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NAPPO Regional Standards for Phytosanitary Measures (RSPM)

RSPM No. 30
Guidelines for the Determination and Designation of Host Status of a Fruit or Vegetable for Fruit Flies (Diptera: Tephritidae)

The Secretariat of the North American Plant Protection Organization
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Contents

Review

NAPPO Standards for Phytosanitary Measures are subject to periodic review and amendment.

The next review date for this NAPPO Standard is 2013. A review of any NAPPO standard may be initiated at any time upon the request of a NAPPO member country.

Approval

This Standard was approved by the North American Plant Protection Organization (NAPPO) Executive Committee on October 20, 2008 and is effective from this date.

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Implementation

See the attached implementation plans for implementation dates in each NAPPO country.

Amendment Record

Amendments to this Standard will be dated and filed with the NAPPO Secretariat.

Distribution

This standard is distributed by the NAPPO Secretariat, to the Industry Advisory Group and Sustaining Associate Members, the International Plant Protection Convention (IPCC) Secretariat, and to other Regional Plant Protection Organizations (RPPOs).

Introduction

Scope

This document describes experimental protocols and comprehensive trials for determining host status of a fruit or vegetable for a particular fruit fly (Diptera: Tephritidae) species and designates categories for host status.

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Definitions, Abbreviations, and Acronyms

conditional host	A fruit or vegetable that is host or a non-host under defined permissive or restrictive conditions, respectively (e.g., stage of maturity, other physiological conditions, physical conditions) (NAPPO)
National Plant Protection Organization (NPPO)	Official service established by a government to discharge functions specified by the IPPC (FAO)
natural host	A fruit or vegetable that becomes infested by a plant pest in nature (e.g., natural, cultivated and/or unmanaged plants) and the plant pest population is sustained on the fruit or vegetable (NAPPO)
natural non-host	A fruit or vegetable that does not become infested by a plant pest in nature (e.g., natural, cultivated and/or unmanaged plants) and the plant pest population is not sustained on the fruit or vegetable (NAPPO)
non-preference	Plant characters and insect responses that lead away from the use of a particular plant or variety for oviposition, food, or shelter, or a combination of all three (Painter 1951)
pest record	A document providing information concerning the presence or absence of a specific pest at a particular location at a certain time, within an area (usually a country) under described circumstances (FAO)
Pest Risk Analysis (PRA)	The process of evaluating biological or other scientific and economic evidence to determine whether an organism is a pest, whether it should be regulated, and the strength of any phytosanitary measures to be taken against it (FAO)
resistance factor	Any condition in plants that protects them from insect infestation, including structures, chemical substances in the plant, or physiological conditions (Torre-Bueno 1978)

Outline of Requirements

This standard describes requirements for determining the host status of a fruit or vegetable for a particular fruit fly (Diptera: Tephritidae) species and designates categories for host status.

Requirements include:

- Selection of the fruit fly, fruit or vegetable, and controls for the trial.
- Definition of parameters for the trial, fruit or vegetable, and fruit fly in order to determine host status and specify the defined condition(s) of the fruit or vegetable to be evaluated as a resistance factor(s).
- Holding of the fruit or vegetable to rear fruit flies after exposure.
- Biological stage of the insect (larvae, pupae or adult) that will be used as the basis for determination of host status.
- Evaluation of experimental data.
- Interpretation of results.

Host status designations outlined in this standard include:

- Natural host – A fruit or vegetable that becomes infested by a plant pest in nature (e.g., natural, cultivated and/or unmanaged plants) and the plant pest population is sustained on the fruit or vegetable. No other trials are necessary to confirm host status.
- Natural non-host – A fruit or vegetable that does not become infested by a plant pest in nature (e.g., natural, cultivated and/or unmanaged plants) and the plant pest population is not sustained on the fruit or vegetable.
- Conditional host – A fruit or vegetable that is host or a non-host under defined permissive or restrictive conditions, respectively (e.g., stage of maturity, other physiological conditions, physical conditions).

