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

[2] **Publication history**

[3]

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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, including analysis of environmental risks and living modified organisms*. 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 being a natural host, non-natural host or non-host for a fruit fly

[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 adults

[24]

non-natural 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 adults under 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 and is not able to sustain its development to 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, non-natural host and non-host.

[28] Requirements for determining host status include:

[29] 1. accurate identification of the fruit fly species, test fruit and, for field trials, control fruit from a known natural host

[30] 2. specification of parameters for adult and larval fruit fly surveillance and experimental design under semi-natural field conditions (i.e. field cages, greenhouses or fruit-bearing bagged branches) to determine host status and specify the conditions of the fruit (including physiological) to be evaluated

[31] 3. observation of fruit fly survival at each stage of development

[32] 4. establishment of procedures for holding and handling the fruit to rear fruit flies

[33] 5. evaluation of experimental data and interpretation of results.

[34] Laboratory tests may be useful for demonstrating non-host status, but field trials are necessary to demonstrate that a fruit is a host to a fruit fly.

[35] **BACKGROUND**

[36] 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 11:2004). Categories of and procedures for determining host status should therefore be harmonized.

[37] When host status is uncertain there is a particular need to provide harmonized guidance to NPPOs for determining the host status of fruit to fruit flies.

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

[39] Historical evidence, pest interception records and scientific literature may provide sufficient information on host status, in which case further surveillance or field trials are not required. However, historical records and published reports may sometimes be unreliable, for example:

[40] 1. Fruit fly species and plant species or cultivars may have been incorrectly identified and reference specimens may not be available for verification.

[41] 2. Collection records may be incomplete, incorrect or dubious (e.g. host status based on the catch from a trap placed on a fruit plant or based on damaged fruit).

[42] 3. Important details may have been omitted (e.g. cultivar and stage of maturity, physical condition of fruit at the time of collection or the sanitary condition of the orchard).

[43] 4. Survival of larvae to adults may not have been verified.

[44] 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.

[45] 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.

[46] When evidence for host status is not conclusive, field trials under semi-natural conditions, with a detailed experimental design and specified statistical confidence, may be performed. Semi-natural field trials allow fruit flies to exhibit natural oviposition behaviour, and because the fruit remains attached to the plant it does not degrade during the trials. However, semi-natural field trials can be resource-intensive and may be compromised by environmental variables.

[47] 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.

[48] **GENERAL REQUIREMENTS**

[49] Determining to which of the three categories of host status (natural host, non-natural host and non-host) a fruit belongs can be done through the following steps, as is outlined in the flow chart (Figure 1):

[50] **A.** When existing biological or historical information provides sufficient evidence that the fruit does not support infestation[1] and development to adults, no further surveys or field trials should be required and the plant should be categorized as a non-host.

[51] **B.** When existing biological and historical information provides sufficient evidence that the fruit supports infestation and development to adults, no further surveys or field trials should be required and the plant should be categorized as a natural host.

[52] **C.** When the evidence is inconclusive, appropriate larval field surveillance by fruit sampling or field trials are necessary to determine susceptibility to infestation. Surveillance and trials may lead to one of the following results:

[53] **C1.** Infestation is found after larval field surveillance (fruit sampling): the plant should therefore be categorized as a natural host.

[54] **C2.** No infestation is found after conducting appropriate larval field surveillance, and no further information indicates that the fruit has the potential to become infested: the plant may therefore be categorized as a non-host.

[55] **C3.** No infestation is found after larval field surveillance, 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 adult stage on the particular fruit species or cultivar.

[56] **C3a.** If the target fruit fly species does not develop to adult stage, the fruit should be categorized as a non-host.

[57] **C3b.** If the target fruit fly species does develop to adult stage, the fruit should be categorized as a non-natural host.

