

**REGIONAL STANDARDS
FOR PHYTOSANITARY MEASURES**

***GUIDELINES FOR THE CONFIRMATION OF
NON-HOST STATUS OF FRUIT AND
VEGETABLES TO TEPHRITID FRUIT FLIES***

APPPC RSPM No. 4



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NON-HOST STATUS OF FRUIT AND
VEGETABLES TO TEPHRITID FRUIT FLIES***

The Asia and Pacific Plant Protection Commission (APPPC)
FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
REGIONAL OFFICE FOR ASIA AND THE PACIFIC
Bangkok 2005

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Endorsement

Regional standards for phytosanitary measures are developed and adopted by the Asia and Pacific Plant Protection Commission as part of the plant protection programme of the Commission's contracting parties. This programme makes available to contracting and other interested parties regional standards for phytosanitary measures to support regional harmonization, with the aim to facilitate trade and avoid the use of unjustifiable measures as barriers to trade.

This standard was endorsed by the twenty-fourth session of the Asia and Pacific Plant Protection Commission in September 2005.



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FAO Regional Representative for
Asia and the Pacific

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Endorsement

This Asia and Pacific Plant Protection Commission (APPPC) Regional Standard for Phytosanitary Measures was endorsed by the twenty-fourth session of the APPPC held from 5 to 9 September 2005 in Bangkok, Thailand.

Review

This standard is a developmental method standard. As such, although it provides the most up-to-date guidelines available as adopted by the 24th Session of the Commission, it will be subject to review at the next meeting of the Commission.

Distribution

APPPC Regional Standards for Phytosanitary Measures are distributed by the Executive Secretariat of the APPPC to all APPPC members, the Administrative Heads of Regional Plant Protection Organizations and the FAO International Plant Protection Convention (IPPC) Secretariat. This standard is available on the APPPC webpage found within the International Phytosanitary Portal: <http://www.ippc.int/En/rppo/jsp>

INTRODUCTION

SCOPE

This standard describes tests for determining the host status of a fruit or vegetable variety at a defined stage of maturity to a particular tephritid fruit fly species. A fruit or vegetable may be classified as a non-host, conditional non-host or potential host on the basis of these tests.

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Fay, H.A.C. 1989. *Multi-host Species of Fruit Fly*, pp. 129-140. *In: Robinson, A.S.; Hooper, G. Fruit Flies – Their Biology, Natural Enemies and Control. Volume 3B, World Crop Pests.* Elsevier Science Publishers, Netherlands.

Glossary of phytosanitary terms, 2002. ISPM No. 5, FAO, Rome.

NZ Ministry of Agriculture and Forestry (MAF) Standard.

DEFINITIONS AND ABBREVIATIONS

commodity	A type of plant, plant product , or other article being moved for trade or other purpose [FAO, 1990; revised ICPM, 2001]
conditional non-host (of a fruit fly species)*	Fruit and vegetables at a specified maturity and specified physical condition that cannot support the development of viable adults of a fruit fly species
eclosion*	The process of larva hatching from an egg or emergence of an adult insect from a pupa
fecundity*	The average number of eggs laid per insect over a specific time period

fruit and vegetables	A commodity class for fresh parts of plants intended for consumption or processing and not for planting [FAO, 1990; revised ICPM, 2001]
fruit fly*	Insect of the order: Diptera: family Tephritidae.
fruit fly host ¹ *	Any fruit or vegetable in which under field conditions fruit flies oviposit, the eggs hatch into larvae, and the larvae acquire sufficient sustenance to form pupae from which viable adults emerge.
gravid female*	Female fruit flies with fertilised eggs
incursion	An isolated population of a pest recently detected in an area, not known to be established, but expected to survive for the immediate future. [ICPM, 2003]
non host (of a fruit fly species)*	Fruit or vegetables that will not support the complete development of a fruit fly species regardless of the stage of maturity and physical characteristics
National Plant Protection Organization	Official service established by a government to discharge the functions specified by the IPPC [FAO, 1990; formerly Plant Protection Organization (National)]
NPPO	National Plant Protection Organization [FAO, 1990; ICPM, 2001]
Outbreak	A recently detected pest population, including an incursion, or a sudden significant increase of an established pest population in an area. [FAO, 1995, revised ICPM, 2003]

¹ Based on Armstrong, J.W. 1986. Pest organism response to potential quarantine treatments. Proceedings 1985 ASEAN PLANTI Regional Conference on Quarantine Support for Agricultural Development 1:25-30. ASEAN Plant Quarantine and Training Institute, Serdang, Selangor, Malaysia.

oviposition*	The act of laying or depositing eggs within a fruit
teneral adults*	Condition of the adult shortly after eclosion when its cuticle is not fully sclerotized or fully mature in colour

* Indicates terms which are not include in ISPM No. 5 Glossary of Phytosanitary terms.