Background

Fruit flies (Diptera: Tephritidae) are important quarantine pests that often prompt the application of phytosanitary measures for movement of host commodities in commerce. The host status of commodities for a particular fruit fly species is an important element of Pest Risk Analysis (*Pest risk analysis for quarantine pests including analysis of environmental risks and living modified organisms*. ISPM No. 11) for assessing the likelihood of pest introduction and spread, as well as determining appropriate risk management options (Appendix 1). The NPPO of the exporting country is required to document the fruit fly distribution and host status of the fruit or vegetable (*Pest reporting*. ISPM No. 17).

Pest Risk Analysis is supported by pest and interception records, the scientific literature, and other relevant evidence. Recent records and literature on host status may be very complete, accurate, and present clear evidence so that host status determination trials are not required. However, the reliability of historical records and literature is frequently in question (*Determination of pest status in an area.* ISPM No. 8) because:

- Fruit fly species may be incorrectly identified and voucher specimens are unavailable for verification.
- Collection records may be incomplete, incorrect, or of dubious value.
- Fruit or vegetable details may not be available, e.g., variety and stage of maturity, collection of the fruit or vegetable from the ground or tree, physical condition at the time of collection, status of the orchard, and prevailing weather conditions.

Protocols and comprehensive trials to determine fruit fly host status have been documented (Aluja *et al*. 2003, Aluja *et a*l. 2004, Enkerlin *et al*. 1993, Willard *et al*. 1929, APPPC 2005; Cowley *et al*. 1992; NASS 1991). However, inconsistencies in terminology and methodologies contribute to variation in interpretation of fruit fly risk and in application of phytosanitary measures (Follett & Hennessey 2007, Aluja & Mangan 2008). Harmonization of terminology and protocols for determination of fruit fly host status will promote consistency among NAPPO member countries and supporting scientific communities. When clear evidence of host status is not available, then required host status trials, detailed experimental design, and the acceptable level of effectiveness and statistical confidence for trials may be detailed in a bilateral agreement.

If a fruit or vegetable is a natural non-host then the importing NPPO may allow import. If the fruit or vegetable is a natural host, then further studies may support designation as a conditional host. Conditional host status may allow mitigations designed to minimize the conditions leading to infestation.

Requirements

The objective of host status trials is to demonstrate host status of a specified fruit or vegetable based on statistically valid data. Trials may include laboratory, field cage, glasshouse (including greenhouse and screenhouse), and natural field infestation components. Field and laboratory experiments should be representative of variability in the fruit or vegetable and fruit fly populations over the entire growing area, harvest and export periods. Experiments should be replicated, statistically analyzed, and the levels of confidence reported based on sample size so that data is verifiable and replicable.

The following items are important in planning host status determination trials:

- Identify the fruit or vegetable (e.g., species, all varieties included, plant parts included). The fruit or vegetable to be used in trials should be the same as that proposed for export.
- Specify the defined condition(s) of the fruit or vegetable to be evaluated as a resistance factor(s).
- Collect and review information, literature, and records regarding host status of the fruit or vegetable and fruit fly species.
- Identify the fruit fly species of concern.
- Describe the origin and rearing status of the fruit fly colony to be used in the trials.

- Describe the known hosts to be used as controls in the trials.
- Conduct separate trials for each fruit fly species for which determination of host status is required.
- Conduct separate trials for each variety of the fruit or vegetable, if varietal differences are the purported source of resistance to fruit fly infestation. Separate trials are not required if the resistance factor has been experimentally demonstrated at a level effective to prevent infestation in all varieties of concern.

1. Experimental Design

The purpose of these experiments is to determine the host status of the fruit or vegetable under specific defined conditions.

