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PM 3/92 (1) Consignment inspection of fresh fruit and vegetables for fruit flies

Specific scope: This Standard describes the procedures for inspection of consignments of susceptible fresh fruit and vegetables at import for infestation of fruit flies with a focus on the family Tephritidae (Diptera). The Standard also provides guidance that may be relevant to exports.

Specific approval and amendment: Approved as an EPPO Standard in 2021–09.

Authors and contributors are given in the Acknowledgements section.

1 | INTRODUCTION

Fruit flies cause damage in many soft-skinned and some harder-skinned fruits and vegetables. For example, consignments of fruits of *Annona* spp., *Citrus* spp., *Mangifera indica*, *Momordica* spp., *Psidium* spp., *Prunus* spp., *Pyrus* spp., *Syzygium* spp. and *Vitis* spp. can be infested. Additionally, some vegetable commodities, for example *Capsicum* spp., *Cucurbita pepo*, *Cucumis sativus*, *Luffa acutangular*, *Solanum lycopersicum*, *S. melongena* and *Trichosanthes cucumerina*, can be infested. These fruits and vegetables (hereafter referred to as 'fruit', as in the biological sense) are a risk for the introduction of fruit flies.

Fruit flies have a global distribution. Many fruit flies of economic concern are native to the Americas, Asia and Pacific areas, and many do not occur in the EPPO region. Importation of fruit is the pathway of most phytosanitary concern. Huge quantities of fruits are traded yearly worldwide, and this poses a high phytosanitary risk of fruit flies spreading to new areas. In addition, the risk can increase with the effect of global climate change.

In the EPPO region, the European Union (EU) is a net importer of fresh fruit. In 2017, the EU imported fruit to the value of 20.1 billion EUR, with imports of fruit accounting for 84.7% of the total value of the fresh fruit market (Eurostat, 2019). The highest import values corresponded to fresh and dried nuts (20.0% of the total value of fresh fruit EU imports), bananas (19.5%), the grouping of dates, figs, pineapples and avocados (14.2%), citrus fruit (9.9%) and grapes (9.1%) (Eurostat, 2019).

1.1 | Tephritidae

Worldwide there are over 4000 species of fruit flies in the family Tephritidae, of which around 350 species are of economic importance (Plant Health Australia, 2018). Most fruit fly species of economic concern belong to the genera *Bactrocera*, *Zeugodacus*, *Anastrepha*, *Ceratitis* and *Rhagoletis*, while some other economically relevant species belong to the genera *Dacus*, *Euphranta*, and others.

Within the major tephritid species, there are populations that are morphologically indistinguishable but are biologically distinct, expressing different life history (e.g. life span, reproduction patterns), behavioural (e.g. mating behaviour; host preference) and genetic traits (EFSA, 2020). Such species complexes can contribute to taxonomic uncertainty concerning the family.

1.2 | Life cycle of fruit flies

It is difficult to generalize the life cycle of species of the large Tephritidae family, but the species of economic and agricultural concern share some important biological attributes.

Adult fruit flies lay eggs under the skin of ripe or ripening fruits. Adult fruit flies can also lay eggs in unripe fruit, causing an early ripening which is often followed by fruit drop. The hatching larva, which are typical Diptera maggots, feed within the fruit during the development of the three larval instar stages and cause brown, rotten marks on the fruit. Each fruit can contain numerous larvae and large fruit can support more than 100 maggots. For most species, at the end of the third-instar stage the maggot exits the fruit and enters the soil to form the puparium. Some fruit fly species, for example *Bactrocera oleae*, can pupate inside the fruit. In infested fruit consignments, the pupae can develop in cracks or other hollow spaces in boxes (e.g. corrugated cardboard). This fact should be taken into account during the inspection of a consignment. The pupal stage usually takes only a few days before the adult emerges.

The larvae of most species are highly polyphagous and pass through the larval stages very quickly. This can cause a very high reproduction rate under ideal conditions.

Appendix 1 provides selected images of life stages of fruit flies. For further images see the EPPO Global Database.

2 | PHYTOSANITARY INSPECTIONS

ISPM 5 *Glossary of phytosanitary terms* (IPPC, 2019) defines inspection as 'Official visual examination of plants, plant products or other regulated articles to determine if pests are present or to determine compliance with phytosanitary regulations'.

General information for carrying out import inspections is included in ISPM 20 *Guidelines for a phytosanitary import regulatory system* (IPPC, 2017a) and ISPM 23 *Guidelines for inspection* (IPPC, 2016). Further information on phytosanitary inspection of consignments is given in the EPPO Standard PM 3/72 *Elements common to inspection of places of production*, *area-wide surveillance*, *inspection of consignments and lot identification* (EPPO, 2009).

Inspection should take place at the point of entry into the EPPO region to reduce the risk of introducing pests. However, if that is not possible, inspection should take place at an approved place of inspection when the consignment is unloaded for the first time.

Inspection will consist of visual examination for all stages of fruit flies or signs of insect activity. However, not all infestations will be visible during inspection of the outside of the fruit, therefore destructive sampling may be needed. When life stages of fruit flies are found, identification of the species during inspection is not possible, therefore sampling for laboratory identification is required.

Risk-based inspections should take into account the following factors:

- origin (pest outbreak areas and other areas with similar climate present the highest risk)
- host commodity [e.g. high risk hosts (see Section 2.2)]
- time of year (e.g. risk of dispersal is lower in the winter)
- destination (transport, storage and intended use)
- compliance record (of the exporting country, exporter, importer and handling facility)
- type and method of phytosanitary treatment carried out on the shipment (e.g. cold treatment, irradiation, fumigation).

Many EPPO countries have established phytosanitary import requirements for fruit flies, including treatments either prior to export or during transport. Knowledge of what treatments have been applied to the consignment should be taken into account during risk-based sampling to determine the intensity of the inspection. Appendix 2 provides further details on types of treatments for fruit flies.

