



[1] **Determination of host status of fruit to fruit flies (Tephritidae) (2006-031)**

[2] **Status box**

[3] ***This is not an official part of the standard and it will be modified by the IPPC Secretariat after adoption.***

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[4] **CONTENTS**

[5] [To be inserted]

[6] **Adoption**

[7] This standard was adopted by the [Xth] Session of the Commission on Phytosanitary Measures in [Month 20-].

[8] **INTRODUCTION**

[9] **Scope**

[10] This standard provides guidelines for the determination of host status of fruit to fruit flies (Tephritidae) and describes three categories of host status of fruit to fruit flies.

[11] Fruit as referred to in this standard covers fruit in the botanical sense, including such fruits that are sometimes called vegetables (e.g. tomato and melon).

[12] This standard includes methodologies for surveillance under natural conditions and field trials under semi-natural conditions that should be used to determine the host status of undamaged fruit to fruit flies for

cases where host status is uncertain. This standard does not address requirements to protect plants against the introduction and spread of fruit flies.

### [13] References

[14] **ISPM 2.** 2007. *Framework for pest risk analysis*. Rome, IPPC, FAO.

[15] **ISPM 5.** *Glossary of phytosanitary terms*. Rome, IPPC, FAO.

[16] **ISPM 11.** 2013. *Pest risk analysis for quarantine pests*. Rome, IPPC, FAO.

[17] **ISPM 26.** 2006. *Establishment of pest free areas for fruit flies (Tephritidae)*. Rome, IPPC, FAO.

[18] **ISPM 30.** 2008. *Establishment of areas of low pest prevalence for fruit flies (Tephritidae)*. Rome, IPPC, FAO.

[19] **ISPM 35.** 2012. *Systems approach for pest risk management of fruit flies (Tephritidae)*. Rome, IPPC, FAO.

### [20] Definitions

[21] Definitions of phytosanitary terms can be found in ISPM 5 (*Glossary of phytosanitary terms*). In this standard, the following additional definitions apply:

[22]	host status (of fruit to a fruit fly)	Classification of a plant species or cultivar as being a natural host, conditional host or non-host for a fruit fly species
[23]	natural host (of fruit to a fruit fly)	A plant species or cultivar that has been scientifically found to be infested by the target fruit fly species under natural conditions and able to sustain its development to viable adults
[24]	conditional host (of fruit to a fruit fly)	A plant species or cultivar that is not a natural host but has been scientifically demonstrated to be infested by the target fruit fly species and able to sustain its development to viable adults under defined permissive conditions as concluded from the semi-natural field conditions set out in this standard
[25]	non-host (of fruit to a fruit fly)	A plant species or cultivar that has not been found to be infested by the target fruit fly species or is not able to sustain its development to viable adults under natural conditions or under the semi-natural field conditions set out in this standard

### [26] Outline of Requirements

[27] This standard describes requirements for determining the host status of a particular fruit to a particular fruit fly species and designates three categories of host status: natural host, conditional host and non-host.

[28] Requirements for determining host status include:

- [29] • accurate identification of the fruit fly species, test fruit and, for field trials, control fruit from a known natural host
- [30] • specification of parameters for adult and larval fruit fly surveillance and experimental design under semi-natural field conditions (i.e. field cages, greenhouses or bagged fruit-bearing branches) to determine host status and specify the conditions of the fruit (including physiological) to be evaluated
- [31] • observation of fruit fly survival at each stage of its development
- [32] • establishment of procedures for holding and handling the fruit for host status determination
- [33] • evaluation of experimental data and interpretation of results.

#### [34] **BACKGROUND**

[35] Fruit flies are economically important pests and the application of phytosanitary measures is often required to allow movement of their host fruit in trade (ISPM 26:2006; ISPM 30:2008; ISPM 35:2012). The host status of fruit is an important element of Pest Risk Analysis (PRA) (ISPM 2: 2007; ISPM 11:2013). Categories of and procedures for determining host status should therefore be harmonized.

[36] It is important to note that host status may change over time because of changes in biological conditions.