1.1 Samples

- Sampling protocols should be based on principles of independence and randomness and be appropriate for the statistics to be computed.
- Trials should be appropriate to evaluate the specified defined condition(s) of the fruit or vegetable as a resistance factor(s) for fruit fly infestation.
- Number of seasons and number of replications per season to account for variability of flies and fruit or vegetable over time. This should account for early and late harvest conditions. At least two years may be needed to meet this requirement.
- Number of replications per trial to account for variability in flies and fruit or vegetable over the production area. This should be representative of the range of actual production and growing conditions, for example, crop grown at high and low elevation. Adjustments may be made based on the biology of the fruit fly or characteristics of the fruit or vegetable.
- Desired level of effectiveness may be the same as the maximum pest limit of less than one reproductive pair per consignment (Mangan et al. 1997). It may be different if other phytosanitary measures are applied or if the likelihood of establishment of the species in the importing country is low based on climate, host availability, or other factors.
- Desired level of confidence should be based on sample size. For stand-alone measures, a level of 95% has been generally used (Follett & Hennessey 2007).
- Number and weight of the fruit or vegetable required per trial to determine effectiveness and confidence level.
- Number of eggs oviposited, resulting immatures, or adults to be required from controls versus treatments to determine effectiveness and confidence level. Infestation level is measured by determining the proportion of the fruit or vegetable that is infested and the number of eggs, larvae, pupae or adults emerging per individual fruit or vegetable. Notes on oviposition behavior of the females on the fruit or vegetable should be recorded to determine if non-preference is occurring.
- Control fruit or vegetable to be used for laboratory and field cage and glasshouse trial.

1.2 Fruit Flies

- When possible colony should originate from the same area as the fruit or vegetable.
- Colony should be no older than three generations at the initiation of the trials, without re-stocking, and maintained on natural hosts to ensure normal oviposition behavior.
- Records on the origin and rearing of the colony should be maintained.
- Identified voucher specimens should be kept.
- The pre-oviposition and oviposition periods should be determined so that sexually-mature, mated females are exposed to the fruit or vegetable at the peak of their reproductive potential.
- The optimum number of females required to infest the fruit or vegetable should be determined. The exact number per replicate should be justified according to fly biology, amount of the fruit or vegetable to be exposed, and other experimental conditions.
- Determine the duration of exposure of females to fruit or vegetable in trials. Exposure period should be determined by degradation of fruit or vegetable quality during the trial and oviposition behavior. Exposure time can be determined by observations on the controls. If females are ovipositing in controls but not in trial fruit or vegetable, then either non-preference is occurring or the females need more time to accept the trial fruit or vegetable. This acceptance and oviposition period should be determined by observation. As the exposure period is lengthened, the harvested fruit or vegetable will begin to degrade, ripen and change physiologically. These changes impact the host status and add uncertainty to the results. The number of eggs oviposited into the fruit or vegetable may be checked by dissection and visual counts of a sample after completion of the period of exposure.
- Trials should be conducted under optimum environmental conditions for fruit fly activity.
  - Cages should be of an appropriate size and construction for trials.
  - Adults should be provided with food and water *ad libitum*.
  - The minimum and maximum temperatures, relative humidity, and photoperiod should be recorded during the period of the trial. Males may be kept in cages or greenhouse with the females, if it is beneficial for encouraging oviposition.
- The number of dead adults occurring during the trial should be recorded and, if it is a small scale trial, dead flies should be replaced with live adults. High adult mortality may indicate that unfavorable conditions (e.g., excessive temperature) or contamination of trial fruit or vegetable (e.g., insecticides) has occurred. In such a case, the trial should be repeated. It should be noted if an individual female is used in more than one trial.

1.3 Fruits and Vegetables

Trials should be appropriate to evaluate the specified defined condition(s) of the fruit or vegetable as a resistance factor(s) for fruit fly infestation.

The fruit or vegetable used in the host status trials should be:

- The same variety and from the same location as that to be exported, and be verified as such (e.g., photographic documentation and identification by a botanist).