2.1 | Fruit flies of concern for the EPPO region

This Standard relates to fruit flies from the family Tephritidae (Diptera) which are of economic importance to fresh fruit, and which are listed in the EPPO A1 and A2 Lists of pests recommended for regulation as quarantine pests (Table 1). It also considers those fruit flies which are regulated by specific EPPO countries, in particular countries in the south of the EPPO region (Table 2). The phytosanitary procedures described in the Standard are primarily aimed at preventing the introduction of these pests into the EPPO region via imported consignments. Details of all these pests can be found in species-specific EPPO datasheets available via Global Database (EPPO, 2021a) and EPPO/CABI (1997). For additional up-to-date information, the respective scientific literature should be consulted [e.g. Australian Scientific Advisory Services/FAO/IAEA (2019)].

EPPO A1 and A2 lists of pests recommended for regulation as quarantine pests are subject to annual additions and deletions. The species listed in Tables 1 and 2 should therefore be revised whenever new pests are identified or species become newly regulated.

2.2 | Symptom description

Fruit flies cause rots and discolouration on infested commodities. Commodities with higher phytosanitary risk include several fruits, such as berries, *Citrus* spp., *Persea americana*, *Musa* spp. (ripened), *Mangifera indica*, *Actinidia* spp., *Carica papaya*, *Passiflora edulis*, *Cucumis* spp., olive, *Litchi chinensis*, *Averrhoa carambola*, *Prunus* spp. (cherries), *Malus* spp., Pyrus spp. (pears), *Vitis* spp., *Solanum lycopersicum*, *Cucumis sativus* and *Capsicum* spp.

Signs of infestation are small spots on the surface of the fruit where the female pierces the skin with the ovipositor and lay eggs. Oviposition punctures are difficult to detect visually, especially in the early stages of infestation, and can depend on the type of fruit (soft-skinned commodities can show more damage than hard-skinned). Sometimes oviposition puncture holes can exude fruit juice and at a more advanced stage of infection the area around the puncture holes becomes soft (larval feeding causes the fruit structure to disintegrate).

In some cases, wounds on fruit can favour the entry of pathogenic fungi, causing rot, with or without the release of juice and exudate. The larvae damage the pulp of the fruits. Considerable damage may occur inside the fruit before symptoms are visible externally, often as networks of tunnels accompanied by rotting. More advanced signs of infestation can be seen on maturing fruit, including

TABLE 1 Fruit flies of quarantine concern from the EPPO A1 and A2 lists

Scientific name	Major hosts	EPPO list
Anastrepha fraterculus	*Mangifera indica*, *Psidium guajava*	EPPO A1 List
Anastrepha ludens	*Citrus* spp., *Mangifera indica*	EPPO A1 List
Anastrepha obliqua	*Mangifera indica*	EPPO A1 List
Anastrepha suspensa	*Eriobotrya japonica*, *Eugenia uniflora*, *Psidium cattleyanum*, *P. guajava*, *Syzygium jambos*, *S. malaccense*, *S. samarangense*	EPPO A1 List
Bactrocera carambolae	*Averrhoa carambola*, *Citrus aurantium*, *Psidium guajava*, *P. guineense*, *Syzygium samarangense*	EPPO A1 List
Bactrocera caryeae	*Mangifera indica*, *Psidium guajava*	EPPO A1 List
Bactrocera dorsalis	Highly polyphagous species[a]	EPPO A1 List
Bactrocera kandiensis	*Mangifera indica*	EPPO A1 List
Bactrocera latifrons	*Capsicum annuum*, *C. chinense*, *C. frutescens*, *Citrullus lanatus*, *Citrus aurantiifolia*, *Cucumis melo*, *C. sativus*, *Punica granatum*, *Solanum lycopersicum*, *S. melongena*	EPPO A1 List
Bactrocera minax	*Citrus maxima*, *C. reticulata*, *C. sinensis*	EPPO A1 List
Bactrocera occipitalis	*Mangifera indica*, *Psidium guajava*	EPPO A1 List
Bactrocera pyrifoliae	*Prunus persica*	EPPO A1 List
Bactrocera tryoni	*Anacardium occidentale*, *Annona glabra*, *A. squamosa*, *A.* × *atemoya*, *Averrhoa carambola*, *Capsicum annuum*, *Carica papaya*, *Casimiroa edulis*, *Chrysophyllum cainito*, *Citrus paradisi*, *C. reticulata*, *C. sinensis*, *Coffea arabica*, *Eriobotrya japonica*, *Eugenia uniflora*, *Fortunella japonica*, *Malus sylvestris*, *Mangifera indica*, *Manilkara zapota*, *Morus nigra*, *Nauclea orientalis*, *Passiflora edulis*, *P. suberosa*, *Prunus persica*, *P. persica* var. *nucipersica*, *Psidium cattleyanum*, *P. guajava*, *Solanum lycopersicum*, *Syzygium aqueum*, *S. forte*, *S. jambos*, *S. malaccense*, *S. suborbiculare*, *S. tierneyanum*, *Terminalia arenicola*, *T. aridicola*, *T. catappa*, *T. muelleri*, *T. platyphylla*, *T. sericocarpa*, *T. subacroptera*	EPPO A1 List
Bactrocera tsuneonis	*Citrus reticulata*	EPPO A1 List
Bactrocera zonata	*Mangifera indica*, *Prunus persica*, *Psidium guajava*	EPPO A2 List
Ceratitis capitata	Highly polyphagous species[a]	EPPO A2 List
Ceratitis rosa	*Citrus reticulata*, *C. sinensis*	EPPO A1 List
Dacus ciliatus	*Cucumis melo*, *C. sativus*, *Cucurbita pepo*	EPPO A2 List
Euphranta canadensis	*Ribes nigrum*, *R. rubrum*	EPPO A1 List
Euphranta japonica	*Prunus cerasifera*	EPPO A1 List
Rhagoletis cingulata	*Prunus avium*, *Prunus salicina*	EPPO A2 List
Rhagoletis fausta	*Prunus avium*	EPPO A1 List
Rhagoletis indifferens	*Prunus avium*	EPPO A1 List
Rhagoletis mendax	*Vaccinium angustifolium*, *V. corymbosum*	EPPO A1 List
Rhagoletis pomonella	*Malus domestica*	EPPO A1 List
Zeugodacus cucumis	*Cucumis melo*, *C. sativus*, *Cucurbita pepo*	EPPO A1 List
Zeugodacus cucurbitae	*Citrullus lanatus*, *Cucumis melo*, *C. sativus*, *Cucurbita pepo*, *Luffa acutangula*, *L. aegyptiaca*, *Momordica charantia*, *Trichosanthes cucumerina*	EPPO A1 List

