosts of the pest are presented in bold.

**Conclusion on host range and main hosts**

For surveillance, *Capsicum* spp. and *Solanum melongena* are the most relevant host plants in the EU that should be targeted by detection surveys. Whereas if there is an outbreak, delimiting surveys should consider all known host plants of *Anthonomus eugenii* that can be found in the survey area.
1.6. Environmental suitability

*Anthonomus eugenii* has no diapause and thus requires the continuous presence of host plants. The species is likely to become established in greenhouses with a nearly continuous *Capsicum* fruit crop (the pest may, however, be eradicated from the greenhouse when the crop is completely removed) and outdoors in *Capsicum* fruit production areas in plant hardiness zones 7–10 and higher and the milder areas in zone 9 (van der Gaag and Loomans, 2013). In the absence of a *Capsicum* crop, wild *Solanum* species can be alternative hosts. The potential distribution area of *A. eugenii* is thus limited to the southernmost parts of the EU when outdoors (EFSA, 2019). This area includes southern Spain, southern Portugal, Madeira, the Azores, southern Italy, Malta, southern Greece and Cyprus (Figure 4).

![Figure 4: The potential outdoor distribution of the pest in the EU NUTS2 regions based on the host distribution (Source: EFSA, 2019)](image)

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7 Plant hardiness zones are based on the average annual extreme minimum temperature (Magarey et al., 2008) and can be used as an indicator of potential establishment for phytosanitary risk analysis.
Conclusion on environmental suitability

*Anthonomus eugenii* is able to become established indoors in all areas of the EU where *Capsicum* crops and *Solanum melongena* are grown in greenhouses, provided that a nearly continuous production cycle is in place. It could become established outdoors in the southernmost parts of the EU.

1.7. Spread capacity

**Natural spread**

*Anthonomus eugenii* is not considered to be a strong flyer, but its spread via wind can still be considerable. Adults may fly one or several kilometres within one season. EFSA (2019) considers, based on expert knowledge elicitation, that the maximum distance expected to be covered is approximately 2 km per year (with a 95% uncertainty range of 119–5,802 m). During the outbreak in the Netherlands, later eradicated, six greenhouses that became infested were all within a 2 km radius. van de Vossenberg et al. (2019) concluded that this was the result of a single introduction and subsequent natural spread.

**Human-assisted spread**

The pest can be spread over larger distances, especially by trade of infested fruit. The pest has been intercepted many times on *Capsicum* and *Solanum* fruit in the EU (EUROPHYT, online). Local movement can also occur, via worker movement between greenhouses and farms.

**Conclusion on spread capacity**

*Anthonomus eugenii* is not a strong flyer, and the maximum distance expected to be covered is about 2 km per year. However, the pest can spread larger distances by trade of infested fruit and plants.

1.8. Risk factor identification

Identification of risk factors and their relative risk estimation is 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 in each Member State. This section presents examples of risk factors for *Anthonomus eugenii* (Table 2) and is not necessarily exhaustive.

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

**Example 1: Distance from packing and sorting stations of *Capsicum* and *Solanum* fruit imported from regions where the pest is present**

*Anthonomus eugenii* has been intercepted on the fruit of *C. frutescens*, but also on other *Capsicum* species and *S. melongena*. Adult weevils can survive prolonged cool conditions (2–5°C) for over 3 weeks and could thus survive long-distance transport (Costello and Gillespie, 1993). Specific import requirements are in place to mitigate the risk of introduction, but infested fruit may still be a pathway of entry. Therefore, the packing and sorting stations that handle such fruit can be considered risk locations. Given the spread capacity of the species, the risk area surrounding the packing and sorting stations is of limited size. In fact, for *A. eugenii* to become established in the EU, it would require close contact between the imported consignment and the production crop.
**Example 2: Host plants**

*Anthonomus eugenii* can reproduce on various species within the genera *Capsicum* and *Solanum*, but has a clear preference for *Capsicum* species (see Table 1). When performing surveys in commercial crops, *Capsicum* spp. cultivation would thus be of higher risk than cultivation of *S. melongena*.

**Table 2:** Example of a risk activity and corresponding risk locations relevant for the surveillance of *Anthonomus eugenii*

| Risk activity                                      | Risk locations                                    | Risk areas                                 |
|----------------------------------------------------|---------------------------------------------------|--------------------------------------------|
| Imports of fruit from countries where the pest is  | Packing and sorting stations where such fruit is  | The area surrounding the packing and       |
| present                                            | handled                                          | sorting stations                           |
| Growing of preferred hosts                         | Fields where such hosts are grown                 | Fields where such hosts are grown          |

**2. Detection, sampling and identification**

**2.1. Detection**

**2.1.1. Visual examination**

The goal of the visual examination is to detect the symptoms caused by *Anthonomus eugenii* or the detection of the pest itself.