[37] When host status is uncertain there is a particular need to provide harmonized guidance to national plant protection organizations (NPPOs) for determining the host status of fruit to fruit flies. Historical evidence, pest interception records and scientific literature generally may provide sufficient information on host status, without the need for additional larval field surveillance or field trials. However, historical records and published reports may sometimes be unreliable, for example:

- [38] • Fruit fly species and plant species or cultivars may have been incorrectly identified and reference specimens may not be available for verification.
- [39] • Collection records may be incorrect or dubious (e.g. host status based (i) the catch from a trap placed on a fruit plant; (ii) damaged fruit; (iii) simply finding larvae inside fruit; or (iv) cross-contamination of samples)."
- [40] • Important details may have been omitted (e.g. cultivar, stage of maturity, physical condition of fruit at the time of collection, sanitary condition of the orchard).
- [41] • Development of larvae to viable adults may not have been verified.

[42] Protocols and comprehensive trials to determine fruit fly host status have been documented in the scientific literature. However, inconsistencies in terminology and methodology contribute to variations in the determination of fruit fly host status. Harmonization of terminology, protocols and evaluation criteria for the determination of fruit fly host status will promote consistency among countries and scientific communities.

[43] Surveillance by fruit sampling is the most reliable method to determine natural host status. Surveillance of natural infestation by fruit sampling does not interfere with the natural behaviour of fruit flies and takes into account high levels of variability in the fruit, fruit fly behaviour and periods of activity. Fruit sampling includes the collection of fruit and the rearing of fruit flies on it to determine if the fruit is a host to the fruit fly (i.e. if the fruit can sustain fruit fly development to viable adults).

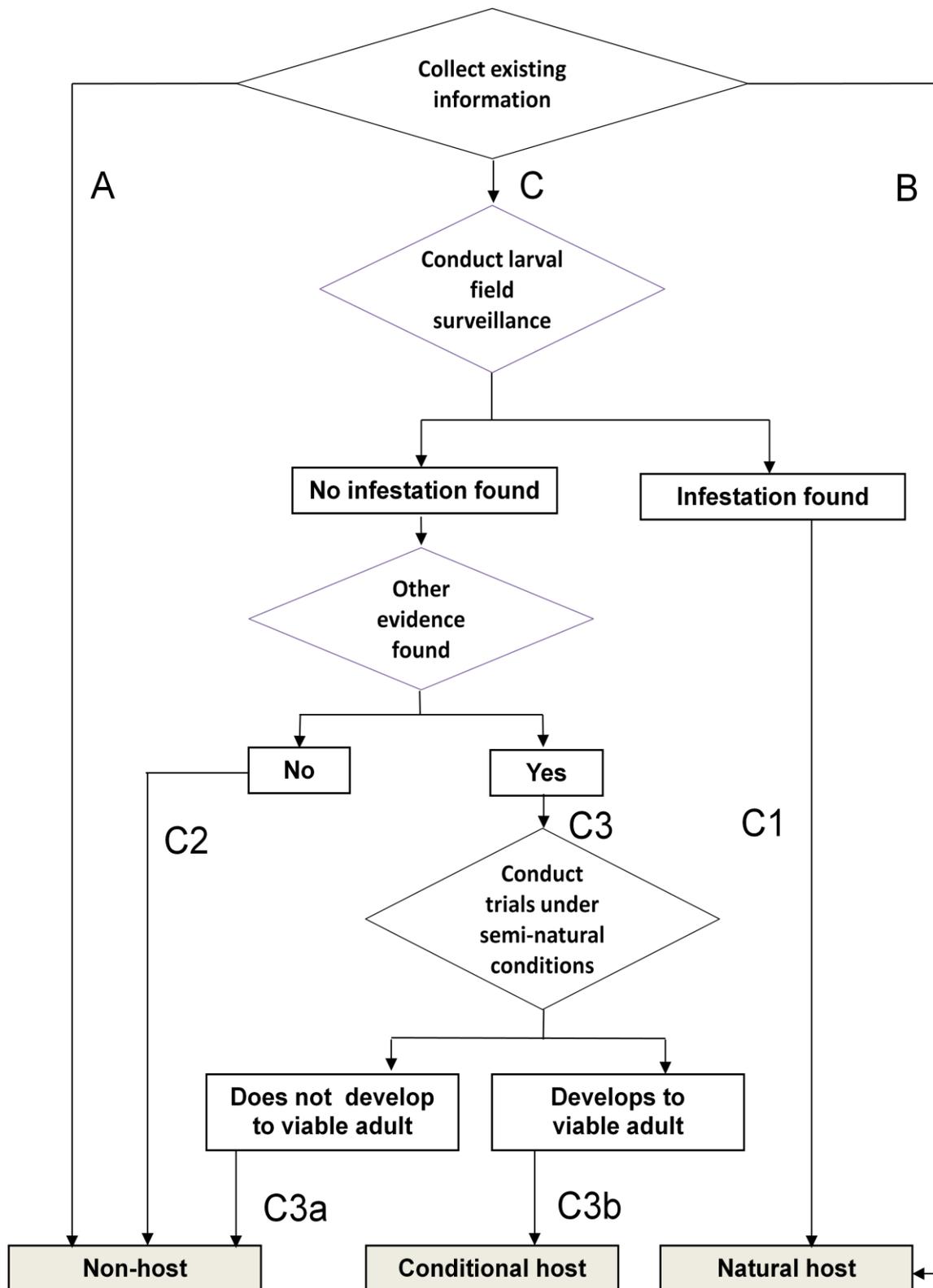
[44] Field trials under semi-natural conditions allow fruit flies to exhibit natural oviposition behaviour, and because the fruit remains attached to the plant it does not degrade rapidly during the trials. However, field trials under semi-natural conditions can be resource-intensive and may be compromised by environmental variables.