[a]For hosts refer to the EPPO Global Database (EPPO, 2021a).

the presence of maggots in the pulp, rotting of attacked parts and scars on surface of the fruit. Attacked fruits are unmarketable.

Appendices 3 and 4 provide images of external and internal symptoms. For further images see the EPPO Global Database.

2.3 | Lot identification

General background information on lot identification is given in EPPO (2009) PM 3/72 *Elements common to inspection of places of production*, *area-wide surveillance*, *inspection of consignments and lot identification*.

According to ISPM 5, a lot is 'a number of units of a single commodity, identifiable by its homogeneity of composition, origin etc., forming part of a consignment' (IPPC, 2019). Criteria for lot identification for consignments of fresh fruit should at least include the species and the country of origin. Variety, area of production, grower, packaging, distinguishing marks (e.g. commercial brand or lot number) and exporter may also be considered. Lots identified on the phytosanitary certificate and declared separately to customs should be the starting

TABLE 2 Other fruit flies regulated by specific EPPO member countries[a]

Scientific name	Examples of hosts [hosts in bold are major hosts as detailed in the EPPO Global Database (EPPO, 2021a)]	Regulated by specific EPPO member country
Anastrepha bistrigata	*Psidium guajava*	Morocco (QP 2018)
Anastrepha distincta	*Inga edulis*	Morocco (QP 2018), Tunisia (QP 2012)
Anastrepha grandis	***Cucumis melo*, *Cucurbita pepo***	Jordan (A1 List 2013)
Anastrepha pseudoparallela	*Passiflora ambigua*, *Passiflora quadrangularis*	Morocco (QP 2018)
Anastrepha serpentina	*Eugenia myrcianthes*, *Malus domestica*, *Mangifera indica*, *Manilkara zapota*, *Persea americana*, *Pouteria caimito*	Jordan (A1 List 2013), Morocco (QP 2018)
Anastrepha sororcula	***Psidium guajava***	Morocco (QP 2018)
Anastrepha striata	*Mangifera indica*, *Psidium acutangulum*, *Psidium guajava*, *Psidium guineense*, *Spondias mombin*	Morocco (QP 2018)
Anastrepha turpiniae	***Psidium guajava***	Morocco (QP 2018)
Bactrocera aquilonis	*Momordica charantia*, *Psidium guajava*	Jordan (A1 List 2013), Morocco (QP 2018)
Bactrocera correcta	***Mangifera indica*, *Manilkara zapota*, *Prunus persica*, *Psidium guajava*, *Syzygium jambos*, *Terminalia catappa*, *Ziziphus jujuba***	Jordan (A1 List 2013), Morocco (QP 2018)
Bactrocera curvipennis	*Annona reticulata*, *Annona squamosa*, *Averrhoa carambola*, *Capsicum annuum*, *Carica papaya*, *Casimira edulis*, *Citrus* spp., *Solanum lycopersicum*, *Mangifera indica*, *Prunus persica*, *Psidium guajava*, *Syzygium* spp.	Morocco (QP 2018)
Bactrocera facialis	*Capsicum annuum*, *Carica papaya*, *Citrus* spp., *Mangifera indica*, *Persea americana*, *Psidium guajava*, *Syzygium* spp.	Morocco (QP 2018)
Bactrocera frauenfeldi	*Mangifera indica*, *Psidium guajava*, *Citrus* spp., *Syzygium malaccense*, *Averrhoa carambola*, *Carica papaya*	Morocco (QP 2018)
Bactrocera jarvisi	*Carica papaya*, *Mangifera indica*, *Musa* spp., *Prunus persica*, *Psidium guajava*	Jordan (A1 List 2013), Morocco (QP 2018)
Bactrocera kirki	*Mangifera indica*, *Psidium guajava*, *Citrus* spp., *Persea americana*, *Syzygium* spp., *Averrhoa carambola*, *Carica papaya*, *Annona reticulata*, *Passiflora edulis*, *Morinda citrifolia*, *Pouteria caimito*, *Capsicum annuum*, *Solanum lycopersicum*, *Solanum melongena*	Morocco (QP 2018)
Bactrocera melanota	*Carica papaya*, *Citrus* spp., *Psidium guajava*, *Mangifera indica*, *Syzygium* spp.	Morocco (QP 2018)
Bactrocera musae	***Musa* x *paradisiaca***	Jordan (A1 List 2013)
Bactrocera neohumeralis	*Psidium guajava*	Jordan (A1 List 2013), Morocco (QP 2018)
Bactrocera passiflorae	*Citrus* spp.	Morocco (QP 2018)
Bactrocera pedestris	*Citrus* spp.	Morocco (QP 2018)
Bactrocera psidii	*Citrus* spp., *Mangifera indica*, *Psidium guajava*	Morocco (QP 2018)
Bactrocera trivialis	*Capsicum frutescens*, *Citrus* × *paradisi*, *Mangifera indica*, *Prunus persica*, *Psidium guajava*	Morocco (QP 2018)
Bactrocera xanthodes	*Artocarpus altilis*, *Carica papaya*	Morocco (QP 2018)
Ceratitis cosyra	***Mangifera indica***	Jordan (A1 List 2013), Morocco (QP 2018), Tunisia (QP 2012), Ukraine (A1 List 2019)
Ceratitis malgassa	*Citrus* spp., *Prunus* spp., *Pyrus* spp.	Morocco (QP 2018)
Ceratitis quinaria	***Prunus armeniaca*, *Prunus persica***	Israel (QP 2009), Jordan (A1 List 2013), Tunisia (QP 2012), Turkey (A1 List 2016), EU (A1 QP (Annex II A 2019))
Dirioxa pornia	Ripe, damaged and fallen fruit from numerous species	Morocco (QP 2018)
Monacrostichus citricola	*Citrus aurantifolia*, *Citrus reticulata*, *Citrus limon*, *Citrus limetta*, *Citrus maxima*	Morocco (QP 2018)