[58]

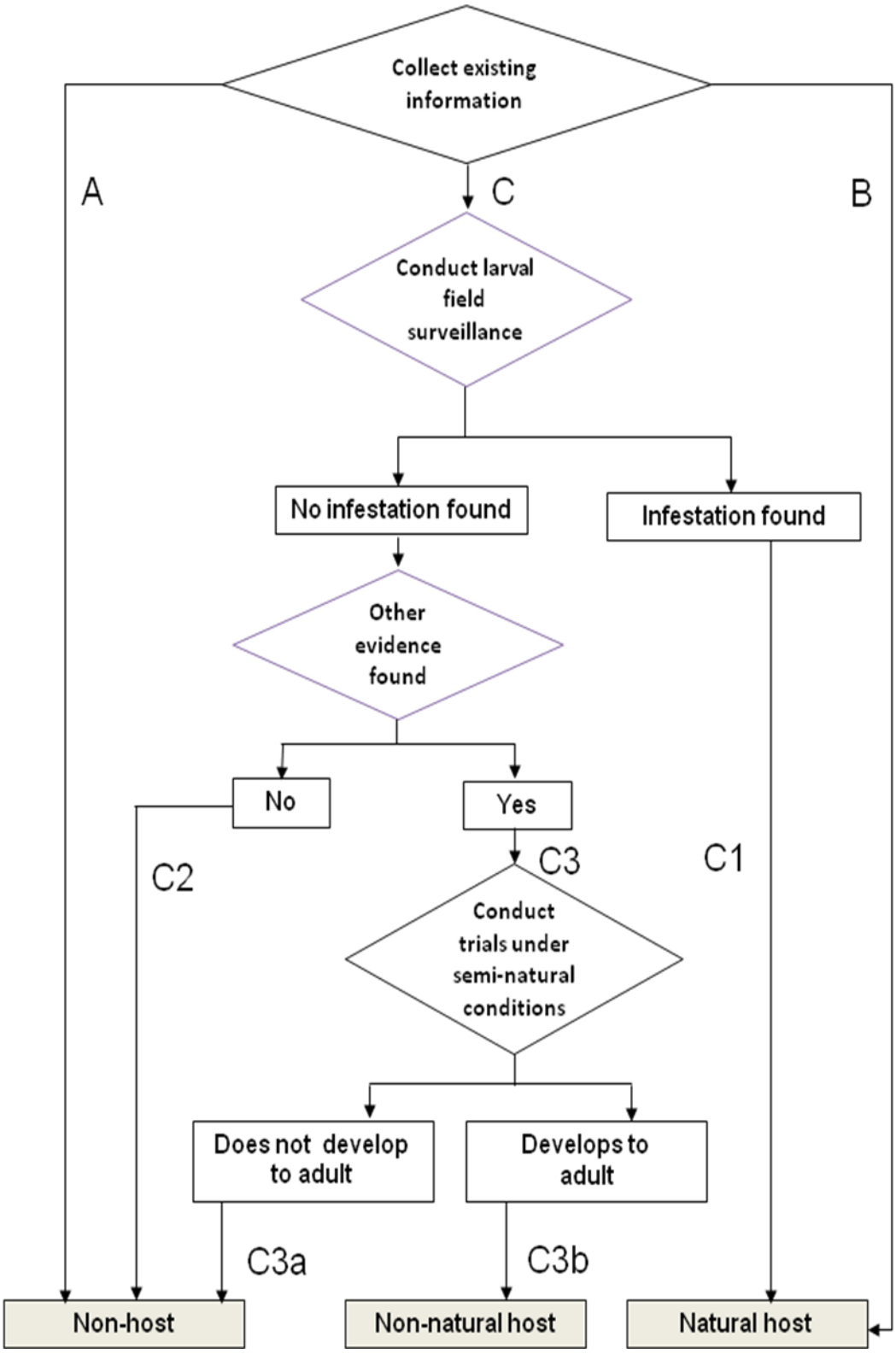

Figure 1. Steps for fruit fly host status determination.