[45] Results of field trials carried out in a certain area may be extrapolated to comparable areas if the target fruit fly species and the physiological condition of the fruit are similar, so that fruit fly host status determined in one area does not need to be repeated in a separate but similar area.

**[46] GENERAL REQUIREMENTS**

- [47]** Determining to which of the three categories of host status (natural host, conditional host and non-host) a fruit belongs can be done through the following steps, as is outlined in the flow chart (Figure 1):
- [48]** **A.** When existing biological or historical information provides sufficient evidence that the fruit does not support infestation<sup>1</sup> and development to viable adults, no further surveys or field trials should be required and the plant should be categorized as a non-host.
- [49]** **B.** When existing biological and historical information provides sufficient evidence that the fruit supports infestation and development to viable adults, no further surveys or field trials should be required and the plant should be categorized as a natural host.
- [50]** **C.** When the evidence is inconclusive, appropriate field surveillance by fruit sampling or field trials should be used to determine host status. Surveillance and trials may lead to one of the following results:
- [51]** **C1.** If infestation with development to viable adults is found after field surveillance by fruit sampling, the plant should be categorized as a natural host.
- [52]** **C2.** If no infestation is found after field surveillance by fruit sampling, and no further information indicates that the fruit has the potential to become infested, the plant may be categorized as a non-host.
- [53]** **C3.** When no infestation is found after field surveillance by fruit sampling, but available biological or historical information indicates that the fruit has the potential to become infested, additional field trials under semi-natural conditions may be needed to assess whether the target fruit fly can develop to viable adults on the particular fruit species or cultivar.
- [54]** **C3a.** If the target fruit fly species does not develop to viable adults, the plant should be categorized as a non-host.
- [55]** **C3b.** If the target fruit fly species does develop to viable adult, the plant should be categorized as a conditional host.

[56]

[57] **Figure 1.** Steps for fruit fly host status determination.[58] **SPECIFIC REQUIREMENTS**

[59] Host status may be determined from historical production records or from trade or interception data indicating natural infestations. Where historical data do not provide clear determination of host status, surveillance by fruit sampling should be conducted to gather evidence of natural infestations and development to viable adults, or trials under semi-natural field conditions may be required. In cases where host status has not been scientifically determined by surveillance, or when there is a particular need to determine if a fruit is a conditional host or a non-host, trials conducted under semi-natural field conditions may be required.