(Continues)

TABLE 2 (Continued)

Scientific name	Examples of hosts [hosts in bold are major hosts as detailed in the EPPO Global Database (EPPO, 2021a)]	Regulated by specific EPPO member country
Monacrostichus malaysiae	*Citrus halimii*	Morocco (QP 2018)
Myiopardalis pardalina	***Cucumis melo***	Kazakhstan (A2 List 2017)
Neoceratitis cyanescens	***Solanum lycopersicum***	Tunisia (QP 2012), Turkey (A1 List 2016), EU (A1 QP (Annex II A 2019))
Rhagoletis cerasi	***Prunus avium***	Jordan (A1 List 2013), Morocco (QP 2018), Tunisia (QP 2012)
Rhagoletis completa	*Prunus persica*	Jordan (A1 List 2013), Morocco (QP 2018), Tunisia (QP 2012), Turkey (A1 List 2016)
Rhagoletis ribicola	***Ribes rubrum*, *Ribes uva-crispa***	Tunisia (QP 2012), Turkey (A1 List 2016), EU (A1 QP (Annex II A 2019))
Rhagoletis suavis	*Prunus persica*	Jordan (A1 List 2013), Tunisia (QP12), Turkey (A1 List 2016), EU (A1 QP (Annex II A 2019))
Zeugodacus tau	*Solanum lycopersicum*	Jordan (A1 List 2013), Morocco (QP 2018)

Abbreviation: QP, quarantine pest.

[a]The European Union regulates all non-European Tephritidae, although this is under revision.

point for planning the inspection. When a consignment comprises more than one lot, the inspection to determine compliance should consist of multiple separate visual examinations of each lot, and each lot should be sampled separately. Packaging normally contains an indication of the country of origin and additional information that may be used to identify individual lots.

2.4 | Sampling and inspection procedures

2.4.1 | Purpose of inspection and sampling

Generally, the ability to detect low levels of infestation with a high degree of confidence level is desirable for phytosanitary inspections.

To support results/outcomes of sampling methods and capacity it is not adequate to determine that a consignment is pest free; monitoring of the pathway and searching for a selected pest along the pathway may still be valuable to detect pests to gain more information on the risks of the pathway through the traceability chain.

Phytosanitary inspections are also an opportunity to check whether the consignment complies with other requirements, for example whether there is evidence to demonstrate that it originates from a pest-free area for a quarantine organism of concern (this might be carried out by checking the phytosanitary certificate and any movement documents for the phytosanitary information).

2.4.2 | Sampling for visual examination (general aspects)

Visual examination of imported consignments of fruit may be combined with destructive sampling to detect the presence of fruit flies or their symptoms. A magnifying lens (at least 10×) or binocular microscope (35×) can be useful to visually detect life stages of fruit flies.

An adequate sample size (see below) of fruit from each lot should be subjected to a systematic examination to detect the presence or signs of fruit flies. In addition, an appropriate number of asymptomatic fruits may be destructively sampled (see Section 2.4.4).

If sampling is undertaken to provide information about the general phytosanitary condition of a consignment, to detect pests or to verify compliance with phytosanitary import requirements, as in the case of inspection of fruit consignments, statistically based methods are appropriate. The sample (as the minimum number of individuals selected from the lot or consignment to be examined) should be determined based on lots, taking into account the statistical background provided in ISPM 31 *Methodologies for sampling of consignments* (IPPC, 2008).

The sampling unit commonly used for fruit is an individual item (e.g. for *Citrus*, mangos, apples etc.). For small items, the sampling unit can be the smallest unit defined as one piece [e.g. a bunch of grapes or a punnet (small box) of berries].

The necessary number of fruit should be selected from the whole lot/consignment and from different places and

depths within a box. If the sampling unit is a box (e.g. punnet) it should be emptied in such a way that all fruits in the box can be checked.

For fruit or vegetable consignments, which are usually large lots sufficiently mixed, and for which the sample size is less than 5% of the lot size, the sample size can be calculated using either the binomial or the Poisson distribution (IPPC, 2008). A confidence level of 95% should normally be used for fruit.

It is up to the NPPO to set the sample size. For example, from a consignment consisting of a large container of fruit (where an item is the sampling unit), 300 items should be inspected to provide a 95% confidence of detecting symptoms present in 1% of items, provided the symptoms are uniformly distributed and the fruits are randomly selected. To detect infestation in a consignment in which 5% of the fruits are displaying symptoms with 95% confidence, 60 items should be inspected provided the symptoms are uniformly distributed and the fruits are randomly selected. For other levels of confidences or other percentages of symptoms consult ISPM 31.

If the inspector suspects the presence of an infestation of fruit flies of quarantine status, the lot or consignment should be detained under official control.

