# IRAC Susceptibility Test Methods Series

*Method No: 019*

*Version: 3.4*

**Details:**

| | |
|---|---|
| Method: | IRAC No. 019 |
| Status: | Approved |
| Species: | Peach-Potato Aphid *(Myzus persicae)*<br>Cotton Aphid *(Aphis gossypii)*<br>Currant-Lettuce Aphid *(Nasonovia ribis nigri)*<br>Soybean Aphid *(Aphis glycines)*<br>Buckthorn Potato Aphid *(Aphis nasturtii)*<br>Pea Aphid *(Acyrthosiphon pisum)*<br>Black Bean Aphid *(Aphis fabae)*<br>Foxglove Aphid *(Aulacorthum solani)*<br>Potato Aphid (*Macrosiphum euphorbiae*) |
| Species Stage | Adult or Nymph |
| Product Class: | Carbamate (1A)*<br>Organophosphate (1B)*<br>Pyrethroid (3A)*<br>Neonicotinoids (4A)*<br>Pymetrozine (9B)**<br>Flonicamid (9C)**<br>Diafenthiuron (12A)*<br>Tetronic and Tetramic acid derivatives (23)** |

Green Peach Aphid
*Myzus persicae* (Sulzer)
Photograph Courtesy of: Scott Bauer, USDA

Comments:
Mortality assessment period may vary depending on insecticide mode of action

The following guidelines may be used:

*72 hours assessment period
**120 hour assessment period

**Objectives:**

Susceptibility Baseline: ☒
Resistance Monitoring: ☒

**Description:**

Materials:
Small (3-5cm diameter) petri-dishes or plastic pots, ventilated petri-dish or plastic pot lids (ventilated with gauze covered ventilation holes),forceps, sharpened metal tube for cutting leaf discs (diameter 2mm less than Petri-dish), agar powder, fine pointed brush, beakers for test liquids, syringes/pipettes for liquids or weighing balance for solids, syringes/pipettes for making dilutions, binocular microscope or hand lens, untreated leaves of a host plant, paper towels, maximum/minimum thermometer, seeking pin or fine forceps, microwave oven or hotplate

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*(a)* Ventilate the Petri-dish or pot lids with small holes with a diameter too small for the aphids to escape or glue a gauze mesh over larger holes.

*(b)* Prepare agar by mixing 1% w/w agar powder with distilled water, heat until boiling and then allow cooling while constantly mixing. After cooling for approximately 10 minutes, pour the warm agar into the bases of the petri-dishes to a depth that is at least 3-4mm. Allow for at least 10mm between the top of the agar and the rim of the petri-dish or container. NOTE: Different brands of agar powder may necessitate using different concentration than 1%, so experiment first to determine the required agar to water ratio for the required level of gelling.

*(c)* Collect sufficient non-infested, untreated host plant leaves. Do not allow leaves to wilt by keeping them in a humid environment (plastic bag). The following host plants are recommended for each species.

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|---|---|
| Peach-Potato Aphid *(Myzus persicae)* | Chinese Cabbage |
| Cotton Aphid *(Aphis gossypii)* | Cotton |
| Lettuce Aphid *(Nasonovia ribi-nigri)* | Lettuce (Romaine variety)* |
| *S*oybean Aphid *(Aphis glycines)* | Soybean |
| Buckthorn Potato Aphid *(Aphis nasturtii)* | Potato |
| Pea Aphid *(Acyrthosiphon pisum)* | Broad bean |
| Black Bean Aphid *(Aphis fabae)* | Broad bean |
| Foxglove Aphid *(Aulacorthum solani)* | Broad bean |
| Potato Aphid (*Macrosiphum euphorbiae*) | Potato or Broad bean |

Choice of host plant should be recorded for future reference.

* Romaine variety is preferred to more hydrated lettuce varieties which wilt quickly once leaves are cut from the plant.

*(d)* Prepare accurate dilutions of the test compound from the identified commercial product. For initial studies, six widely spaced rates are recommended; the rate range can be narrowed once an appropriate dose response has been identified. The use of additional wetter is only recommended for highly waxed leaf material, in which case this wetter solution is used for the “untreated” (control) solution in place of water alone. As the addition of a wetting agent can significantly affect the performance of an insecticide product in a bioassay, it is essential that details of the wetting agent are recorded with any summary data and that only data generated with the same agent and concentration are compared for susceptibility measurements.