OUTLINE OF REQUIREMENTS

Non-host or conditional non-host status at a particular stage of harvest maturity can be used as a phytosanitary measure to ensure freedom from fruit fly infestation. To facilitate the determination of this status, this standard describes the general and specific requirements for testing the response of a fruit or vegetable variety at a defined stage of maturity to a particular tephritid fruit fly species.

A series of laboratory and field trials, using a specific fruit damaging technique, are used to determine host status, the results of which are interpreted in the following way:

- ◆ if punctured fruit (used in the botanical sense) show no sign of fruit fly infestation then the host is described as a non-host
- ◆ if unpunctured fruit, from either laboratory or field trials, are not infested by a fruit fly species but damaged fruit is, the host is described as a conditional non-host
- ◆ if both punctured and unpunctured fruit become infested, the commodity is described as a potential host.

Specific requirements should be followed for each stage of the testing – using punctured fruit in laboratory tests, unpunctured fruit in laboratory tests and unpunctured fruit in field or glasshouse tests. These requirements concern the testing of the fecundity of the fruit flies, the development of fruit fly populations, the selection of fruit used for the tests, the holding of the fruit after exposure to fruit flies and the assessment of the tests.

GENERAL REQUIREMENTS

1. Background

Non-host or conditional non-host status at a particular stage of harvest maturity can be used as a phytosanitary measure to ensure freedom from fruit fly infestation. However, published records of hosts for particular fruit fly species may not always be reliable for determining non-host status for phytosanitary purposes. It is frequently difficult or impossible to validate old records. Fruit fly species may be correctly identified, but in many cases host details such as the fruit or vegetable variety, the stage of maturity, and the skin condition (damaged or undamaged) at collection were not recorded. Thus, published host records may be misleading, incomplete or incorrect for negotiating market access protocols and the development of a standard method for determining host status under defined, reproducible conditions was highly desirable. Such a method has important ramifications for international trade in many fresh fruit and vegetable commodities.

This standard uses well-known techniques in the form of a standard to provide a regular methodology for solving the problem of inaccurate host status records or the absence of host status information. These guidelines are “new” in this format and trading partners will need consultation before using them. It is likely further information will be available in the near future, so the standard will be reviewed at the next meeting of the Commission.

Where APPPC members use these guidelines, they are encouraged to inform the APPPC Executive Secretary particularly when improvements or additions are made to the techniques.