2.4.3 | Sampling for visual examination (specific aspects)

For visual examination of fresh fruit consignments, plant health inspectors should be equipped with a torch, a knife and a magnifying lens (10×).

The place where the inspection is conducted should be well lit. The visual examination should begin with an overall examination of the consignment. Visual examination of the container, packaging and means of conveyance can provide indications of adverse conditions during transport (e.g. adverse temperatures or signs of damp or wetness) which may affect the physical condition of the fruit. An inspection table can be used to inspect fruit (Figure 1). Any emptied boxes should be inspected for signs of pests. Any wrapping on individual fruit should be removed.

Fruit flies are mostly detected as larvae in fruits. Holes are visible on the fruits. Eggs might be found inside the fruit at the point where oviposition puncture marks are visible on the surface. Larvae will leave the fruits to pupate, and so consequently pupae may also be detected in packaging.

Each item examined should be gently pressed to detect soft areas of the fruit indicative of Tephritidae larvae presence. Inspection of fresh fruit should look for soft areas, dark spots, rot, holes or lesions that may have originated from oviposition or larval activity. Some symptoms are characteristic of fruit fly infestation (see Appendix 3 for supporting images).

FIGURE 1 Example of a specific inspection table. (Courtesy: Vañó García.)

2.4.4 | Destructive sampling

Given that signs of infestation can be difficult to detect by inspection of the whole fruit, it may be needed to cut fruit to examine the inside. A risk-based approach may be adopted for destructive sampling where consideration is given to the factors detailed in Section 2 and the size of the lot. An appropriate number of asymptomatic fruit should be randomly selected and cut in half to look for larvae. Larger fruit can be peeled and cut into pieces. From the sample taken for inspection, random sampling of a minimum of 30 fruit for destructive examination gives a 95% confidence level of detecting the pest at a 10% infestation level. Sixty fruit for destructive examination need to be sampled to achieve this level of confidence at a 5% infestation level. For smaller items (e.g. a punnet), the same numbers apply to the sampling unit.

3 | SAMPLING FOR LABORATORY IDENTIFICATION

Visual examination does not allow identification to the species level and laboratory diagnostics is necessary. Morphological identification is the most widely used method for Tephritidae adults using morphological keys. Molecular techniques are also available.

If the fruit shows symptoms of fruit fly damage, the inspector should look for larvae or adults. In the case of detection of a life stage of a fruit fly, a sample should be taken and sent to the laboratory to confirm the identity of the pest. This is important because not all countries have regulated all Tephritidae species, but also because some species are already present in the EPPO region.

Larvae can be sent alive along with a piece of the fruit in an airtight, secure container. If collected larvae are to be preserved, they should be placed in boiling water for a few seconds (until they become immobile) and then transferred to 70% ethanol (if a molecular test is to be carried out subsequently, 95–100% ethanol is recommended).

Adults can be killed in 70% ethanol. Adults can be sent for identification in a hermetic tube or container in 70% ethanol (if a molecular test is to be carried out subsequently, 95–100% ethanol is recommended). Placing the adults live in a hermetic tube allows for the colour and pattern of the body and wings to develop, which can aid identification. It is recommended to send several adults for identification. When adult specimens are needed for identification, larvae can be reared to adult on infested fruit placed in a secure container with a pupation medium (e.g. damp vermiculite, sand or sawdust). Once the adults emerge, they must be kept alive for several days to ensure that the tegument and wings acquire the rigidity and characteristic coloration of the species.

Morphological identification should be performed by a taxonomic specialist. Morphological identification with a binocular microscope can be performed for some Tephritidae species according to White and Elson-Harris (1992) or ISPM 27 *Diagnostic protocols for regulated pests* (IPPC, 2006), DP 5: DP 09: Genus *Anastrepha* Schiner or available EPPO Standards: *Bactrocera latifrons* PM 7/142 (EPPO, 2020), *Bactrocera zonata* PM 7/114 (EPPO, 2013), *Ceratitis capitata* PM 7/104 (EPPO, 2011a), *Ceratitis cosyra* PM 7/105 (EPPO, 2011b), *Dacus ciliatus* PM 7/134 (EPPO, 2018a), *Rhagoletis completa* PM 7/107 (EPPO, 2018b) and *Zeugodacus cucurbitae* PM 7/135 (EPPO, 2016).

Molecular diagnostic techniques are available that may be used as complementary tools for supporting the morphological identification (see for example the Standards detailed above). They can be used to identify early larval stages (which are hard to identify reliably on morphological features) and eggs. They can also be used for incomplete adults that may be missing specific anatomical features required to use morphological keys, or specimens that have not fully developed their features (especially colour patterns) (from EFSA, 2020).

LAMP assays are available and can be used for the rapid detection of fruit fly species in the laboratory and field. LAMP assays have been developed for several species of fruit flies of economic concern, such as *Ceratitis capitata* and species of the genera *Bactrocera*, *Dacus* and *Zeugodacus* (Blacket et al., 2020).

4 | TRAPS

Pheromone/attractant traps can be an effective means of monitoring for the presence of fruit flies at the place of inspection. If a fruit fly is detected in a trap, although infested consignments have not been found during inspection, this can signal that inspections should be intensified.

Adult fruit flies are lured using food attractants, pheromones and parapheromones, and host odours, as well as visual stimuli. Sex pheromones and male lures have been explored as trap baits, in fruit fly detection efforts, for the major species of *Anastrepha*, *Bactrocera*, *Ceratitis*, *Dacus*, *Rhagoletis* and *Toxotrypana*.

Compared to food-based baits relatively little research has been conducted into developing pheromonal baits. This is due to inconsistency in the results of studies testing the effects of pheromone-based trapping (using live males or male pheromones), as well as to the chemical complexity of pheromones and the unknown levels of sexual communication. For several species, male lures are strong attractants, most of them having a simple chemical structure that allow a rather low-cost production (EFSA, 2020).