[60] Artificial conditions are inherent in laboratory tests in which fruit flies are presented with harvested fruit that undergoes rapid physiological changes and thereby may become more susceptible to infestation. The detection of infestation in laboratory tests for the determination of host status may therefore be misleading. In addition, it has been widely documented that under artificial conditions, females of polyphagous species will lay eggs in almost any fruit presented to them and, in most cases, the larvae will develop into viable adults. Therefore, laboratory tests may be sufficient for demonstrating non-host status, but are inappropriate for demonstrating natural or conditional host status.

[61] The following elements are important considerations in planning field trials:

- [62] • the identity of the plant species (including cultivars where appropriate) and the target fruit fly species
- [63] • the physical and physiological variability of the fruit in the growing area
- [64] • past chemical usage in the fruit production area
- [65] • target fruit fly incidence over the entire growing area, and relevant harvest and export periods
- [66] • relevant information, literature and records regarding host status of the fruit and fruit fly species, including a critical review of such information
- [67] • the origin and rearing status of the fruit fly colony to be used
- [68] • known natural host species and cultivars to be used as controls
- [69] • separate field trials where appropriate for each fruit fly species for which determination of host status is required
- [70] • separate field trials for each cultivar of the fruit if cultivar differences are the purported source of host variability to infestation
- [71] • the placing of field trials in the fruit production areas
- [72] • all field trials should comply with sound statistical practice.

### [73] **1. Natural Host Status Determination Using Surveillance by Fruit Sampling**

[74] Fruit sampling is the most reliable method to determine natural host status. The status of a natural host can be determined based on confirmation of natural infestation and development to viable adults by sampling fruit during the harvest period.

[75] Fruit samples should be representative of the range of production areas and environmental conditions, as well as of physiological and physical stages.

### [76] **2. Host Status Determination Using Field Trials under Semi-natural Conditions**

- [77] The objective of field trials is to determine host status under specified conditions of a fruit that has been determined not to be a natural host. Trials may include the use of field cages, greenhouses (including glass, plastic and screen houses) and bagged fruit-bearing branches.
- [78] The emergence of a viable adult in any one replicate of a field trial under semi-natural conditions indicates that the fruit is a conditional host.
- [79] The following subsections outline elements that should be taken into account when designing field trials.
- [80] **2.1 Fruit sampling**
- [81] The following requirements apply to fruit sampling in field trials:
- [82] • Where possible, sampling should target fruit suspected of being infested. Otherwise, sampling protocols should be based on principles of randomness and replication and be appropriate for any statistical analysis performed.
  - [83] • Period of time, the number of repetitions per growing season and the number of replicates should account for the variability of target fruit flies and fruit over time and over the production area. They should also account for early and late harvest conditions and be representative of the proposed area from where the fruit will be moved. The number and weight of the fruit required and replicates per trial to determine effectiveness, and appropriate confidence level, should be specified.
- [84] **2.2 Fruit flies**
- [85] The following requirements apply to operational procedures pertaining to the fruit flies used in field trials:
- [86] • Taxonomic identification of the fruit flies used for the field trials should be performed and voucher specimens be preserved.
  - [87] • Basic information on target fruit fly species, including normal period of development and known hosts in the specific production area, should be compiled.
  - [88] • The use of wild populations for the field trials is desirable. If wild flies cannot be obtained in sufficient numbers, the colony used should not be older than five generations at the initiation of the trial, whenever possible. The fruit fly population may be maintained on substrate, but the generation to be used in the trial should be reared on the natural host to ensure normal oviposition behaviour. Flies used in experimental replicates should all come from the same population and generation (i.e. cohort).
  - [89] • The fruit fly colony should originate from the same area as the target fruit whenever possible.
  - [90] • Pre-oviposition, oviposition and mating periods should be determined before the field trials so that mated female flies are exposed to the fruit at the peak of their reproductive potential.
  - [91] • The age of the adult female and male flies should be recorded on the mating date and at the beginning of the field trials.
  - [92] • The number of mated female flies required per fruit should be determined according to fruit size, female fecundity and field trial conditions. The number of fruit flies per replicate trial should be determined according to fruit fly biology, amount of fruit to be exposed, and other field trial conditions.
  - [93] • The exposure time of the fruit to the target fruit fly species should be based on fruit fly oviposition behaviour.
  - [94] • An individual female fly should be used only once.

