Pest survey card on *Rhagoletis pomonella*

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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 *Rhagoletis pomonella* surveys. These are required to design statistically sound and risk-based pest surveys, in line with current international standards. *Rhagoletis pomonella* is part of a species complex of closely related and morphologically similar sibling species that have distinct host plant affiliations. It is most clearly defined by its capacity to infest apples. Import of plants for planting of the major host plants is currently prohibited, while apples are subject to special import requirements that aim to prevent the entry of *R. pomonella*. Nevertheless, the most likely introduction of *R. pomonella* would be via transport of infested fruit, followed by gradual natural spread. *Rhagoletis pomonella* is currently absent from the EU and surveys would thus be aimed at substantiating pest freedom. Adult trapping is the preferred survey method for detecting *R. pomonella*. The best timing for trapping is limited to the presence of adults, which generally overlaps with the period in which the host fruit are available. Since the pupae undergo a long dormancy period, adults usually emerge several months following pupation. The primary host for detection surveys in the EU would be cultivated apples, whereas *Crataegus* species should be included for delimiting surveys. *Rhagoletis pomonella* is expected to be able to become established in most or all areas of the EU where apple and hawthorn species grow. Identification of *R. pomonella* at the species level requires morphological examination of the adults. Identification is most accurate when adults are obtained from flies reared in apples.

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Introduction

The information presented in this pest survey card was summarised from a pest risk analysis on *Rhagoletis pomonella* (WSDA et al., 2016), a pest report to support the ranking of EU priority pests (EFSA, 2019), the pest categorisation of non-EU Tephritidae (EFSA PLH Panel, 2020), the European and Mediterranean Plant Protection Organisation (EPPO) Global Database, the Centre for Agriculture and Bioscience International (CABI) datasheet on *R. pomonella* (CABI, 2019) and other documents.

The objective of this pest survey card is to provide the relevant biological information needed to prepare surveys for *R. pomonella* in EU Member States (MSs) following the methodology described by EFSA (2018). It is part of a toolkit that has been developed to assist the MSs with planning a statistically sound and risk-based pest survey approach in line with International standards for phytosanitary measures (ISPM 6: FAO 2018; ISPM 31: FAO, 2016a) and International Plant Protection Convention guidelines for surveillance (FAO, 2016b). The EFSA toolkit 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 on *Rhagoletis pomonella*.

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: *Rhagoletis pomonella* (Walsh)

Class: Insecta, Order: Diptera, Family: Tephritidae, Genus: *Rhagoletis* Species: *Rhagoletis pomonella* (Walsh)

Synonym(s): *Trypeta pomonella* Walsh 1867; *Spilographa pomonella* (Walsh), *Zonosema pomonella* (Walsh)

EPPO Code: RHAGPO

Common name: apple maggot fly, apple maggot

The family Tephritidae consists of a vast number of species and includes many species that are significant agricultural pests. In particular, the genus *Rhagoletis* contains 77 described species that are distributed throughout Europe, Asia and America and includes several species of economic importance (EFSA PLH Panel, 2020). *Rhagoletis pomonella* was first reported after the species expanded its natural host range from fruit of the hawthorn (*Crataegus* spp.) to fruit of domesticated apple trees when this fruit crop was introduced into North America (Bush, 1993).

*Rhagoletis pomonella* (Figure 1) is part of a group of closely related species – the *pomonella* species group or complex – which originally comprised the sibling species *R. pomonella*, *R. mendax*, *R. zephyria* and *R. cornivora* (Bush, 1966). The undescribed flowering dogwood fly is also considered to be part of this species complex (Berlocher, 1999). These species have a very similar morphology but have distinct host plant affiliations. Moreover, several allozyme loci display frequency patterns that are useful in discriminating the species (Berlocher, 2000). In addition, the species *R. pomonella* has been differentiated into host races (apple and hawthorn-infesting populations). The exact taxonomic