Traps in use for detection of fruit flies are detailed in FAO/IAEA (2013) and in the ISPM 26 (IPPC, 2015). See the same references on how to use the different types of traps.

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APPENDIX 1 SELECTED IMAGES OF FRUIT FLY LIFE STAGES

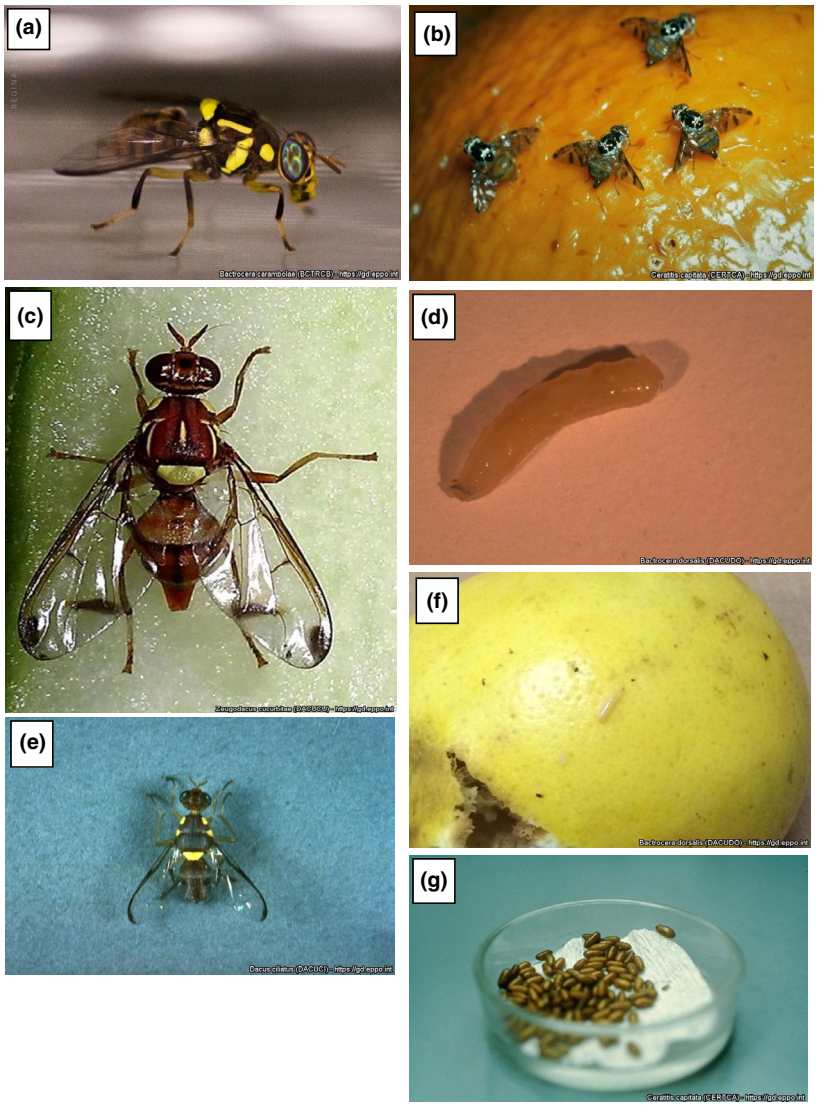

FIGURE A1 (a) Adult *Bactrocera carambolae*. Courtesy: Regina Sugayama. (b) Adults of *Ceratitis capitata* on an orange. Courtesy Agroscope FAW, Wädenswil (CH). (c) *Zeugodacus cucurbitae*. Courtesy Sajad Hussain Mir. (d) Larva of *Bactrocera dorsalis*. Courtesy Paride Missio, Swiss Federal Plant Protection Service. (e) *Dacus ciliates*. Courtesy Central Science Laboratory, York (GB) – British Crown. (f) Larva of *Bactrocera dorsalis* on grapefruit. Courtesy Paride Missio, Swiss Federal Plant Protection Service. (g) Pupae of *Ceratitis capitata*. Courtesy M. Muñiz, Centro de Ciencias Medioambientales (ES). All images from the EPPO Global Database

APPENDIX 2 TREATMENTS USED ON SUSCEPTIBLE FRESH FRUIT CONSIGNMENTS FOR FRUIT FLIES

Temperature treatments

Cold treatment pre-shipment or during transport is widely used and recommended to eliminate fruit flies in commodities originating from areas where fruit flies are present. Cold treatment is also possible at final destination when treatment is incorrectly applied during transport. Cold treatment can be considered as a risk reduction measure itself, as it will kill many eggs and larvae concealed in the infested products (Lin et al., 2020).

Cold treatments are often required by importing countries as a phytosanitary measure for fruits such as citrus, kiwi and grapes (e.g. United States, China, Japan), especially for treating fresh commodities from areas which are infested with *Ceratitis capitata*, the Mediterranean fruit fly. In such case, fruits are exposed to a combination of temperatures and exposure times, including from 10 days at 0°C, 11 days at 0.6°C, 12 days at 1.1°C, 14 days at 1.7°C or 16 days at 2.2°C (see Yahia, 2019). In fruit

quality assessment, fruits exposed to cold treatment still maintained their market value.

Certain types of fruits can be treated with hot water (e.g. for mango, 46°C for 65 to 110 min depending on fruit size) or with vapor heat (e.g. 43°C for 4–6 h) (EPPO, 2021b) or forced hot-air treatment (Mangan & Ingle, 1994).

Irradiation treatments

Irradiation is only applicable if the importing country accepts irradiated food products. Irradiation can be used for phytosanitary treatments, including treatments against fruit flies (IPPC, 2003; Hallman et al., 2016). Irradiation of Mexican guava is regularly used before export to the United States (Hallman & Blackburn, 2016). Irradiation at 70 Gy is also considered an effective treatment for immature stages of *Anastrepha ludens* (EPPO, 2021b).