- Free from contaminants, pesticides, wax, dirt, defects, fruit flies and other pests (also applies to controls)
  - o If trial fruit or vegetable or host controls are sprayed just before or during trials, then data from those trials must not be considered.
- Commercial export grade of a defined color, size, and physiological condition from which the resistance factor should be evaluated.
  - o Appropriate defined stage of maturity
  - o Artificially-damaged fruit or vegetable should be punctured uniformly a predetermined number of times to a predetermined depth, as described in the experimental design.

1.4 Control Hosts

Control hosts are required for all laboratory, cage or glasshouse trials. The control host should be a fruit or vegetable which is a known host and free of prior infestation. Control hosts can be exposed to females as a single layer in choice or no-choice situations in laboratory, field cage, and glasshouse trials. In natural infestation sampling, control hosts may be placed in the field alongside the trial fruit or vegetable at a rate appropriate to determine period and rate of natural oviposition. Fruit flies used in a control and experimental replication should all come from the same group, colony, or population and be all of the appropriate age and condition to encourage oviposition.

Controls are used to:

- Verify that females are sexually-mature, mated, and exhibiting normal oviposition behavior.
- Indicate the high level of infestation that may occur in a host.
- Indicate the normal timeframe for development to the adult.
- Confirm that environmental conditions were appropriate for infestation and rearing.
- In the case of natural infestation samples, confirm that wild females were ovipositing in the area where the fruit or vegetable is grown during the trial period.
- In the case of monophagous flies, controls should be a known host and host status trials should be done at the appropriate stage of maturity.

1.5 Data Analysis

- Calculate levels of infestation, effectiveness of the resistance factor(s), and levels of confidence which will support a host status determination and designation.
- The sample size used to determine the level of effectiveness and confidence should be determined by the number of fruit flies (eggs, larvae or adults) exposed to the fruit or vegetable, or the amount of fruit or vegetable exposed to fruit flies, depending on the type of trial. In laboratory and field cage host status experiments, sexually-mature mated females are typically exposed to the fruit or vegetable. The number of females and the number of eggs they lay in the fruit or vegetable can be determined. To determine the natural infestation rate, fruits or vegetables are collected from the field and dissected to count eggs and larvae, or held for adult fruit fly emergence. The numbers of adult fruit flies present in the orchard and the numbers of fruits or vegetables visited by female flies during a defined period or phenological fruit stage is unknown. Therefore, the sampled

number of the fruit or vegetable is used to determine the level of confidence (Follett & Hennessey 2007).

- The effectiveness of the resistance factor in the fruit or vegetable and its confidence level should be calculated from the level of infestation, which is the number of third instar larvae, pupae and fertile adults which develop relative to the control (Appendix 2).
- Corrections for mortality control of the treatments should be calculated according to Abbott (1925).

2. Trials

Natural infestation, field cage, glasshouse, and laboratory cage trials to determine host status are described. Trials may be conducted in sequence. However it may be more practical to conduct trials simultaneously while the fruit or vegetable is available. Trials should be appropriate to evaluate the specified defined condition(s) of the fruit or vegetable as a resistance factor(s) for fruit fly infestation (Aluja *et al.* 2003).

2.1 Natural Infestation Determined from Field Collected Fruit or Vegetable

Host status can be determined and designated based on confirmation of natural infestation during the export harvest period without any other trials (Santiago *et al.* 1993). This trial is mandatory regardless of data from field cage, glasshouse, or laboratory cage trials. However, if status as a natural host is confirmed from natural infestation trials, then field cage, glasshouse, and laboratory trials may not be necessary.