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1The 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=f91d6e95376fda5da206eb1815ad1489

2https://zenodo.org/record/2541541/preview/ribess-manual.pdf

3https://shiny-efsa.openanalytics.eu/
Rhagoletis pomonella survey card

The status of the entities in the pomonella complex is somewhat uncertain. However, sequence data analysis has shown that *R. cornivora* is clearly distinct from the other species, but neither nuclear nor mtDNA loci resolved the phylogenetic relationships among populations of *R. pomonella, R. mendax*, *R. zephyria* and flowering dogwood fly in the USA (Xie et al., 2008). Despite this complexity and uncertainty, *R. pomonella* can be considered a clearly defined entity given its capacity to infest apples (i.e. because of their host fidelity a finding of *Rhagoletis* larvae in apples would be a very strong indication of the presence of *R. pomonella*).

![Rhagoletis pomonella, the apple maggot fly](Source: Harvey Schmidt)

**Figure 1:** *Rhagoletis pomonella*, the apple maggot fly (Source: Harvey Schmidt)

### Conclusions on taxonomy

*Rhagoletis pomonella* is part of a species complex of closely related and morphologically similar sibling species that have distinct host plant affiliations. The species is most clearly defined by its capacity to infest apples.

### 1.2. EU pest regulatory status

*Rhagoletis pomonella* is a Union quarantine pest listed in Annex II of Commission Implementing Regulation (EU) 2019/2072.4

*Rhagoletis pomonella* is also listed as a priority pest under Commission Delegated Regulation (EU) 2019/17025 which imposes, among other measures, the obligation to conduct annual surveys of the pest.

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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.
Special import requirements are laid down in Annex VII of Commission Implementing Regulation (EU) 2019/2072 regarding the fruit of Malus to ensure pest freedom from R. pomonella. In addition, this Annex lays down special import requirements for growing medium in general that would also mitigate the risk for entry of pupae of R. pomonella should plants for planting of hosts be imported.

Plants for planting of the main host plants of the genera Malus and Crataegus are included in the list of high-risk plants under Commission Implementing Regulation (EU) 2018/2019².

The general requirements for surveys of quarantine organisms in the EU territory are laid down in Regulation (EU) 2016/2031⁷.

### Overview of the EU regulatory status

*Rhagoletis pomonella* is a Union quarantine pest, also listed as a priority pest. Import of plants for planting of the major host plants is currently prohibited, while the fruit of Malus is subject to specific import requirements that aim to prevent the entry of R. pomonella.

#### 1.3. Pest distribution

*Rhagoletis pomonella* is endemic to eastern North America from Canada via the USA to Mexico (CABI, 2019; Yee et al. 2014a; Michel et al. 2007; Rull et al. 2006). The species shifted from its native hawthorn host (Crataegus spp.) to domesticated apple in the mid-1800s (Hood et al. 2013). Nowadays, the species is also present in the western United States (Figure 2). Overall, *R. pomonella* is currently distributed in several states of the US, Canada and Mexico. No outbreaks have been reported in the EU so far.

![Image](https://gd.eppo.int)

**Figure 2:** Global distribution of *Rhagoletis pomonella*. (Source: EPPO Global Database, https://gd.eppo.int)

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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 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. OJ L 323, 19.12.2018, p. 10–15.

⁷ 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. 4–104.
1.4. Life cycle

Rhagoletis pomonella is a univoltine species, meaning that one generation of flies complete their life cycle in a single year (Dean and Chapman, 1973) (Figure 3). Flies overwinter in an obligate pupal diapause under the surface in the top 5 cm of soil beneath the host trees (mainly Malus spp. and Crataegus spp.), but can sporadically overwinter on the soil surface (within fallen leaves and dry grasses) or in fallen fruit. A small proportion of pupae may yield adults within the same season. Occasionally, flies delay emergence from the pupae in the soil for one or two years, while a few pupae may refrain from entering diapause causing flies to emerge before the winter in the same year.