Chemical treatments

The availability of chemical compounds to be applied as chemical fumigants is very restricted.

Ethylene dibromide was previously widely used as a fumigant but is now generally withdrawn because of its carcinogenicity (EPPO, 2021b).

Fumigation with methyl bromide is severely restricted under the Montreal Protocol because of its impact on the ozone layer (IPPC, 2017b). For this reason, it is not a recommended option and is approved on a very limited basis, for example one treatment schedule is currently approved by the USDA to treat *Citrus* from Mexico under pre-clearance (USDA, 2016).

APPENDIX 3 OVIPOSITION DAMAGE ON FRUIT

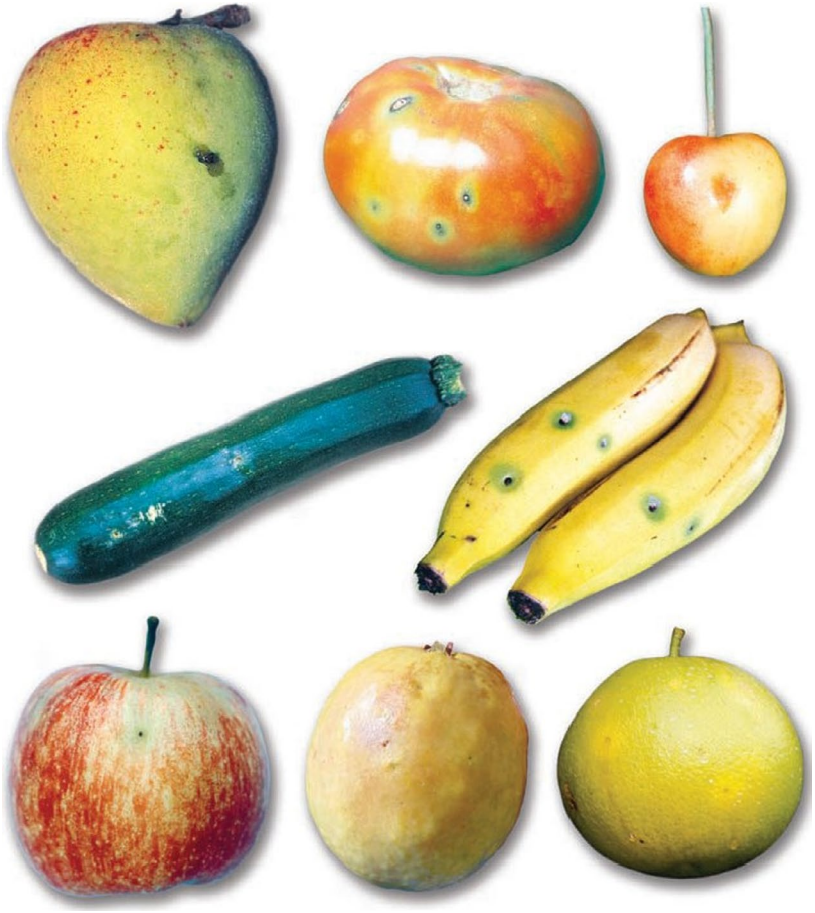

FIGURE A2 Oviposition damage on the surface of the fruit can lead to the entry of pathogenic fungi, causing rot. Fruits clockwise from top left: mango, tomato, cherry, banana, grapefruit, common guava, apple and courgette. Images courtesy of Richard Piper Australian Scientific Advisory Services/FAO/IAEA (2019)

APPENDIX 4 LARVAL DAMAGE

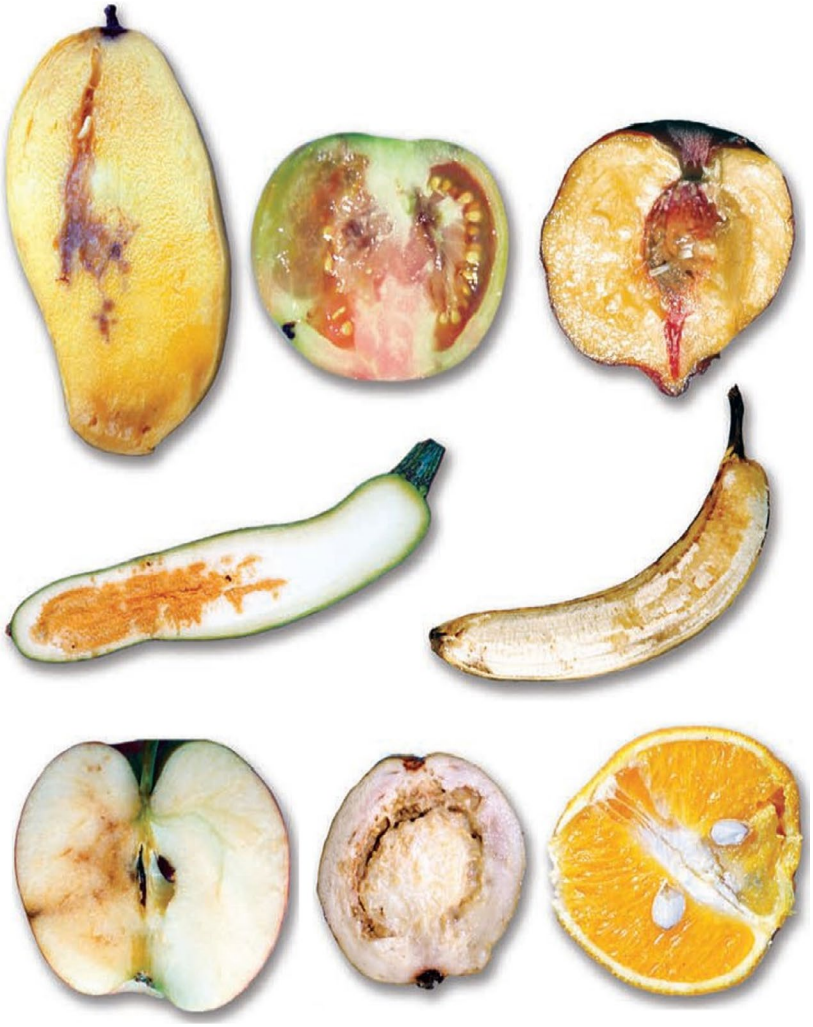

FIGURE A3 Fruits clockwise from top left: mango, tomato, peach, banana, orange, common guava, apple and courgette. Images courtesy of Richard Piper Australian Scientific Advisory Services/FAO/IAEA (2019)

