


Details:

Method:	No. 015 (Formally Method No 12a)	 <p>Photograph Courtesy of: Central Science Laboratory, Harpenden Archives, British Crown</p>
Status:	Approved	
Species:	Whiteflies, <i>Trialeurodes vaporariorum</i> and <i>Bemisia tabaci</i>	
Species Stage	Adults	
Product Class:	Neonicotinoids, pymetrozine, pyrifluquinazon, flonicamid, pyrethroids, organophosphates and other whitefly adulticides	
<p>Comments: This method is suitable for use with commercial formulations of insecticides. This method was developed by Rothamsted Research, UK and validated for endorsement as an IRAC approved method by BASF and Syngenta.</p>		

Description:

Materials:

Small (3-5 cm diameter) Petri-dishes with gauze-covered ventilation holes in lids, sharpened metal tube for cutting leaf discs (diameter 2 mm less than Petri-dish diameter), agar powder, aspirator for transferring whiteflies, carbon dioxide (cylinder), glass flasks or disposable plastic cups (150-200 ml) for serial dilutions of insecticides, syringes/pipettes for making dilutions, binocular microscope or hand lens, paper towels, maximum/minimum thermometer.

Method:

- (a) Ventilate the Petri-dish lids by making small holes (~10) with a diameter small enough to prevent escape of whiteflies or glue gauze mesh over larger holes.
- (b) Prepare agar by mixing 1% w/w agar powder with distilled water, heat until boiling and then allow to cool while constantly mixing. After cooling for approximately 10 minutes, pour the warm agar into the bases of the Petri dishes to a depth of 3-4 mm. NOTE: Different brands of agar powder may require different concentrations from 1%, so experiment first to determine a proper agar to water ratio for the required level of gelling (the purpose of the agar layer is to keep the leaf discs in place and stay fresh for the duration of the test).

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- (c) Use the metal tube to cut leaf-discs from clean, untreated host plant leaves (jack bean *Canavalia ensiformis*, or cotton are recommended).
- (d) Immerse the leaf discs into serial water dilutions of a formulated insecticide for 20 seconds (use of additional wetter is not recommended) and air dry on paper towels. Control discs are dipped in distilled water only.
- (e) Prepare at least three leaf discs (replicates) per concentration, including the water control.
- (f) Once the agar has cooled and set, lay a treated leaf disc adaxial (upper) surface down, into each Petri-dish.
- (g) Place 15-20 healthy whiteflies using a small mouth or venturi operated aspirator onto each leaf disc. If necessary, lightly anesthetize the whiteflies using carbon dioxide to facilitate transfer. Place the ventilated lid onto the Petri-dish base and ensure a close seal to prevent the whiteflies escaping.
- (h) When the whiteflies have recovered from narcosis (if carbon dioxide is used), invert the dishes so that the adults are oriented normally.
- (i) Keep the dishes under natural light or in a light cycling (16L/8D) incubator at $25 \pm 2^\circ\text{C}$.
- (j) Assess mortality at 48 hours (72 h for Group 4, and 120 h for Group 9 and 29 products) after treatment using a binocular microscope or hand lens.
- (k) Express results as percentage mortalities. Untreated mortality should be recorded (if $>20\%$, the study should be considered as invalid and be repeated). When necessary, correct for “untreated” (control) mortalities using Abbott’s formula: Corrected % mortality = $(\% \text{ alive control} - \% \text{ alive treated}) \times 100\% / (\% \text{ alive control})$.
- (l) The mortality data can be subjected to a probit or logistic dose response analysis to calculate an LC_{50} or LC_{90} .

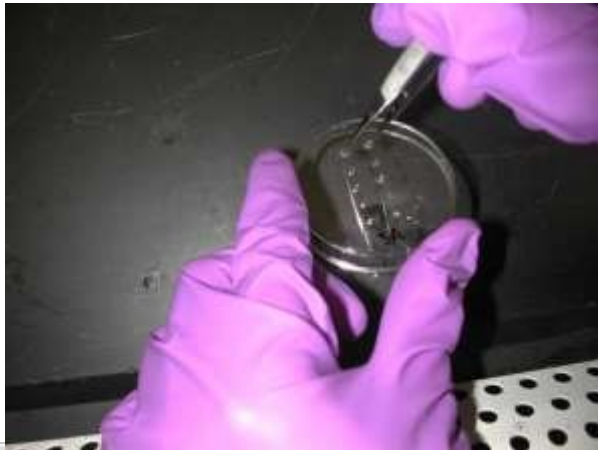


Figure 1. Preparing Petri dishes with ventilated lids

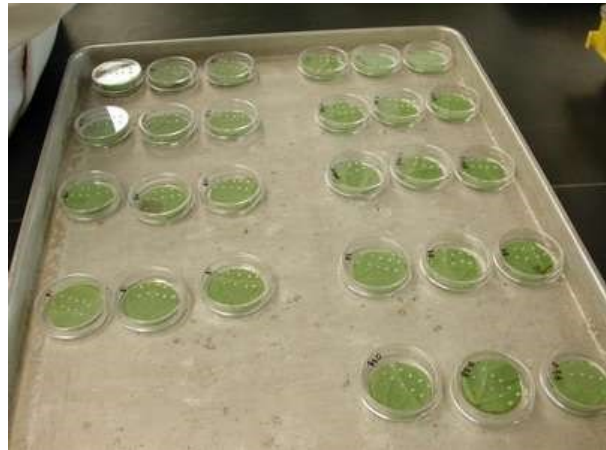


Figure 2. Bioassay in progress showing Petri dishes containing treated leaf disks and whiteflies

Precautions & Notes:

Group 9 product pyrifluquinazon and Group 29 product flonicamid were not validated directly, but assumed applicable.

Where glass equipment is used it must be adequately cleaned with an appropriate organic solvent before re-use to prevent cross-contamination.

References & Acknowledgements:

Thanks to Ian Denholm and Kevin Gorman of Rothamsted Research for providing the method and for allowing publication by IRAC.

Figures 1 - 2 are courtesy of BASF.