*(e)* Use the metal tube to cut leaf-discs from clean, untreated host plant leaves. Leaf disc should be 2mm less in diameter compared to the petri-dish or pot. The metal tube should be sharpened and cleaned regularly to ensure the clean cutting of the leaf discs.

*(f)* Dip leaf discs (cut before or after dipping) individually in the test liquid for 10 seconds with gentle agitation and place to surface-dry on paper toweling (abaxial surface facing skywards). Ensure the entire leaf surface is emerged equally and do not allow the leaves to wilt. Dip the same number of leaves per treatment (a minimum of three replicate leaves per concentration is recommended),

*(g)* Place the dipped leaf discs on paper towels (abaxial surface facing skywards) to air dry.

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*(h)* Make sure that the agar has cooled and set. Once the insecticide deposit is dry, lay individual leaf discs (abaxial surface facing skywards) into each petri-dish or pot. Ensure treatment details (Insecticide and concentration) are recorded on each container. A small drop of distilled water placed on the surface of the agar prior to laying the leaf on the surface may help to stick the leaf to the agar surface.

*(i)* **Adults:** 20-30 apterous adults are transferred onto each of the leaf discs using the paint brush. Each unit is sealed with a close-fitting, ventilated lid. Petri-dishes are stored upright.

**Nymphs:** 5 apterous adults are transferred onto each of the leaf discs using the paint brush. Each unit is sealed with a close-fitting, ventilated lid. 24 hours later all insects are removed except 10-15 nymphs. Lids are replaced and Petri-dishes are stored upright.

*(j)* Store the containers in an area where they are not exposed to direct sunlight or extremes of temperature. Record maximum and minimum temperatures. If possible a mean temperature of 20°C, 60% RH and 16:8 light/dark regime is preferred.

*(k)* An assessment of mortality (dead and live aphids) is made after 72 hours (120 hours for pymetrozine, flonicamid and tetronic & tetramic acid derivatives). Aphids which are unable to right themselves within 10 seconds once turned on their back are to be considered as dead (combination of dead and seriously affected).

*(l)* Express results as percentage mortalities, correcting for “untreated” (control) mortalities using Abbott’s formula. It is recommended that the mortality data is utilised to perform a probit or logit dose response analysis to provide LC50 and LC90 estimates for each insecticide or insect population tested.

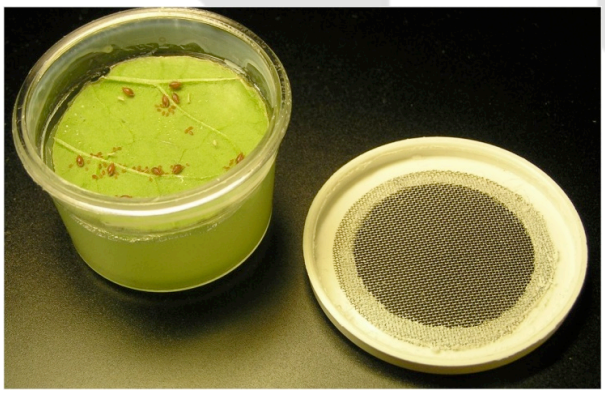

Fig 1: Aphid infested test container and ventilated lid
Photograph courtesy of Rothamsted Research

Fig 2: Aphid Infested test containers
Photograph courtesy of Rothamsted Research

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**Percautions & Notes:**

1. Disposable plastic equipment is preferred provided that it is not affected by the formulation constituents; glass equipment may be used but must be adequately cleaned with an appropriate organic solvent before re-use.

2. Insecticide products contain varied concentrations active ingredient(s). Ensure insecticide dilutions are based on active ingredient content (g a.i.).

3. Where possible, bioassays to measure the variation in insecticide susceptibility should run in parallel with a bioassay to measure the susceptibility of a known susceptible standard population of the target insect.

**References & Acknowledgements:**

This methodology is based on a similar method developed for *Myzus persicae* and *Aphis gossypii* by Rothamsted Research. Thanks to Ian Denholm and Kevin Gorman of Rothamsted Research for providing the original method and for allowing publication by IRAC.