2. Methodology

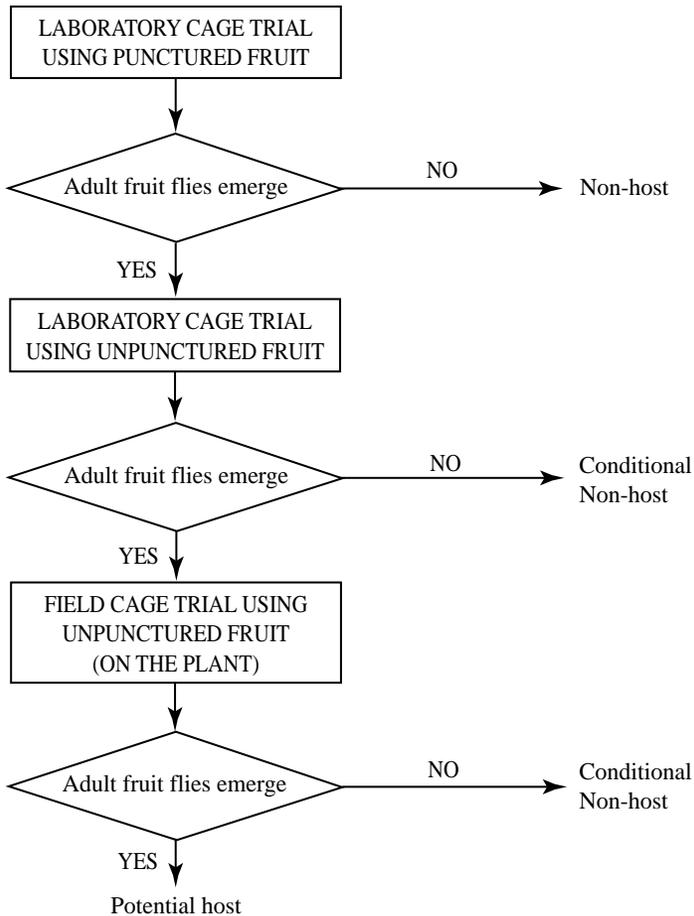
2.1 Basic guidance

Basic guidance for host status testing includes the following:

- ◆ in determining the host status of a fruit variety at a particular (described) stage of maturity, the methods outlined in this document should be adhered to;

- ◆ the host status of each variety of fruit (at the described stage of maturity) should be determined separately;
- ◆ each fruit fly species for which determination of host status studies are required should be tested separately;
- ◆ the three stages noted in Figure 1 can be conducted sequentially or concurrently.

Figure 1: Diagram of the host testing stages



2.2 Pre-requisites for host status testing

The following points should be considered as prerequisites to the commencement of host status trials:

- ◆ a list of all fruit fly species occurring in the exporting country (list i)
- ◆ a list of fruit fly species for which the exporting country considers host-status testing to be necessary (list ii)
- ◆ information supporting the non-host status of the fruit variety concerned to some of the fruit flies found in the exporting country. Survey data should show:
 - that the fruit variety is not a recorded host of those fruit fly species excluded from list (ii)
 - that the fruit fly species excluded from list (ii) is highly host specific on one host species (ie is recorded from only one host species).

For each fruit fly species listed as requiring host-status testing, the exporting contracting party should provide the importing contracting party with reports giving the results of host-status testing in accordance with this standard.

Consultation with trading partners prior to and during trials will increase the understanding of NPPOs and their confidence in the trial results.

2.3 Overview of methodology

The standard describes tests for determining the host status of a fruit or vegetable variety at a defined stage of maturity to a particular fruit fly species (Diptera: Tephritidae). A fruit variety may be classified as a non-host, conditional non-host or a potential host on the basis of these tests.

There is a series of tests involving laboratory cages trials and field trials. Laboratory cage trials using punctured and unpunctured fruit provide a robust test and are mandatory. This system can be

supplemented by field trials using punctured fruit if required. The results of trials are interpreted in the following manner:

- ◆ if no survival is recorded in a damaged fruit, regardless of maturity, then the fruit is described as a non-host to the fruit fly species tested
- ◆ if no survival is recorded in a damaged fruit at a specific maturity, then the fruit is described as a conditional non-host to the fruit fly species tested
- ◆ if no survival is recorded in undamaged fruit at a particular stage of maturity then the fruit is described as a conditional non-host
- ◆ if either damaged or undamaged fruit become infested, then the fruit is described as a potential host.

The term potential host is used because the trials are forced, no-choice tests using laboratory reared flies and these may over represent the population pressure actually found in the field situation.

Physical damage to fruit (i.e. breaks in the skin surface) may provide fruit flies the opportunity to oviposition where this opportunity is precluded by undamaged skin. Therefore, when determining host-status of a fruit, consideration should be given to both physically damaged and undamaged states of the fruit.

For the purpose of these trials physical damage to fruit is achieved by puncturing fruit with entomological pins. The terms punctured and unpunctured fruit are used to describe damaged and undamaged fruit in this standard, as these terms reflect the actual methods used to damage fruit in the trials.

The suggested sequence of tests is as follows:

The first test is of punctured fruit in a laboratory cage to determine if a commodity can be a host to a fruit fly species if it is punctured. The results of trials are interpreted in the following manner:

- ◆ if the fruits do not become infested, then the commodity is regarded as a non-host to that fruit fly species

- ◆ if the commodity does become infested, even if only one adult of that fruit fly species develops, then the commodity is either a host or conditional non-host to that fruit fly species.

The second test is a laboratory cage test using unpunctured fruit to determine if fruit may be a conditional host. The results of trials are interpreted in the following manner:

- ◆ if the unpunctured fruit does not become infested, then the commodity is regarded as a conditional non-host
- ◆ if the unpunctured fruit does become infested, even if only one adult fruit fly develops, then the commodity is regarded as a host unless the third test (as noted below) shows it to be a conditional non-host.