A new generation of adults emerges from early June (Mattsson et al., 2015) onwards to September and adults live for approximately 30 to 40 days (Dean and Chapman, 1973). After emergence, they feed for 7 to 10 days before reaching sexual maturity. Adults feed on a variety of food sources, including honeydew, pollen and liquid from the plant glands, wounds and oviposition stings (Boller and Prokopy, 1976). Adults mate on or near the host fruit and the female lays a single fertilised egg just under the outer skin of ripe fruit on the tree. A single R. pomonella female can lay more than 200 eggs during her life. Eggs hatch after 3 to 7 days, while the emerging larvae take about 2 to 3 weeks to develop within the fruit (Christenson and Foote, 1960), undergoing three larval instar stages. Development times depend on the host species, fruit softness and temperature (Christenson and Foote, 1960; Messina and Jones, 1990; Dean and Chapman, 1973). The larvae tunnel through the flesh of the fruit while feeding, leaving a brown trail and causing the fruit to deteriorate. This often results in the premature abscission of infested fruit. Mature larvae emerge from the fruit to pupate after it has dropped to the ground or emerge from the fruit while still on the tree. The larvae burrow into the soil to pupate and enter the diapause.

Because adult trapping is the preferred survey method, the timing of the surveys should coincide with the period in which adult flies are present, which is aligned with the period in which host fruit is present on the host tree in North America (from June to September) (Mattsson et al., 2015). The presence of ripe or ripening fruit greatly increases the probability of trapping an adult at a given survey site, so the timing should be fine-tuned for specific host species, apple cultivars and local conditions.
**1.5. Host range and main hosts**

*Rhagoletis pomonella* has been reported to attack a number of host plants, several of which are agricultural hosts. Hawthorn species are its native host plants. The most heavily infested hosts are species of the *Malus* and *Crataegus* genera, which are largely distributed across the EU, while many species are considered incidental hosts. Yee et al. (2014a) consider that only apple (*M. domestica*) and various hawthorn species (*C. aestivalis*, *C. brachyacantha*, *C. crus-galli*, *C. douglasii*, *C. flabellate*, *C. flava*, *C. gracilior*, *C. greggiana*, *C. holmesiana*, *C. macroperma*, *C. mexicana*, *C. mollis*, *C. monogyna*, *C. opaca*, *C. pruinosa*, *C. punctate*, *C. rivularis*, *C. rosei*, and *C. viridis*) are the most important potential host species. Species in the genera *Amelanchier*, *Aronia*, *Cotoneaster*, *Prunus*, *Pyracantha*, *Pyrus*, *Rosa* and *Sorbus* can also be infested, but are considered to be of low importance (Yee et al., 2014a), whereas *Pyrus pyrifolia* (Asian pear) is considered to be of medium importance. For example, Bush (1966) indicates that larvae of *R. pomonella* have been found in pears (*Pyrus communis*), but that no adults have emerged.

The main commercial host is *Malus domestica*, in which the pest completes its life cycle and causes severe damage (CABI, 2019). In the EU, apples are cultivated on a large scale and these host trees are the main target for detection surveys. The existence of native *Crataegus* species in the EU, including *C. laevigata*, *C. monogyna*, and *C. orientalis*, and introduced ornamental ones (e.g. *C. aestivalis*, *C. brachyacantha*, *C. crus-galli*, *C. douglasii*, *C. flabellate*, *C. flava*, *C. gracilior*, *C. greggiana*, *C. holmesiana*, *C. macroperma*, *C. mexicana*, *C. mollis*, *C. monogyna*, *C. opaca*, *C. pruinosa*, *C. punctate*, *C. rivularis*, *C. rosei*, and *C. viridis*) are the most important potential host species. Species in the genera *Amelanchier*, *Aronia*, *Cotoneaster*, *Prunus*, *Pyracantha*, *Pyrus*, *Rosa* and *Sorbus* can also be infested, but are considered to be of low importance (Yee et al., 2014a), whereas *Pyrus pyrifolia* (Asian pear) is considered to be of medium importance. For example, Bush (1966) indicates that larvae of *R. pomonella* have been found in pears (*Pyrus communis*), but that no adults have emerged.
Rhagoletis pomonella highlights the importance of conducting survey activities in areas where those species are present. These species can be found in (semi-)natural conditions or urban areas, but can also be found as a hedge species in agricultural areas across all temperate environments in the EU. Crataegus is very likely to be infested should R. pomonella be introduced, but is a more challenging target for detection surveys given the difficulties of localising the scattered locations of these trees and shrubs. Nonetheless, for delimiting surveys, Crataegus spp. should be included as a target for surveillance.