- [95]           • The number of adults dying during the field trials should be recorded and dead fruit flies should be replaced with live adults of the same population and generation (i.e. cohort). High adult mortality may indicate unfavourable conditions (e.g. excessive temperature) or contamination of field trial fruit (e.g. residual pesticides). In such cases, the trials should be repeated under more favourable conditions.
- [96]   In repeated field trials, fruit flies should be of a similar physiological age and have been reared under the same conditions.
- [97]   **2.3 Fruit**
- [98]   The following requirements apply to the fruit used in field trials. The fruit should be:
- [99]           • of the same species and cultivar as the fruit to be moved
- [100]           • from the same production area, or an area representative of it, as the fruit to be moved
- [101]           • practically free from pesticides deleterious to fruit flies and from baits, dirt, other fruit flies and pests
- [102]           • free from any mechanical or natural damage
- [103]           • of a specified commercial grade regarding colour, size and physiological condition
- [104]           • at an appropriate, specified stage of maturity (e.g. dry weight or sugar content).
- [105]   **2.4 Controls**
- [106]   Fruit of known natural hosts at known stage of maturity are required as controls for all field trials. These may be of different species or genera from the target fruit species. Fruit should be free of prior infestation (e.g. by bagging or from a pest free area). Fruit flies used in controls and experimental replicates (including control) should all come from the same population and generation (i.e. cohort).
- [107]   Controls are used to:
- [108]           • verify that female flies are sexually mature, mated and exhibiting normal oviposition behaviour
- [109]           • indicate the level of infestation that may occur in a natural host
- [110]           • indicate the time frame for development to the adult stage under the field trial conditions in a natural host
- [111]           • confirm that environmental conditions for infestation are appropriate
- [112]   **2.5 Field trial design**
- [113]   For this standard, field trials use field cages, greenhouses or bagged fruit-bearing branches. Trials should be appropriate for evaluating how the physical and physiological condition of the fruit may affect host status.
- [114]   Flies are released into large mesh field cages that enclose whole fruit-bearing plants or mesh bags that enclose the parts of plants with the fruit. Alternatively, fruit-bearing plants may be placed in greenhouses into which flies are released. The fruit-bearing plants can be grown in the enclosures or be introduced as potted plants for the trials. It is important to note that because female fruit flies are artificially confined within the specific enclosure under observation, they may be forced to lay eggs in the fruit of a conditional

host.