[59] **SPECIFIC REQUIREMENTS**

[60] Host status may be determined from historical production, trade or interception data indicating natural infestations. Where historical data do not provide clear determination of host status, larval surveillance by fruit sampling should be conducted.through surveillance by fruit sampling to gather evidence of natural infestations, or through trials under semi-natural field conditions Field trials under semi-natural conditions may be conducted 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 non-natural host or a non-host.

[61] 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 substrate presented to them and, in most cases, the larvae will develop into adults. Laboratory tests may be useful for demonstrating non-host status, but field trials are necessary to demonstrate that a fruit is a host.

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

[63] 1. the identity of the plant species (including cultivars where appropriate) and the target fruit fly species

[64] 2. the physical and physiological variability of the fruit in the growing area

[65] 3. target fruit fly incidence over the entire growing area, and relevant harvest and export periods

[66] 4. relevant information, literature and records regarding host status of the fruit and fruit fly species, including a critical review of such information

[67] 5. origin and rearing status of the fruit fly colony to be used

[68] 6. known natural host species and cultivars to be used as controls

[69] 7. separate field trials where appropriate for each fruit fly species for which determination of host status is required

[70] 8. separate field trials for each cultivar of the fruit if cultivar differences are the purported source of host variability to infestation

[71] 9. the placing of field trials in the fruit production areas.

[72] 10. all field trials should comply with sound statistical practice

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

[74] Larval field surveillance by 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 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 to not be a natural host. Trials may include the use of field cages, greenhouses (including glass, plastic and screen houses) and fruit-bearing bagged branches.

[78] The emergence of an adult in any one replicate of a field trial under semi-natural conditions indicates that the fruit is a non-natural 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] 1. 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] 2. 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] 3. The number of eggs oviposited by controls and the resulting immatures or adults should be determined.

[85] **2.2 Fruit flies**

[86] The following requirements apply to operational procedures pertaining to the fruit flies used in field trials:

[87] 1. Taxonomic identification of the fruit flies used for the field trials should be performed and voucher specimens be preserved.

[88] 2. Basic information on target fruit fly species, including normal period of development and known hosts in the specific production area, should be compiled.

[89] 3. The use of wild populations for the field trials is desirable. If wild flies cannot be obtained in sufficient numbers, the colony used should be no older than three generations at the initiation of the trial, whenever possible, and it should be maintained on natural hosts to ensure normal oviposition behaviour.

[90] 4. The fruit fly colony should originate from the same area as the target fruit whenever possible.

[91] 5. Pre-oviposition, oviposition and mating periods should be determined before the field trials so that sexually mature, mated female flies are exposed to the fruit at the peak of their reproductive potential.

[92] 6. The age of the adult female and male flies should be recorded on the mating date and at the beginning of the field trials.

[93] 7. The number of mated female flies required per fruit should be determined according to fruit size 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.

[94] 8. The exposure time of the fruit to the target fruit fly species should be based on fruit fly oviposition behaviour.

[95] 9. An individual female fly should preferably be used only once.

[96] 10. 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.

[97] In repeated field trials , fruit flies should be of a similar physiological age and should have been exposed to the same conditions.

[98] **2.3 Fruit**

[99] The following requirements apply to the fruit used in field trials. The fruit should be:

[100] 1. of the same species and cultivar as the fruit to be moved

[101] 2. from the same production area, or an area representative of it, as the fruit to be moved

[102] 3. practically free from pesticides deleterious to fruit flies and from baits, dirt, other fruit flies and pests

[103] 4. free from any mechanical or natural damage

[104] 5. of a specified commercial grade regarding colour, size, and physiological condition

[105] 6. at an appropriate, specified stage of maturity (e.g. dry weight or sugar content).

[106] **2.4 Controls**

[107] Known natural hosts are required as controls for all field trials. These may be of different species or genera from the target fruit fly species. Fruit should be free of prior infestation (e.g. by bagging or from a pest free area). Fruit flies used in control and experimental replicates should all come from the same population and generation (i.e. cohort).

