

Draft APPPC RSPM No. 4

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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Introduction

Scope

This standard describes host status 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 host on the basis of these tests.

References

Drew, R.A.I.; Lloyd, A.C. 1989. Bacteria associated with Fruit Flies and their Host Plants, pp. 131-140. *In*: Robinson, A. S.; Hooper, G. Fruit Flies – Their Biology, Natural Enemies and Control. Volume 3A, World Crop Pests. Elsevier Science Publishers, Netherlands.

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 [ISPM No 5?]

NZ Ministry of Agriculture and Forestry (MAF) Standard

Definitions

Commodity

A type of plant, plant product or other article being moved for trade or other purpose (FAO, 1999)

Conditional non-host of fruit flies

Fruit and vegetables at a specified maturity and specified physical condition that cannot support the development of viable adult fruit fly

Eclosion

The process of larva hatching from the eggs or the adult insect leaving from the last nymphal skin or puparium

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 FAO, 1995]

Fruit fly

Insect of the order: Diptera: family Tephritidae.

Fruit fly host¹

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]

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]

Non host of fruit flies

Fruit or vegetables that will not support the complete development of fruit fly regardless of the stage of maturity and physical characteristics

National Plant Protection Organization

Official service established by a government to discharge functions specified by the International Plant Protection Convention (FAO, 1999)

NPPO

Acronym for National Plant Protection Organization

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

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.

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 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 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. Hence the development of a standard method for determining host status under defined, reproducible conditions is highly desirable and has important ramifications for international trade in many fresh fruit and vegetable commodities.

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.

2.2 Pre-requisites for host status testing

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

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

Physical damage to fruit (ie. 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.

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. In this standard trials are described to determine if the fruit or vegetable plants under test can be given the status of non-hosts, conditional non-hosts or hosts to a fruit fly species. The results of trials are interpreted in the following manner:

- if a punctured fruit (used in the botanical sense) shows no sign of fruit fly infestation then the host is described as a non-host
- if an unpunctured fruit is not infested by a fruit fly species but punctured 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 host.

The following is the suggested sequence of tests:

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 may indicate:

- if the fruits do not become infested, then the commodity can be 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, the commodity may be a host or conditional non-host to that fruit fly species.

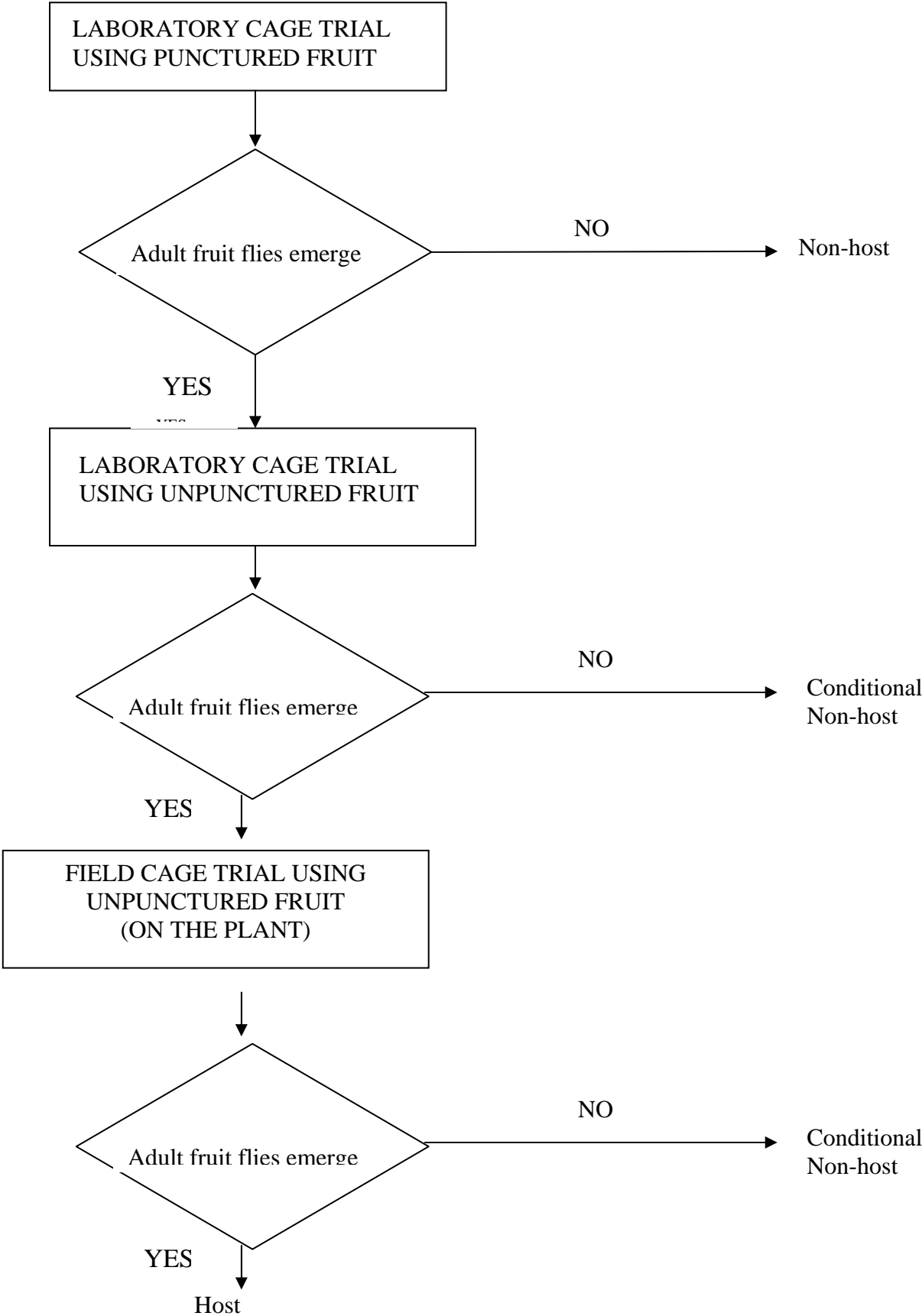
The second test is a laboratory cage test using unpunctured fruit to determine if fruit may be a conditional host. The results may indicate:

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

Figure 1: Diagram of the host testing stages



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 with 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 the edges of the dome 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 250 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.

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.

Five replicates, each with a minimum of 500g of whole 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.

A control replicate using a minimum of 500 g of a known 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 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 that will be exposed to the flies.

Test fruit should be contained as a single layer for 24 hours with the flies in fine meshed cages of minimum dimensions of 300 mm x 300 mm x 300 mm. Measures should be taken to prevent access by ants and *Drosophila*. Each cage should contain a source of sugar and water.

Trials should 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 gourd, cucumber, tomato, most citrus

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

and banana) 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 five trial replicates, then the commodity at the described stage of maturity can be accepted as a non-host to the fruit fly species tested.

If one or more adults emerge from trial replicates, then the commodity is considered to have potential to be a host. This result necessitates a laboratory cage trial using unpunctured fruit.

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 fruits are not punctured. The control replicate should be punctured as per section 3.4.

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 accepted 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 undertaken.

5. Field cage/glasshouse trials using unpunctured fruit

A field 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 fruits are not punctured and remain attached to the test host plant. The fruiting host plants may be exposed to the test 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 to 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 320 viable eggs.

In this standard a minimum potential oviposition load of 250 viable eggs per replicate was chosen for laboratory trials. For field/glasshouse trials a minimum potential oviposition load of 320 viable eggs per replicate was chosen. The higher rate of 320 eggs per replicate for field/glasshouse trials was chosen to compensate for higher adult mortality that may be experienced when laboratory flies are released in the field. Additionally, the exposure period for field/glasshouse trials is 48 hours compared to 24 hours for laboratory trials. The longer exposure period for field/glasshouse trials is to allow the laboratory reared flies to acclimatise to field conditions.

5.3 Field cage trial

Five replicates of approximately 500g 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 (320 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 x 300 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 replicate using approximately 500g 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 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 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 replicates of approximately 500g of whole fruit attached to parent plants should be used for each replicate. Each replicate 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 500g of fruit per replicate. Regardless of the number of cages and plants used to house 500g 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 500g of a known host should be run concurrently with the 5 trial replicates and under exactly the same glasshouse conditions. Control fruit should be punctured as per section 3.4 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 replicate, the field or glasshouse 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 fruit at the described stage of export harvest maturity can be accepted 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 host.