- [115] Field trials should be conducted under conditions appropriate for fruit fly activity, especially oviposition, as follows:
- [116] Field cages and greenhouses should be of an appropriate size and a design to ensure confinement of the adults and trial plants, allow adequate airflow, and allow conditions that facilitate natural oviposition behaviour.
- [117] • Adults should be provided with satisfactory and sufficient food and water.
- [118] • Environmental conditions should be optimal and be recorded during the period of the field trials.
- [119] • Male flies may be kept in cages or greenhouses with the female flies if it is beneficial for encouraging oviposition.
- [120] • Natural enemies to the target fruit fly species should be removed from the cages before initiating the trials and re-entry should be prevented.
- [121] • Cages should be secured from other consumers of fruits (e.g. birds and monkeys).
- [122] • For controls, fruit from known natural hosts can be hung on branches of plants (not on the branches with test fruit). Controls must be separated from tested fruits (in separate field cages, greenhouses or bagged fruit-bearing branches) to ensure the trial is not a choice test.
- [123] • The test fruit should remain naturally attached to plants and may be exposed to the fruit flies in field cages, bags or greenhouses.
- [124] • The plants should be grown under conditions that exclude as far as possible any interference from chemicals deleterious to fruit flies.
- [125] • A replicate should be a bag or cage, preferably on one plant at the experimental unit.
- [126] • Fruit fly mortality should be monitored and recorded and dead flies immediately replaced with live flies from the same population and generation (i.e. cohort) to maintain the same fruit fly incidence.
- [127] • The fruit should be grown under commercial conditions or in containers of a size that allows normal plant and fruit development.
- [128] • After the designated exposure period for oviposition, the fruit should be removed from the plant and weighed and the number and weight of fruit recorded.
- [129] The sample size to be used to achieve the confidence level should be pre-determined using scientific references.
- [130] **3. Fruit Handling for Fruit Fly Development and Emergence**
- [131] Fruit collected under natural conditions (surveillance by fruit sampling) and semi-natural conditions (field trials), as well as control fruit, should be kept until larval development is complete. This period may vary with temperature and host status. Fruit handling and holding conditions should maximize fruit fly survival and be specified in the sampling protocol or experimental design of the field trial.
- [132] Fruit should be kept in an insect-proof facility or container under conditions that ensure pupal survival, including:
- [133] • appropriate temperature and relative humidity
- [134] • suitable pupation medium.

[135] Furthermore, conditions should facilitate accurate collection of larvae and pupae, and viable adults emerging from the fruit.

[136] Data to be recorded include:

[137] 1. daily physical conditions (e.g. temperature, relative humidity) in the fruit holding facility

[138] 2. dates and numbers of larvae and pupae collected from the test fruit and the control fruit, noting that:

[139]       • the medium may be sieved at the end of the holding period

[140]       • at the end of the holding period, the fruit should be dissected before being discarded, to determine the presence of live and dead larvae or pupae; depending on the fruit decay stage, it may be necessary to transfer the larvae to an adequate pupation medium

[141]       • all or a subsample of pupae should be weighed and abnormalities recorded

[142] 3. emergence dates and numbers of all adults by species, including any abnormal adult flies.

#### [143] 4. Data Analysis

[144] Data from larval surveillance and field trials may be analysed quantitatively to determine, for example:

[145]       • levels of infestation (e.g. number of larvae per fruit, number of larvae per kilogram of fruit, percentage of infested fruit) at a specific confidence level

[146]       • development time of larvae and pupae, and number of viable adults

[147]       • percentage of adult emergence.

#### [148] 5. Record-Keeping and Publication

[149] The NPPO should keep appropriate records of larval field surveillance and field trials to determine host status, including:

[150]       • scientific name of the target fruit fly

[151]       • scientific name of the plant species or name of the cultivar

[152]       • location of the production area of the fruit (including geographic coordinates)

[153]       • location of voucher specimens of the target fruit fly (to be kept in an official collection)

[154]       • origin and rearing of the fruit fly colony used for the field trials

[155]       • physical and physiological condition of the fruit tested for infestation by fruit flies

[156]       • experimental design, trials conducted, dates, locations

- [157] • raw data, statistical calculations and interpretation of results
- [158] • key scientific references used
- [159] • additional information, including photographs, that may be specific to the fruit fly, the fruit or host status.
- [160] Records should be made available to the NPPO of the importing country upon request.
- [161] Research should, as far as possible, be peer reviewed and published in a scientific journal or otherwise made available.
- [162] **This appendix is for reference purposes only and is not a prescriptive part of the standard.**
- [163] **APPENDIX 1: Bibliography**
- [164] **Aluja, M. & Mangan, R.L.** 2008. Fruit fly (Diptera: Tephritidae) host status determination: critical conceptual and methodological considerations. *Annual Review of Entomology*, 53: 473–502.
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- [177] **NAPPO RSPM No. 30.** 2008. *Guidelines for the determination and designation of host status of a fruit or vegetable for fruit flies (Diptera: Tephritidae)*. Ottawa, NAPPO.

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- [185] **Footnote 1:** Henceforward, “infestation” refers to infestation of a fruit by a target fruit fly species.