The third test is a field cage trial using unpunctured fruit to determine if a fruit found to be a host under laboratory conditions (as in the second test) may be a conditional non-host under field conditions. The laboratory cage trial are recognized as stringent tests that may not duplicate what happens in the field. The results may indicate:

- ◆ if the unpunctured fruit under field conditions does not become infested, then the commodity is regarded as a conditional non-host
- ◆ if the unpunctured fruit under field conditions does become infested, even if only one adult fruit fly develops, then the commodity is regarded as a host.

The number of flies that should be used in host status trials is selected to try to truly reflect field populations. This has been the subject of debate for many years. The only country that has established a standard for host testing in New Zealand. Their standard states that the number of gravid females to be used per cage should be adequate to ensure 250-500 viable eggs are laid per 500 gm of fruit. To assess the potential oviposition load that trial fruit may be exposed to, fecundity tests on colony flies are undertaken. The average fecundity per female is then used to calculate the required number of females per cage.

In this standard a minimum potential oviposition load of 1 000 viable eggs per replicate was chosen for laboratory trials. However, for field/glasshouse trials a minimum potential oviposition load of 1 500 viable eggs per replicate was chosen. The higher rate of 1 500 eggs per replicate is to compensate for higher adult mortality that may be experienced when laboratory reared flies are released in the field. Additionally, the exposure period for field/glasshouse trials is 48 hours compared to 24 hours for laboratory trials to allow laboratory reared flies to acclimatise to field conditions.

SPECIFIC REQUIREMENTS

3. Laboratory cage trial using punctured fruit

The following basic components are required to conduct a laboratory cage trial:

- ◆ adult fruit flies for oviposition
- ◆ fruit of the defined variety and harvest maturity to be tested and
- ◆ conditions/facilities for fruit holding.

3.1. Adult fruit flies

Adult fruit flies should be obtained from laboratory colonies. The laboratory colonies of multivoltine species used should be no more than one year old or, if older than one year, they should have been replenished with wild flies at least once every 12 months. Records of colony performance and replenishment will be required in addition to host status results.

3.2 Fecundity test

Prior to conducting host status trials, a fecundity test should be conducted on gravid females from the laboratory colonies. This allows the estimation of the potential oviposition load to which the replicates of the test fruit may be exposed.

At least five replicates, each of 10 gravid females per cage, should be used for the fecundity tests. Cages should have fine mesh of

minimum dimensions of 300 mm x 300 mm x 300 mm. Measures should be taken to prevent access by ants and *Drosophila* spp. Each cage should contain a source of sugar and water.

Oviposition receptacles can be either a hollowed, punctured dome of a known host or an artificial eggging device. If a dome is used, its edges should be sealed to prevent flies from getting under the dome. Oviposition receptacles should be exposed to gravid females for a period of 24 hours.

After 24 hours exposure, the eggs should be washed from the dome or the artificial eggging device. Those embedded in the dome should be carefully eased out of the fruit tissue and washed from the dome. The eggs should then be placed on moist filter paper, counted and held for a sufficient period to determine egg hatch. This allows the calculation of the mean number of viable eggs per gravid female over a 24-hour period.

The number of gravid females to be used per replicate should be adequate to ensure that each replicate is exposed to a potential oviposition load of a minimum of 1 000 viable eggs.

3.3 Fruit flies used in the trials

Each fruit fly species for which host-status studies are required should be tested separately.

The determined number of gravid females should be caged with test fruit for 24 hours. The trial will consist of 5 replicates each with the same number of gravid females per cage.

Gravid females for the laboratory cage trials should be obtained from the same cage of flies used in the fecundity test. Flies should be at their peak fecundity.

3.4 Test fruit

The host status of each fruit variety should be tested separately. A variety may be described formally in an application for

proprietary rights² or, where this is not the case, a variety should be described including distinctive commodity characteristics when present. Colour photographs of the trial commodity are required if a variety has not been formally described under proprietary rights.

Test fruit of the described variety should be grown under conditions that exclude the use of chemicals that may deleterious to fruit flies (e.g. insecticides, miticides).

Test fruit should be collected at the stage of maturity accepted for export harvest. The stage of maturity should be described by the grower/supplier.