[108] Controls are used to:

[109] 1. verify that female flies are sexually mature, mated and exhibiting normal oviposition behaviour

[110] 2. indicate the level of infestation that may occur in a natural host

[111] 3. indicate the time frame for development to the adult stage under the field trial conditions in a natural host

[112] 4. confirm that environmental conditions for infestation are appropriate

[113] 5. in the case of natural infestation controls, confirm that wild female flies were ovipositing in the area where the fruit is grown during the trial period.

[114] **2.5 Field trial design**

[115] For this standard, field trials include the use of field cages, greenhouses and fruit-bearing bagged branches. Trials may be conducted in sequence; however, it may be more practical to conduct them simultaneously while the fruit is available. Trials should be appropriate for evaluating how the physical and physiological condition of the fruit may affect host status.

[116] 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 non-natural host.

[117] Field trials should be conducted under conditions appropriate for fruit fly activity, especially oviposition, as follows:

[118] 1. Field cages and greenhouses should be of an appropriate size and design to ensure confinement of the adults and trial plants, allow adequate airflow, and allow conditions that facilitate natural oviposition behaviour.

[119] 2. Adults should be provided with satisfactory and sufficient food and water.

[120] 3. Environmental conditions should be optimal and be recorded during the period of the field trials.

[121] 4. Male flies may be kept in cages or greenhouses with the female flies if it is beneficial for encouraging oviposition.

[122] 5. Natural enemies to the target fruit fly species should be removed from the cages before initiating the trials and re-entry should be prevented.

[123] 6. Cages should be secured from other consumers of fruits (e.g. birds and monkeys).

[124] 7. For controls, fruit from known natural hosts can be hung on branches of plants (not on the branches with test fruit).

[125] 8. The test fruit should remain naturally attached to plants and may be exposed to the fruit flies in field cages, bags or greenhouses.

[126] 9. The plants should be grown under conditions that exclude as far as possible any interference from chemicals deleterious to fruit flies.

[127] 10. A replicate should be a bag or cage, preferably on one plant at the experimental unit.

[128] 11. 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.

[129] 12. For greenhouse trials, the fruit should be grown under commercial conditions or in containers of a size that allows normal plant and fruit development.

[130] 13. 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.

[131] The sample size to be used to achieve the confidence level should be pre-determined using scientific references.

[132] **3. Fruit Handling for Fruit Fly Emergence**

[133] 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 field trial design.

[134] Fruit should be kept in an insect-proof facility or container under conditions that ensure pupal survival, including:

[135] 1. appropriate temperature and relative humidity

[136] 2. suitable pupation medium.

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

[138] Data to be recorded include:

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

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

[141] • the medium may be sieved at intervals before all larvae have left the fruit and at the end of the holding period

[142] • 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 – if live larvae are present, the fruit should be kept until all mature larvae have exited

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

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

[145] **4. Data Analysis**

[146] Data from larval surveillance and field trials may be analysed quantitativelyto determine, for example:

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

[148] 2. development time of larvae and pupae, and number of viable adults

[149] 3. percentage of adult emergence.

[150] **5. Record-Keeping and Publication**

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

[152] 1. scientific name of the target fruit fly

[153] 2. scientific name of the plant species or cultivar

[154] 3. location of the production area of the fruit (including geographic coordinates)

[155] 4. location of voucher specimens of the target fruit fly (to be kept in an official collection)

[156] 5. origin and rearing of the fruit fly colony for the field trials

[157] 6. physical and physiological condition of the fruit tested for infestation by fruit flies

[158] 7. experimental design, trials conducted, dates, locations

[159] 8. raw data, statistical calculations and interpretation of results

[160] 9. key scientific references used

[161] 10. additional information, including photographs, that may be specific to the fruit fly, the fruit or host status.

[162] Records should be made available to the NPPO of the importing country upon request.

[163] Research should, as far as possible, be peer reviewed and published in a scientific journal or otherwise made available.

[164] This appendix is for reference purposes only and is not a prescriptive part of the standard.

[165] **APPENDIX 1: Bibliography**

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[187] **Footnote 1:** Henceforward, "infestation" refers to infestation of a fruit by a target fruit fly species.