Natural infestation trials should include, but are not limited to, the following:

- Surveillance in fruit or vegetable growing areas to verify the presence of the target fruit fly species in the area during the trial and export harvest periods. The trap density and minimum acceptable level of adult activity in the trial orchards or fields may be species specific and should be delineated in a bilateral work plan (IAEA 2003; *Establishment of pest free areas for fruit flies (Tephritidae).* ISPM No. 26).
- Data from multiple harvest seasons may be required to account for annual variability in fruit fly populations and production quality (Robertson et al. 1995).
- Natural infestation samples should be representative of the range of production areas and environmental conditions, maturity stages and natural damage levels. Data from natural infestation samples should be analyzed individually to determine the significance of experimental variables. Natural samples may include, but are not limited to:
    - Fruits or vegetables for export from packinghouses immediately after harvest.
    - Fruits or vegetables harvested directly from orchards by commercial pickers.
    - Fruits or vegetables that have been through export processing (e.g., culling, washing, cooling).
    - Fruits or vegetables from packinghouse culls, damaged, or overripe pieces from the field.
    - Fruits or vegetables with the specified defined condition(s) to be evaluated as a resistance factor(s) for fruit fly infestation.

- A known host of the fruit fly species should be exposed in the harvest area to confirm fruit fly presence and oviposition during the trial and export harvest periods. Control hosts can be collected from naturally-occurring plants in the same area during the trial period.
- A record of processing and other condition of samples and control hosts should be maintained.

Advantages of natural infestation trials include:

- Provides most accurate assessment of host status of all trials.
- Accounts for high level of variability in the fruit or vegetable, fly behavior, and periods of activity.
- No interference with host preference and non-preference.

Disadvantages of natural infestation trials include:

- Variability in flies is not completely known or controlled.
- Variability in the fruit or vegetable is not completely known or controlled.
- Data may be insufficient if the confidence level of the sample is low.

2.2 Field Cage and Glasshouse Trials

Field cage or glasshouse trials should be conducted when data from natural infestation trials do not establish host status of the fruit or vegetable. Data from field cage and glasshouse trials conducted under defined conditions may be used to support results obtained from natural infestation and laboratory cage trials.

Field cages can be mesh cages that enclose whole plants or parts of plants including the fruit or vegetable and into which the flies are released. Alternatively, plants may also be exposed in glasshouses into which flies are released. The fruit or vegetable can be grown in the enclosure or be introduced as potted plants for the trials. The results of the trials are interpreted in the same manner as for laboratory cage trials.

Field cage and glasshouse trials should include, but are not limited to, the following:

- Monitor minimum and maximum temperatures, relative humidity, and other relevant environmental conditions daily for the duration of the trial.
- Food and water should be provided in each cage for the females.
- Consideration should be given to the size of the cage or glasshouse to ensure containment of the adults, allow adequate airflow, and the designated oviposition pressure.
- The cage should prevent entry of ants and predators. Predators should be removed from cages before initiating the trial.
- A control replicate using a known host should be run concurrently alongside the trial of the fruit or vegetable. Control hosts should be exposed to same the oviposition pressure as the trial.
- Known control hosts do not need to be attached to plants.
- Fruits or vegetables should have the specified defined condition(s) to be evaluated as a resistance factor(s) for fruit fly infestation.

- The fruit or vegetable remains attached to plants and may be exposed to the fruit flies either by caging the fruit or vegetable in the field or by using potted plants in a glasshouse. Mesh bags may be used as cages in the field.
- The plants should be grown under conditions that exclude the use of chemicals that may be deleterious to fruit flies.
- A replicate may be composed of multiple cages preferably on one plant but if not possible, on adjacent plants. If the replicate is divided into multiple cages, the number of females per cage should be evenly distributed between cages to maintain the designated oviposition pressure. Fly mortality should be monitored and it may be necessary to replace dead flies with live flies to ensure adequate infestation pressure.
- For glasshouse trials, the fruit or vegetable should be grown under commercial conditions or in containers of a size that allows normal plant and fruit or vegetable development.
- After the designated exposure period for oviposition, the fruit or vegetable should be removed from the plant and each replicate weighed and the number recorded. The number of dead flies, escaped flies, and predators per cage should also be recorded.