The trials should be replicated three times with trial fruit sourced from different producers for each replicate. For each replicate five batches, each with a minimum of 500 g of fruit, should be used. Whole fruit should be used, irrespective of the weight of individual fruit. The weight and number of fruit used per replicate should be recorded just prior to exposure to the flies.

A control using a minimum of 500 g of a known primary/preferred host should be run concurrently with the 5 trial replicates. This provides evidence that the experimental procedures adopted do not prevent the successful emergence of fruit flies. The control replicate should be exposed to the same number of gravid females as determined in section 3.1.

Before exposure of a fruit to female flies, the skin of the trial fruit and control fruit should be punctured 50 times penetrating through and puncturing the pericarp of the fruit using entomological pins of size 3. The punctures should be distributed evenly across the surface of the fruit. When placed in the trial cage, fruit should be randomly orientated (e.g. stem end up, blossom end up) in a single layer. Fruit should remain in the cages for a period of 24 hours.

² International Code of Nomenclature for Cultivated Plants 1980; International Union for the Protection of New Varieties of Plants 1991.

Cages should have minimum dimensions of 300 mm x 300 mm x 300 mm and be covered with a fine mesh. Measures should be taken to prevent access by ants and *Drosophila* spp. Each cage should contain a source of sugar and water.

Trials should be conducted under optimum conditions for fruit fly activity. The minimum and maximum temperatures and relative humidity should be recorded during the period of caging.

At the end of the 24-hour period the number of dead flies per cage should be recorded. High adult mortality may indicate unfavourable conditions (e.g. excessive temperature) or contamination of trial fruit (e.g. insecticides).

3.5 Fruit holding

After exposure to gravid females for 24 hours, the fruit should be removed from the cage and held over a suitable pupation medium. Sawdust, sand or vermiculite may be used. The medium should be obtained from untreated sources and be sterilised (e.g. 120°C for a minimum of two hours).

Each replicate of fruit should be held separately so that the number of pupae and adults emerging can be recorded per weight of fruit for each replicate.

Fruit that breaks down rapidly (such as eggplant, bitter melon, cucumber, tomato, banana and most citrus) should be held above the pupation medium on a container covered by fine mesh which allows the passage of juice into the container but prevents larvae entering the container.

Each replicate should be held in individual containers that allow adequate ventilation yet prevent the access of ants and *Drosophila* spp.

The minimum and maximum temperatures and relative humidity should be recorded each day during the period of fruit holding.

After an appropriate holding period (which may vary with temperature and host) the pupation medium should be sieved to extract pupae. Fruit should be dissected (but not discarded) to determine the presence of larvae. If larvae are present, the fruit should be held until all larvae have pupated.

The numbers of pupae should be recorded and pupae held in a moistened pupation medium until eclosion. All emerging adults should be counted and identified after morphological characteristics have developed (teneral adults should not be used for identification).

3.6 Assessment and interpretation

If no adults emerge from the control replicate, the laboratory cage trial should be repeated.

If adults emerge from the control replicate and no adults emerge from the replicates of trial fruit, then the trial commodity at the described stage of maturity is regarded as a non-host to the fruit fly species tested.

If one or more adults are reared from the trial replicates, then the commodity is considered to be a potential host. A laboratory cage trial using unpunctured fruit should be undertaken.

4. Laboratory cage trial using unpunctured fruit

A laboratory cage trial using unpunctured fruit should be conducted if flies have emerged from the punctured test fruit in the laboratory cage trial described in section 3. Trial methodology and procedures are identical to that described in section 3, except that fruits are not punctured.

Each fruit fly species for which host-status studies are required should be tested separately.

4.1 Assessment and interpretation

If no adults emerge from the control replicate, the laboratory cage trial using undamaged fruit should be repeated.

If adults emerge from the control replicate and no adults emerge from any of the replicates of trial fruit, then the trial commodity at the described stage of maturity can be regarded as a conditional non-host to the fruit fly species tested.

If adults of one or more of the fruit fly species to be tested emerge from trial replicates, then field trials should be undertaken.

5. Field cage/glasshouse trials using unpunctured fruit

A field cage or glasshouse trial using unpunctured fruit should be conducted if flies have emerged from the undamaged test fruit in the laboratory trial described in section 4.

Trial methodology and procedures are basically similar to those described in section 3, except that fruits are not punctured and remain attached to the test host plant. The fruiting host plants may be exposed to the test fruit fly species either by caging fruit in the field or by using potted fruiting host plants in a glasshouse.