**Pest**

Identification of *A. eugenii* to the species level is extremely difficult in the field because *Anthonomus* species are highly similar in their external morphology. To aid the identification, it is important to record from which crop or plant species the specimens are collected. *Anthonomus* species that do naturally occur in the EU are not known to attack plant species that are known to be host plants of *A. eugenii*. When beetles are collected from a pepper crop that is affected by the signs of a pepper weevil infestation as described below, it is a sign that the pest involved is in fact *A. eugenii*. Nevertheless, species identification will still be needed and requires the use of a stereomicroscope.

Morphological species identification can only be performed on adult beetles and not by the examination of eggs, larvae or pupae. Larvae are white to grey in colour, with a yellowish-brown head and – depending on whether they are in the first, second or third instar stage – they are between 1 and 5 mm long (Capinera, 2014). The pupae are initially white and eventually become yellowish with brown eyes. They resemble the adults in form, except that the wings are not fully developed and they have large setae on the prothorax and abdomen (Capinera, 2014). Adult pepper weevils are oval in shape, and are quite small, being 2.0 to 3.5 mm in length and 1.5 to 1.8 mm in width (Figure 5). The dark mahogany to nearly black body is strongly arched, while the head has a long stout beak. Both the thorax and elytra are mostly covered with small scales. The antennae are long and markedly expanded at the tip (Capinera, 2014). *Anthonomus eugenii* is the only weevil present on pepper that has a small spur on the underside of the femur near the joint with the tibia (Ghidii et al., 2008). This spur is present on all the insects’ legs.
Symptoms

In the early stages after the arrival of *A. eugenii* in a crop, detection will be challenging. Early signs of infestation are not specific, such as small holes in immature fruit and flowers and small circular or oval holes (2–5 mm in diameter) in leaves which can be mistaken for slug or caterpillar damage. Infested *Capsicum* fruits can have apparent symptoms, but the pest may be present in seemingly healthy peppers. As the population of *A. eugenii* builds up, signs of infestation become more abundant. Particularly, premature ripening and abscission of young fruit – as a consequence of feeding and developing inside the buds and fruit – is a clear sign of infestation by *A. eugenii* (Riley and Sparks, 1995). The presence of multiple abscised fruit at the base of a plant is therefore a good indicator that the pest is present. Other external signs and symptoms on fruit include egg-laying scars and the emergence holes of the adults (Figure 6 and 8). Larval feeding may also result in discoloration (Figure 7) and deformation of the fruit. When an affected fruit is cut open, signs of larval feeding – and the larva itself – can be observed. Peppers that are damaged by pepper weevils become more susceptible to fungal pathogens, such as *Alternaria alternata*, causing fungal growth within the fruit (Bruton et al., 1989).

Adult feeding punctures on the fruit appear as dark specks, but are not as apparent as the larval damage. Other signs of the presence of *A. eugenii* are bud drop and adult feeding punctures in the flowers (Figure 6). In the absence of fruit and flowers, the adults feed on the leaves and stems of the plants without causing significant damage. Examples of the signs and symptoms on plants and fruit are provided, for example, by van der Gaag and Loomans (2013) and Capinera (2014).
**Figure 6:** Signs of a pepper weevil infestation: feeding punctures in flowers (left) and egg-laying scars in young fruit (right) (Source: NVWA; van der Gaag and Loomans, 2013)

**Figure 7:** Signs of a pepper weevil infestation: fruit discolouration (left) and aborted fruit with dried calyx (right) (Source: NVWA; van der Gaag and Loomans, 2013)
Figure 8: Signs of a pepper weevil infestation: emergence hole (left) and damage inside to fruit seeds (right) present in young fruit due to feeding (Source: NVWA; van der Gaag and Loomans, 2013)

2.1.2. Trapping

Yellow sticky traps can be used to catch pepper weevil adults (Riley and Schuster, 1994) both outdoors and in greenhouses. Pheromone traps are also available. Mellinger and Bottenberg (2000) studied the efficacy of pheromone traps. The traps may attract beetles from over several hundred metres in the absence of a Capsicum crop, but in the presence of crops with blooms and fruit, weevils may be attracted from within a distance of 6–9 m only. This is because ‘pepper plants with blooms and fruit produce olfactory compounds that attract weevils, and therefore compete with the pheromone traps’. More beetles were caught during crop destruction activities.

Low infestation levels are difficult to detect by visual inspection so traps are recommended (Riley and Schuster, 1994). Within each survey site traps should be placed near the edges of the crop at the height of the plant and especially during crop destruction activities or shortly after planting, when the number of buds and fruit is still limited. Traps should be regularly checked for the presence of adults. If an adult is found in a trap the crop should be visually inspected to confirm the infestation. When adults are captured during the intercropping period, the immediate surroundings of the greenhouse or field should be inspected for host weed plants and pest symptoms.

Trapping among Solanum weeds around fields that have previously produced Capsicum or S. melongena fruit are only relevant if there is an outbreak and the National Plant Protection Organisation needs to check whether the pest is present in field edges especially during intercropping periods.

Conclusions for detection methods

Detection of Anthonomus eugenii should either be performed by visual examination of the host plants to detect signs of infestation in the growing crop or by observing the pest instars themselves. Pheromone or colour traps are effective at detecting adult beetles in the absence of host plant fruit and buds.