C. crusgalli, C. pedicellata and C. persimilis highlights the importance of conducting survey activities in areas where those species are present. These species can be found in (semi-)natural conditions or urban areas, but can also be found as a hedge species in agricultural areas across all temperate environments in the EU. Crataegus is very likely to be infested should R. pomonella be introduced, but is a more challenging target for detection surveys given the difficulties of localising the scattered locations of these trees and shrubs. Nonetheless, for delimiting surveys, Crataegus spp. should be included as a target for surveillance.

Conclusion on host range and main hosts
The main host for detection surveys in the EU would be cultivated apple trees, while in addition Crataegus species should be included for surveillance in the case of delimiting surveys. The host range covers all the EU.

1.6. Environmental suitability

Rhagoletis pomonella is currently present in a considerable part of North America across a range of ecoclimatic conditions that closely resemble the climatic conditions in large parts of the EU. The pest seems to have a preference for moderate temperatures and high precipitation (WSDA et al., 2016).

Based on the present distribution in North America, Geng et al. (2011) used CLIMEX 3.0 to determine the potential distribution of R. pomonella in China. Hot and dry summers were found to have a negative effect on pest prevalence in the model. Geng et al. (2011) suggested that the determining factor for the predicted distribution was the environmental conditions (e.g. temperature, humidity) that the pupae experience, whereas eggs and larvae are protected from environmental fluctuations inside the host fruit. Kumar et al. (2016) used both MaxEnt and CLIMEX models, which indicated that most parts of central and southern Europe were highly favourable for the establishment of R. pomonella. Large parts of Scandinavia and the northern edges of the UK were estimated to be unsuitable for establishment (EFSA PLH Panel, 2020). Establishment is conditional on the presence of suitable host plants, which are largely absent from the areas considered unsuitable for R. pomonella. Overall, most or all areas of the EU in which apple and hawthorn species grow are at risk for the establishment of R. pomonella.

Conclusion on environmental suitability
Since host availability is not a limit for the spread of the pest, Rhagoletis pomonella is expected to be able to become established in most or all areas in the EU where apple and hawthorn species grow.

1.7. Spread capacity

Natural spread

Rhagoletis pomonella is not considered to be a long-distance flier. The flies normally travel relatively short distances when food resources and breeding sites are abundant. However, they may travel longer distances when this is not the case. All dispersal studies that have been carried out under field conditions were conducted in areas with abundant host plants. Release–recapture studies have found maximum flight distances up to approximately 1.5 km (Maxwell and Parsons, 1968), but when apple trees are more abundant nearby, reported maximum flight distances are much shorter (76–665 m) (Maxwell, 1968; Neilson, 1971; Bourne et al., 1934; WSDA et al., 2016).

Based on expert knowledge elicitation, EFSA (2019) estimated that the maximum distance expected to be covered in one year by R. pomonella is approximately 230 m (with a 95% uncertainty range of 24 m to 2.3 km).
Human-assisted spread

The most likely human-assisted spread of *R. pomonella* is through the transport of infested fruit (imports of fruit commodities or fruit in passenger luggage) because the development of eggs and larvae takes place in the fruit, making the infestation difficult to detect. Despite the fact that around 4,400 interceptions were generally attributed to Tephritidae specimens in the period 1995–2020 (EUROPHYT, online), no interceptions or outbreaks of *R. pomonella* have been reported in the EU so far.