Each fruit fly species for which host-status studies are required should be tested separately.

5.1 Adult fruit flies

Adult fruit flies should be prepared as in 3.1.

5.2 Fecundity test

Prior to conducting host status trials a fecundity test should be conducted on gravid females from the laboratory colonies. Test should be made as per section 3.2 except that the exposure period is 48 hours.

The number of gravid females to be used per replicate should be adequate to ensure that replicates are exposed to a potential oviposition pressure of at least 1 500 viable eggs.

5.3 Field cage trial

The trials should be replicated three times. For each replicate five batches of approximately 500 g of undamaged fruit attached to the parent plant should be used. The plants should be grown under conditions that exclude the use of chemicals that may be deleterious to fruit flies.

A cage should be placed around the selected fruit be it a single fruit, group of fruits or a whole plant. A replicate of a minimum of 500 g of fruit may comprise more than one cage preferably on one plant but if not possible, on adjacent plants. Should the replicate be divided into multiple cages, the number of gravid females per cage should be evenly distributed between cages to maintain the potential oviposition pressure (1 500 viable eggs) as specified in 5.2.

A suitable cage shall consist of a supporting frame enclosed by a fine gauze cage with minimum dimensions of 300 mm x 300 mm x 300 mm. The mesh should be of a size to ensure containment of the flies and allow airflow.

Where the cage is in place on a tree/plant branch, the cage end(s) should be securely fastened around the branch or stem to prevent escape of the flies and the entry of ants and predators. A source of sugar and water should be provided in each cage for the gravid females.

The minimum and maximum temperatures and relative humidity should be recorded each day for the duration of the trial.

Gravid females for the trial should be obtained from the same cage of flies used for the fecundity test in section 5.2.

A control using approximately 500 g of a known host should be run concurrently with the 5 trial replicates and under exactly the same field conditions. Control fruit should be punctured as per section 3.4 whilst under the same experimental conditions and exposed to the same number of gravid females as the trial fruit as determined in section 5.2.

After exposure to gravid females for 48 hours, the fruit should be removed from the plant and each replicate weighed and the number of fruit recorded. The number of dead flies per cage should also be recorded.

5.4 Glasshouse trials

For glasshouse trials, test fruit should be grown in containers (e.g. pots) of a size that allows normal plant development, including fruit production. The plants should be grown under conditions that exclude the use of chemicals that may be deleterious to fruit flies.

Cages dimensions should be slightly larger than the height and width of the trial plants.

The frame of the cage should be covered by gauze fine enough to exclude *Drosophila* spp. and other fruit infesting insects. It should be constructed to ensure that flies introduced into the cage would not escape.

Plants in containers are placed in the cage immediately before the trial commences and should be protected from ants. Fruit should be at the described stage of export harvest maturity.

Five batches of approximately 500 g of whole fruit attached to parent plants should be used for each of three replicates. Each batch should be in separate cages. Whole fruit should be used, irrespective of the weight of individual fruit. The weight and number of fruit used per replicate should be recorded subsequent to exposure to gravid females and immediately after harvest.

Depending on the weight of fruit produced per plant, it may be necessary to use multiple plants/cages to achieve the minimum of 500 g of fruit per replicate. Regardless of the number of cages and plants used to house 500 g of fruit, the number of flies/replicate should be evenly distributed amongst the cages.

Gravid females for the trial should be obtained from the same cage of flies used for the fecundity test in section 5.2.

A control replicate using approximately 500 g of a known host should be run concurrently with the 5 trial batches and under exactly the same glasshouse conditions. Control fruit should be punctured as per section 3.4 whilst under the same experimental conditions and exposed to the same number of gravid females as the trial fruit as determined in section 5.2.

After exposure to gravid females for 48 hours, the fruit should be removed from the plant and each replicate weighed and the number of fruit recorded. The number of dead flies per cage should also be recorded.

5.5 Fruit holding

Fruit should be held as described in section 3.5.

5.6 Assessment and interpretation

If no adults emerge from the control fruit, the field or glasshouse trial using undamaged fruit should be repeated.

If adults emerge from the control fruit and no adults emerge from any of the replicates of trial fruit, then the trial fruit at the described stage of export harvest maturity is regarded as a conditional non-host to the fruit fly species tested.

If adults of the fruit fly species in the trial emerge from test fruit in any one replicate, then the fruit is considered a potential host for quarantine purposes.