APPENDIX 5 SHORT PROCEDURE FOR INSPECTORS

General

For visual examination of fresh fruit consignments, plant health inspectors should be equipped with a torch, a knife and a magnifying lens (10×). The place where the inspection is conducted should be well lit.

Hygiene measures

Inspection and sampling can themselves be a pathway for spreading pests, therefore inspectors should take appropriate precautions during inspection and sampling, such as wearing gloves and disinfecting hands and tools. Good hygiene procedures when collecting samples for the laboratory should be followed by decontaminating tools and hands.

Sample size

The sampling unit commonly used for fruit is an individual item (e.g. for *Citrus*, mangos, apples etc.). For small items, the sampling unit can be the smallest unit defined as one piece [e.g. a bunch of grapes or a punnet (small box) of berries].

The necessary number of fruit should be selected from the whole lot/consignment and from different places and depths within a box. If the sampling unit is a box, it should be emptied in such a way that all fruits in the box can be checked.

The number of individual units that has to be inspected should be determined on the basis of lots, taking into account the statistical background provided in ISPM 31 *Methodologies for sampling of consignment* (IPPC, 2008).

It is up to the NPPO to set the sample size. For example, from a consignment consisting of a container of fruit (where an item is the sampling unit), 300 items should be inspected to provide a 95% confidence of detecting symptoms present in 1% of items, provided the symptoms are uniformly distributed and the fruits are randomly selected. To detect infestation in a consignment in which 5% of the fruits are displaying symptoms with 95% confidence, 60 items should be inspected provided the symptoms are uniformly distributed and the fruits are randomly selected. For other levels of confidences or other percentages of symptoms consult ISPM 31.

Visual examination

The visual examination should begin with an overall examination of the consignment. Visual examination of the container, packaging and means of conveyance can provide indications of adverse conditions during transport (e.g. adverse temperatures or signs of damp or wetness) which may affect the physical condition of the fruit. An inspection table can be used to inspect fruit. Any emptied boxes should be inspected for signs of pests. Any wrapping on individual fruit should be removed.

Fruit flies are mostly detected as larvae in fruits. Holes are visible on the fruits. Eggs might be found inside the fruit at the point where oviposition puncture marks are visible on the surface. Although some species can pupate inside the fruit, mostly larvae will leave the fruit to pupate, and so consequently pupae may also be detected in packaging.

Each item examined should be gently pressed to detect soft areas of the fruit indicative of Tephritidae larvae presence. Inspection of fresh fruit should look for soft areas, dark spots, rot, holes or lesions that may have originated from oviposition, holes with juice exuding or larval activity. Some symptoms are characteristic of fruit fly infestation (see Appendix 3 for supporting images).

Fruit flies cause rot and discolouration on infested commodities. Commodities with higher phytosanitary risk include several fruits, such as berries, *Citrus* spp., *Persea americana*, *Musa* spp. (ripened), *Mangifera indica*, *Actinidia* spp., *Carica papaya*, *Passiflora edulis*, *Cucumis* spp., olive, *Litchi chinensis*, *Averrhoa carambola*, *Prunus* spp. (cherries), *Malus* spp., Pyrus spp. (pears), *Vitis* spp., *Solanum lycopersicum*, *Cucumis sativus* and *Capsicum* spp.

Destructive sampling

Given that signs of infestation can be difficult to detect by inspection of the whole fruit, it may be necessary to cut fruit to examine the inside. A risk-based approach may be adopted for destructive sampling where consideration is given to the factors detailed in Section 2 and the size of the lot. An appropriate number of asymptomatic fruit should be randomly selected and cut in half to look for larvae. Larger fruit can be peeled and cut into pieces. From the sample taken for inspection, random sampling of a minimum of 30 fruit for destructive examination gives a 95% confidence level of detecting the pest at a 10% infestation level. Sixty fruit for destructive examination need to be sampled to achieve this level of confidence at a 5% infestation level. For smaller items (e.g. a punnet), the same numbers apply to the sampling unit.

Sampling for laboratory identification

Visual examination does not allow identification of the species and laboratory diagnostics is necessary. Morphological identification is the most widely used method for Tephritidae adults using morphological keys. Molecular techniques are also available.

If the fruit shows symptoms of fruit fly damage, the inspector should look for larvae or adults. In the case of detection of a life stage of a fruit fly, a sample should be taken and sent to the laboratory to confirm the identity of the pest. This is important because not all countries have regulated all Tephritidae species, but also because some species are already present in the EPPO region.

Larvae can be sent alive along with a piece of the fruit in an airtight, secure container. If collected larvae are to be preserved, they should be placed in boiling water for a few seconds (until they become immobile) and then transferred to 70% ethanol (if a molecular test is to be carried out subsequently, 95–100% ethanol is recommended). Adults can be killed in 70% ethanol. Adults can be sent for identification in a hermetic tube or container in 70% ethanol (if a molecular test is to be carried out subsequently, 95–100% ethanol is recommended). Placing the adults live in a hermetic tube allows for the colour and pattern of the body and wings to develop, which can aid identification. It is recommended that several adults are sent for identification.