Advantages of field cage trials include:

- Oviposition level is high
- The fruit or vegetable remains attached to the plant and does not degrade during the trial
- Environmental conditions are closer to nature than in a laboratory cage trial

Disadvantages of field cage trials include:

- Host preference behavior of females is more limited than in natural infestation trials

2.3 Laboratory Cage Trials

Laboratory trials should be conducted when data from natural infestation and field cage trials do not establish that the fruit or vegetable is a natural host. Data from laboratory cage trials conducted under defined conditions may be used to support results obtained from natural infestation, field cage, and glasshouse trials. Defined fruit or vegetable condition(s) will be specified in the case of conditional host evaluation.

Laboratory cage trials should include, but are not limited to, the following:

- The fruit or vegetable should be exposed as soon after harvest as possible to avoid any changes (e.g., ripening) that may alter host status.
- The fruit or vegetable should have the specified defined condition(s) to be evaluated as a resistance factor(s) for fruit fly infestation, including, but not limited to:
  - artificial damage
  - days postharvest
  - stage of maturity, size, color, grade
  - other important physiological conditions (e.g., acidity, turgor pressure)
  - other physical conditions
- Sexually-mature, mated females for oviposition.
- Environmentally controlled facilities for trials and fruit or vegetable holding.
- Cages to hold fruit flies and fruit or vegetable during trials.
- Food and water to maintain fruit flies during trials.

Advantages of laboratory trials include:
- Conditions are highly controlled.
- Survival of flies is high.
- Oviposition level is high.

Disadvantages of laboratory trials include:
- Host preference behavior of flies is more limited than for the natural infestation, field cage or glasshouse trials.
- The fruit or vegetable degrades rapidly.

3. Fruit or Vegetable Handling for Insect Emergence

Fruits or vegetables collected for natural infestation, field cage, glasshouse, and laboratory trials as well as control fruit or vegetable, must be held until fruit fly larvae emerge. Fruit or vegetable holding conditions should maximize fruit fly survival and be specified in the experimental design.

Fruit or vegetable holding criteria that should be considered include, but are not limited to:
- Temperature
- Relative humidity
- Photoperiod
- Suitability of pupation medium
    - pesticide-free
    - sterile
    - well-drained to prevent larval or pupal mortality from excess moisture
    - of no nutritive value.
- Restricted access by insects which can interfere with any of the fruit fly stages such as ants, cockroaches, and *Drosophila* spp.
- Facilitate accurate recording of the number of larvae, pupae and adults emerging from each piece of fruit or vegetable for each replicate.
- Appropriate number of pieces of fruit or vegetable in each aggregate.

Data to be recorded includes, but is not limited to:
- Daily environmental conditions during the period of fruit or vegetable holding
- Number and emergence dates of larvae exiting the fruit or vegetable and control host.
    - The medium may also be sieved at intervals before all larvae have emerged and at the end of the holding period (which may vary with temperature and host).
    - The normal period of development should be determined from the controls and colony. At the end of the holding period, the fruit or vegetable should be dissected (but not discarded) to determine the presence of live and dead larvae or pupae remaining inside and if larvae have had enough time to emerge. A conditional host may require an extended period for larvae to emerge, relative to a control host, but they eventually emerge. If live larvae are present, the fruit or vegetable should be held until all mature larvae have exited or been removed.
    - Dissecting or cutting the fruit or vegetable to count larvae may be used as an alternative to holding it. Dissecting has the disadvantages that host status will then be based on larval counts, instead of on adults, and the efficiency of larvae

detection in the fruit or vegetable and control host must be determined prior to beginning the evaluation.

- Number of pupae and pupation dates
    - o The numbers of pupae should be recorded and pupae held in a pupation medium under appropriate environmental conditions, alongside control pupae, until adults emerge.
- Number and emergence dates of adults by sex
    - o All emerging adults should be counted, sexed and identified after morphological characteristics have developed. Abnormalities should be recorded.
- Ability of adults to reproduce. If this is not done, and adults appear normal, then it is assumed adults can reproduce normally and that the fruit or vegetable cannot be designated as a non-host.
- Deviation from normal larval, pupal, and adult morphology and behavior that may indicate effects of a resistance factor in the fruit or vegetable
- Numbers of larvae, pupae, and adults emerging from the fruit or vegetable should be compared to those from controls.
- Percentage adult emergence from control and trial fruit or vegetable pupae should be compared to those of a colony, if a colony is available.