The likelihood of importing adults can be considered negligible. The main pathway would be the introduction of immature life stages of *R. pomonella* through infested fruit, but this would not result in outbreaks in the majority of cases. This is because, even if eggs or larvae survive the transport, larvae still need to pupate and survive the long dormancy period before reaching the adult stage. Adult females subsequently need to locate both a suitable mate and a suitable host plant with fresh fruit in order to lay eggs for the next generation of flies. Note that in general, any findings of adults or larvae in locally grown fruit should thus be related to an introduction in the previous year.

Pupae may also be transported in the soil or other growing medium with the host plants. However, the import of plants for planting of the main host genera (*Malus* and *Crataegus*) is currently prohibited as these genera are included in the list of high-risk plants under Commission Implementing Regulation (EU) 2018/2019. The risk for entry of pupae of *R. pomonella* in growing medium (i.e. other than soil, consisting in whole or in part of solid organic substances) is also mitigated by the general import requirements for growing medium under Commission Implementing Regulation (EU) 2019/2072.

### Conclusion on spread capacity

The maximum distance expected to be covered by natural spread in one year by *Rhagoletis pomonella* is approximately 230 m (with a 95% uncertainty range of 24 m to 2.3 km). Regarding human-assisted spread, the pest could be introduced into new areas, most likely via transport of infested fruit.

### 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 in each Member State. This section presents examples of risk factors for *R. pomonella* and is not necessarily exhaustive (Table 1).

To identify risk areas, it is first necessary to identify the activities that could contribute to the introduction or spread of *R. pomonella*. These activities should then be connected to specific locations. Risk areas can be defined around these locations, bearing in mind that their size depends on the spread capacity of the target pest and the availability of host plants around these locations.

### Example 1: Entry points, packing and sorting stations for apples imported from regions where the pest is present

Despite the fact that the import of apples is subject to special import requirements, the risk of introduction of *R. pomonella* via the import of infested fruit cannot be excluded. Entry points (e.g. seaports, airports) of apple commodities, the packing and sorting stations, and processing industries that handle apples originating from areas where *R. pomonella* is present would be locations with a higher probability of finding the pest. Risk areas are then the areas in the vicinity of those risk locations where *Malus* and *Crataegus* host plants are present.

The actual risk of a location depends on the storage facilities and the waste disposal procedures. If fruit is stored and handled on site while being cooled, and waste is disposed of in closed containers, the risk of introduction at that site will not be high. Because *R. pomonella* is not considered to be a long-distance flier (i.e. the maximum distance expected to be covered in one year is estimated to be
approximately 230 m; see Section 1.7), the risk area will be of relatively limited size. It should, however, be noted that in the local absence of suitable hosts, the flight distance may become longer. The relative risk of the specific area will depend on the nature of the activities that are taking place at the risk location, e.g. the timing and specific origin of the import, and the storage conditions.

**Example 2: Urban areas**

Given that import of infested fruit constitutes the most likely pathway for introduction, the households that buy apples originating from areas where *R. pomonella* is present and fresh markets where such fruit is sold would be locations with a higher probability of finding the pest. When apples are damaged by *R. pomonella* larvae they are likely to be disposed of in containers or compost heaps. Waste collection centres should also be considered as risk locations. The 'density' of apple consumption (and thus disposal) would be higher in urban areas than in less densely populated areas and the preferred survey sites would then be locations with cultivated apple trees in or near to urban areas. Surveillance may include *Crataegus* spp. or crab apples that are grown as ornamental plants.