2.2. Sampling

Although a standard sampling protocol has not yet been developed, sticky traps and plant material with Anthonomus eugenii signs of infestation should be collected and preserved at a controlled low temperature until laboratory processing. Adult specimens should ideally be preserved in absolute ethanol.
Anthonomus eugenii survey card

for more efficient molecular analysis. Information on time, site, host plant and collector should be recorded and reported for each sample.

2.3. Identification

2.3.1. Laboratory testing

After the collected beetles have been taken to the laboratory, morphological identification of *Anthonomus eugenii* requires the use of a stereomicroscope. A key to the Mexican and Central American genera of *Anthonomini* (Curculionidae, Curculioninae) has been published by Hernández et al. (2013). When the specimen belongs to the genus *Anthonomus*, the key by Clark and Burke (1996) can be used to determine the sampled species. This key includes the *Anthonomus* species that are associated with Solanaceae, so knowledge on the host plant species is also needed for identification.

In addition to the morphological identification, a protocol for DNA barcoding on cytochrome oxidase I (COI) is available and allows the identification of *A. eugenii* (EPPO, 2016). It can also be used to differentiate some major haplogroups (van de Vossenberg et al., 2019).

**Conclusion for pest identification**

Keys for the morphological identification of *Anthonomus eugenii* are available and require the use of a stereomicroscope. However, it is recommended that the identification is confirmed by DNA barcoding.

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 several pests have to be surveyed in the same crop, it is recommended that the same epidemiological and inspection units are used for each pest in order to optimise the survey programme as much as possible. This would optimise field inspections since they are organised per crop visit and not by pest. Table 3 shows an example of these definitions.

**Table 3:** Example of definitions of the target population, epidemiological unit and inspection unit for *Anthonomus eugenii*

| Definition                  |                     |
|-----------------------------|---------------------|
| **Target population**       | All fields or greenhouses in a Member State in which *Capsicum* spp. or *Solanum melongena* plants are grown |
| **Epidemiological units**   | A single field or greenhouse in a Member State in which a *Capsicum* spp. or *Solanum melongena* plants is grown |
| **Inspection unit**         | A host plant of *Capsicum* spp. or *Solanum melongena* or a trap |

To design a plant pest survey on *Anthonomus eugenii* the general guidelines provide further details on the following steps that will generally be necessary:
1/ Determine the type of survey based on its objectives. For *A. eugenii*, the type of survey will depend on the pest status (according to International Standards for Phytosanitary Measures 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 have to be decided by the risk managers before designing the surveys as they reflect the acceptable level of the risk of infestation of the host plants by *A. eugenii*. The 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. eugenii*, 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 plants in a Member State.

3/ Define the epidemiological units. The epidemiological units should be single homogeneous areas that each contain each at least one individual host plant.

4/ Determine the inspection unit. In the case of a field or greenhouse with *Capsicum* spp. and *Solanum melongena* fruit crops, the inspection unit could be the individual plants or could be individual traps placed there.

5/ Determine the number of inspection units per epidemiological unit. For a field or greenhouse with *Capsicum* spp. and *Solanum melongena* fruit crops, this is the average number of plants or traps 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 when using 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 are inspected the higher the confidence will be. 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. 1%), 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 a Member State in order to state with 95% confidence that the prevalence of *A. eugenii* will be at 1% 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, etc.).

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, 2020). |
| **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, 2020). |
| **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).

*analogous to the term **level of detection** used in ‘Methodologies for sampling of consignments’ (ISPM 31: FAO 2016b)* |
| **Detection survey**                           | Survey conducted in an area to determine whether pests are present (ISPM 5: FAO, 2020). |
| **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**                       | 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 to which statistics are applied (e.g. a tree, orchard, field, glasshouse, or nursery) (EFSA, 2018). |

*analogous to the term **lot** used in ‘Methodologies for sampling of consignments’ (ISPM 31: FAO 2016b)*
| **Expected prevalence** | In prevalence estimation approaches, it is the proportion of epidemiological units expected to be infested 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, 2020). This definition is limited to an array of host plant species and does not include the 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 insect 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, 2020). |
| **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, 2020). |
| **Method sensitivity** | The conditional probability of testing positive given that the individual is diseased (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 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 epidemiological unit 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 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 diagnosis** | The process of detection and identification of a pest (ISPM 5: FAO, 2020). |
|-------------------|-------------------------------------------------------------------------|
| **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). |
| **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 (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 freedom from disease. Free access to the software with prior user registration is available at [https://shiny-efsa.openanalytics.eu/](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, 2020). |
| **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/](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 *Phyllosticta citricarpa*, the list of specified plants, which includes host plants and all plants for planting, other than seeds, belonging to the genera or species, can be found in Annex I of Decision (EU) 2015/789. |
| **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, 2020). |
| **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: |

* analoguous to *consignment* used in 'Methodologies for
| **sampling of consignments’**  
* (ISPM 31: FAO 2016b) | • 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, 2020). |
| **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 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, 2020). |

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