4. Interpretation of Results

The following items, among others, should be considered in interpretation of data from the host status determination trials and designation of status:

- The host status conclusions that can be drawn from statistically validated laboratory cage, field cage, glasshouse, and natural infestation trials are natural host, natural non-host, and conditional host.
- The specified defined condition(s) of the fruit or vegetable (e.g., the resistance factor(s) for fruit fly infestation) evaluated and confirmed in the trials can be designated as a requirement for export.
- If no viable adults emerge from control replicates, the trial should be repeated and test conditions may require modification.
- Low emergence of larvae or adults from control hosts may indicate a problem with the experimental conditions, the need to increase the sample size of the fruit or vegetable, the number of females in the trial, or the quality of the females at the time of infestation trials.
- Observation of no adult emergence across all fruit or vegetable trials or across replicates of trials of fruit or vegetable of specified condition(s) may indicate that it is a natural non-host or conditional host.
- Emergence of an adult fruit fly from trial fruits or vegetables in any one replicate indicates that it may be a host.

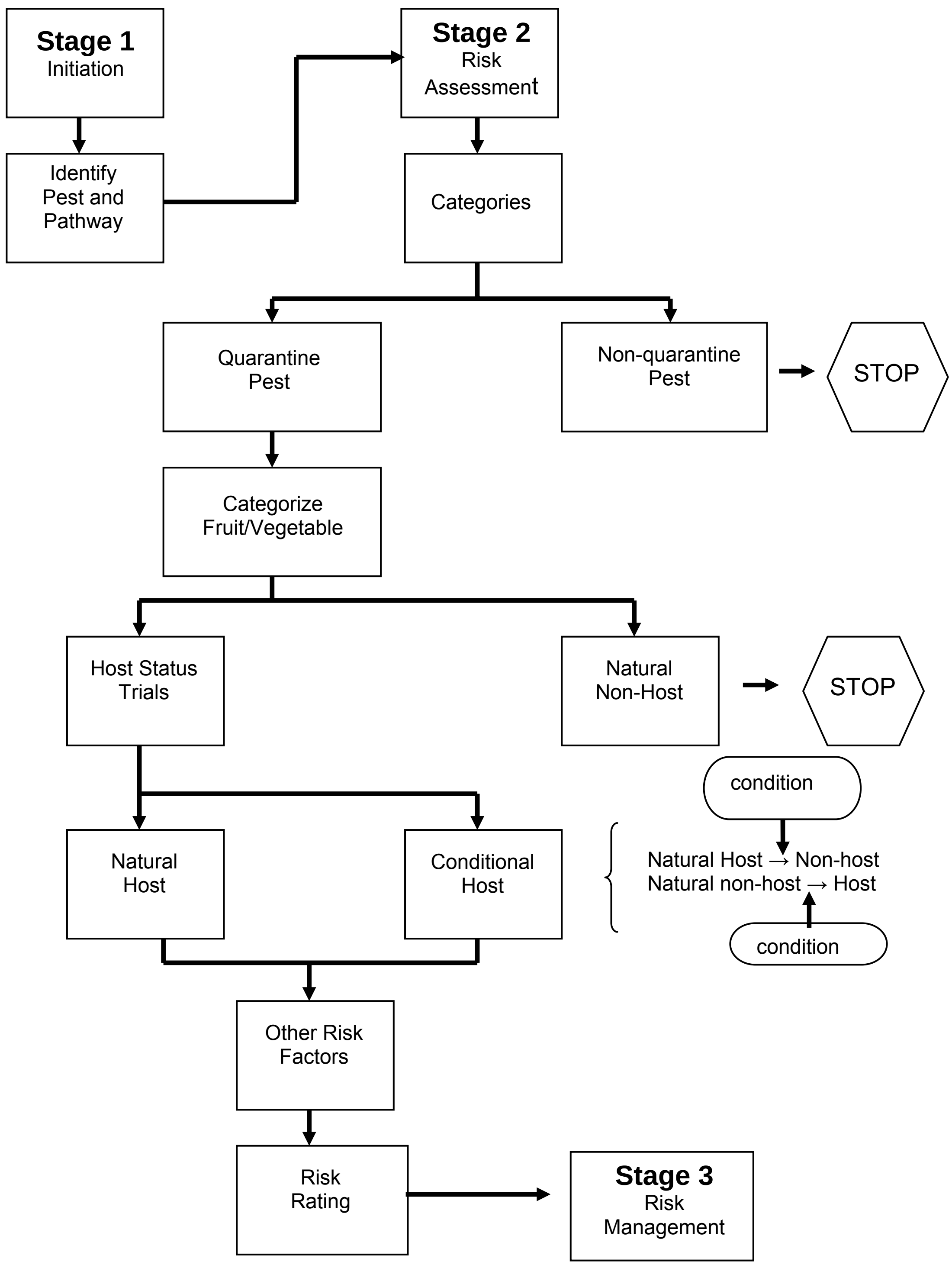
5. Recordkeeping

The NPPO should keep appropriate records of host trials. Information kept should be appropriate for the intended purpose of determination of host status. Information in the records should include, but is not limited to:

- scientific name of fruit fly
- scientific name and variety of fruit or vegetable
- location of voucher specimens
- the specified defined condition(s) of the fruit or vegetable as a resistance factor(s) for fruit fly infestation
- trials conducted, defined conditions, experimental design, dates, locations, data, statistical calculations, and results
- references
- additional information, including photographs, which may be specific to the fruit fly, the fruit or vegetable, or host status

For each fruit fly species and fruit or vegetable, the exporter should provide the importer with reports on results of host-status trials in accordance with this standard.