| Table 1: Examples of a risk activity and corresponding risk locations relevant for the surveillance of *Rhagoletis pomonella* |
|---|---|---|
| **Risk activity** | **Risk locations** | **Risk areas** |
| Imports of apples (and subsequent disposal of damaged fruit) from countries where the pest occurs | Entry points, packing and sorting stations, and processing industries where such fruit is handled | Areas surrounding the risk locations where *Malus* and *Crataegus* trees are present |
| | Households, fresh markets and waste collection centres where apples are being consumed, sold and disposed of | Residential areas with host plants receiving homemade compost |

### 2. Detection, sampling and identification

#### 2.1. Detection

Detection of *Rhagoletis pomonella* can be performed either by trapping adults or by examining fruit in order to detect oviposition stings and/or immature stages (mainly larvae). Trapping would be the recommended method for surveillance and detection, but when examinations of fruit are already performed to detect other pests, it might be worthwhile to include *R. pomonella* in that respective surveillance protocol.

#### 2.1.1. Visual examination

Visual examination should focus on the detection of symptoms cause by *R pomonella*. For completeness, the simplified description of the pest in the CABI datasheet on *R. pomonella* (CABI, 2019) is given below.

**Pest**

According to CABI (2019): 'The eggs are elliptical, semi-opaque and creamy white, with both ends slightly yellow and more opaque, about 0.9 mm long and 0.23 mm wide. The legless larva when fully grown are usually 6.5–8 mm long and 1.5–2 mm wide at the widest point. The cream coloured body consists of 11 apparent segments. The oval, yellow-brown pupae are approximately 5 mm long and 2.3 mm wide.' A detailed description of the eggs, larvae and pupae can be found in Bush (1966).

*Rhagoletis pomonella* can be recognised by four irregular or zig-zag black bands on the wings with the three distal bands forming an F-shape. The body generally has a black colour, while the head and legs are yellowish-brown. The eyes are greenish. The last segment of thorax 'scutellum' is white, in contrast to the European cherry fruit fly, *R. cerasi*. Males and females have three and four white
bands on their abdomen, respectively (Figure 4). Adult flies (also of other closely related *Rhagoletis* species) are about 2 to 4 mm in size and the females are larger than the males (CABI, 2019).

![Adult specimen of Rhagoletis pomonella (Source: Tom Murray)](image)

**Figure 4:** Adult specimen of *Rhagoletis pomonella* (Source: Tom Murray)

**Symptoms**

Fruit can be examined to detect the signs that indicate the presence of *R. pomonella*. When the female flies lay eggs, this leaves a puncture mark (i.e. oviposition sting) on the skin of the apple. Puncture marks can be recognised by a sunken spot on the surface of the fruit. Fruit may become irregular in appearance due to this sunken spot or as a consequence of the feeding of the larvae (Figure 5). Larvae can be detected when opening the fruit as they leave a brown trail while moving through the flesh of the fruit during feeding (Figures 6 and 7). Premature abscission of the fruit can be a clear sign of the presence of *R. pomonella*, causing the fruit to rot on the ground.
Figure 5: Damage by *Rhagoletis pomonella* on an apple (Source: Whitney Cranshaw, Colorado State University, Bugwood.org)

Figure 6: Larval tunnelling on apples (source: Whitney Cranshaw, Colorado State University, Bugwood.org)
2.1.2. Trapping

In general, a wide variety of systems is available to trap *R. pomonella*, similar to other fruit fly (Tephritidae) species. Most trapping systems are used in combination with a lure (FAO, 2018b). For *R. pomonella*, a common adult trapping system consists of a yellow sticky trap (Figure 8) combined with carbonate lures (WSDA et al., 2016). Alternatives include a red sticky sphere with a combination of lures based on fruit volatiles (Reissig et al., 1982; Zhang et al., 1999).