Appendix 1: Relative position of host status determination in the Pest Risk Analysis process.



Appendix 2: Optional statistical analyses in the determination of host status and the effectiveness of the specified defined conditions in host designation

The effectiveness of the resistance factor in the fruit or vegetable and its confidence level should be calculated from the level of infestation, which is the number of third instar larvae, pupae and flies developing relative to the control (Couey & Chew 1986). In developing fruit or vegetable quarantine treatments, such as hot water treatments, the level of confidence associated with treating a number of insects with zero survivors is given by the equation,

$$C = 1 - (1 - p_u)^n \qquad (1)$$

where p_u is the maximum allowable infestation proportion (e.g. 0.0001 for 99.99% mortality) and n is the number of trial insects (Couey & Chew 1986). Equation 1 can be rearranged to determine the number of insects that are required for trials for a given level of confidence.

$$n = [\log(1\text{-}0.95)/\log(1\text{-} p_u)] \qquad (2)$$

Equation 2 calculates how many insects or how much fruit or vegetable (n) there must be in trials with no survivors so that we will have 95% confidence (C, as a proportion) that the survival proportion is below a predetermined level (p_u) (Couey & Chew 1986).

Couey and Chew (1986) provide an equation to estimate the confidence levels for effectiveness when only a few insects survive on a host,

$$\sum_{X=0}^{X=S} e^{-m} m^{x}/x! = 1 - C \qquad (3)$$

where m is $n \times p_u$, n is the number of insects or fruit or vegetable sampled, s is the number of survivors, and C is the confidence level. This equation uses the Poisson distribution law and assumes large n and small p_u (Couey & Chew 1986). It is expected that most fruits and vegetables in host determination trials will have 0 or 1 survivors and a Poisson distribution, which these models assume, may be the most appropriate (Baker et al. 1990). Under different conditions, e.g., high and uniform infestation, a binomial distribution may be more appropriate (Patil 1960). Follett and Hennessey (2007) outline, with examples from the literature, how to determine confidence levels based on the sample size used during host status trials so that its equivalency to traditional quarantine treatments can be demonstrated.