In the western USA, traps baited with ammonium carbonate were more attractive than the fruit volatile blends that were developed specifically for apple and various hawthorn species (Yee et al. 2005, 2014b). Note that in its native range in the eastern USA, there was no added benefit to using ammonium carbonate when traps were baited with the synthetic host fruit odour butyl hexanoate (Rull and Prokopy, 2000). Ammonium carbonate can be applied in capped vials that subsequently release 5–7 mg of ammonia per hour through holes in the cap for a period of about a month (Yee et al. 2005).

Traps should be placed in fruiting host plants and have a limited range of attraction, since *R. pomonella* is not attracted to traps further away than circa 2 m (WSDA et al., 2016). In practice, this means that flies are attracted from the tree in which the trap is placed. The trapping density for *R. pomonella* would need to be very high in order to obtain a reasonable reliability on the absence of the fly in an orchard that is being targeted by the survey.

All traps should be inspected at regular intervals (i.e. weekly) and taken to the laboratory for further confirmation, and lures should be replaced at regular intervals.

Figure 7: Damage by *Rhagoletis pomonella* can be clearly observed inside the apple (Source: H.J. Larsen, Bugwood.org)
2.2. Sampling

Living larvae should be collected from infested fruit and then reared to adulthood for confirmation based on adult morphology. Infested fruit should be taken to the laboratory and kept in controlled conditions until the larvae emerge from the fruit to pupate. It should be noted that to obtain adults from larvae that were retrieved from infested fruit, a period of several months might be required. This is because of the obligate diapause that pupae undergo and needs to be terminated following standard protocols. Living larvae can be dissected from infested fruit but only third instar larvae can be easily located. The collection of living larvae is particularly important for an initial finding but would not be needed when dealing with known outbreaks.

Dead larvae from potentially infested fruit should be transferred to 70% ethanol (for morphological identification at the genus level) or to 95% ethanol (for molecular tests to support identification at the genus level).

Apple samples, living larvae, samples stored on ethanol and trapped insects should be taken to the lab for confirmation.

Conclusions for sampling

Damaged apples and sampled young instars and adult insects should be taken to the lab for confirmation.
2.3. Identification

Identification of *Rhagoletis pomonella* at the species level requires morphological examination of adult flies, as is generally the case for Tephritidae. This implies that larvae (Figure 9) should be reared to the adult stage to enable confirmation of their identity. Because of their host fidelity, a finding of *Rhagoletis* larvae in apples would be a very strong indication of the presence of *R. pomonella*. Identification should be performed by a taxonomic specialist and, particularly in the case of a first finding, the identity should preferably be confirmed by a specialist from the current distribution area of the species.

Once the collected adult *Rhagoletis* flies have been taken to the laboratory or have been reared in the laboratory, keys are available for morphological identification to the genus level (e.g. in White and Elson-Harris, 1992; Foote et al., 1993; Bush, 1966). The information in Bush (1966) can be used for identification within the genus *Rhagoletis*.

*Rhagoletis pomonella* is part of the *pomonella* species group or complex. Its members have a very similar morphology but have distinct host plant affiliations. As a consequence, *R. pomonella* cannot easily be distinguished from the other species in the complex based on morphology alone (Berlocher, 2000; Bi et al. 2007). Particularly, the separation between *R. pomonella* and *R. mendax* (Figure 10) is considered very difficult based on morphology but becomes quite straightforward when information on the host plant is included. When adult specimens have been collected from a sticky trap, the tree species in which the trap was placed can be considered as a proxy for the host plant given the limited range of attraction. However, if it is a first finding it is recommended that living larvae are sought and collected from apples and reared to the adult stage to have the most accurate identification.

Since all non-European Tephritidae are regulated as Union quarantine pests and all sibling species in the *pomonella* complex are non-European, the finding of a member of the complex would always warrant phytosanitary action, even if there is uncertainty about the identity. Nevertheless, accurate identification is desirable for a quarantine pest.

A protocol for DNA barcoding based on the cytochrome oxidase I (COI) gene is described in EPPO Standard PM 7/129 on DNA barcoding as an identification tool for a number of regulated pests (EPPO, 2016) and can be used for all life stages. This protocol can provide additional information on a specimen, but molecular identification by DNA sequencing of a mitochondrial locus and several nuclear loci did not provide sufficient resolution to unequivocally resolve the identity of *R. pomonella*, *R. mendax*, *R. zephyria* and the undescribed flowering dogwood fly (Xie et al., 2008).

Figure 9: Larvae of *Rhagoletis pomonella* in a plum (Source: Whitney Cranshaw, Colorado State University, Bugwood.org)
3. Key elements for survey design

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

When several pests have to be surveyed in the same crop, it is recommended that the same epidemiological and inspection units are used for each pest in order to optimise the survey programme as much as possible. This would optimise field inspections since they are organised per crop visit and not by pest. Table 2 shows an example of these definitions.

Table 2: Example of definitions of the target population, epidemiological unit and inspection unit for *Rhagoletis pomonella*

| Definition                      | Target population                                      | Epidemiological unit                                                                 | Inspection unit                      |
|---------------------------------|--------------------------------------------------------|---------------------------------------------------------------------------------------|--------------------------------------|
| Target population               | All apple trees and hawthorn shrubs/trees in a Member State | A single homogeneous area with apple trees and/or hawthorn shrubs (e.g. an orchard, hectare, NUTS area) | A single apple tree or hawthorn shrub or trap |
| Epidemiological unit            |                                                        |                                                                                      |                                      |
| Inspection unit                 |                                                        |                                                                                      |                                      |
Rhagoletis pomonella survey card

To design a plant pest survey on *Rhagoletis pomonella* 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 *R. pomonella*, 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 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 *R. pomonella*. 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 *R. pomonella*, 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 each contain at least one individual host plant (e.g. an orchard, hectare, NUTS area).

4/ Determine the inspection unit. For an apple orchard, for example, the inspection unit is either an individual apple tree or hawthorn shrub/tree or a trap.

5/ Determine the number of inspection units per epidemiological unit. In the case of an apple orchard, this is the number of host plants or traps 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 *R. pomonella* 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, 2019). |
| **Component**                     | A component is a survey entity which can be distinguished based on its target population, the detection method (e.g. visual examination, laboratory testing, trapping) and the inspection unit (e.g. vectors, branches, twigs, leaves, fruits). A pest survey comprises various components. The overall confidence of the survey will result from the combination of the different components. |
| **Confidence**                    | 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**             | 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**          | 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). |
| **Expected prevalence**           | In prevalence estimation approaches, it is the proportion of |
| **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 array of host plants 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, 2019). |
| **Inspection unit**           | The inspection units are the plants, plant parts, commodities or pest vectors that will be scrutinised to identify and detect the pests. They are the units within the epidemiological units that could potentially host the pests and on which the pest diagnosis takes place (EFSA, 2018). |
| **Inspector**                 | Person authorised by a national plant protection organisation to discharge its functions (ISPM 5: FAO, 2019). |
| **Method sensitivity**        | The conditional probability of testing positive given that the individual is diseased (Dohoo et al., 2010). The method 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. |

**Notes:**
- Rhagoletis pomonella survey card
- Epidemiological units expected to be infected or infested.
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, 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 | 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 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/ |
| 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/](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. In the case of 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, 2019). |
| **Target population** | The set of individual plants or commodities or vectors in which the pest under scrutiny can be detected directly (e.g. looking for the pest) or indirectly (e.g. looking for symptoms suggesting the presence of the pest) in a given habitat or area of interest. The different components pertaining to the target population that need to be specified are:  
  - definition of the target population: the target population has to be clearly identified;  
  - target population size and geographic boundary. (EFSA, 2018)  
  **analogous to consignment used in ‘Methodologies for sampling of consignments’ (ISPM 31: FAO 2016b)** |
### 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 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, 2019).

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