

IRAC Susceptibility Test Methods Series Version: 1.0

Method No: 032

Details:

Method:	No: 032	 <p><i>Credit: David Hall/USDA</i></p>
Status:	Under Review	
Species:	<i>Diaphorina citri</i>	
Species Stage	Adults/4 th instar nymphs	
Product Class:	Organophosphates, Carbamates Pyrethroids, Neonicotinoids Spinosyns, Avermectins, Diamides	
Comments:		

Description:

Materials:

Aspirators, sweep nets, vials, and coolers (for insect collection); Petri dishes (6-cm diameter); leaf disk cutter (6-cm diameter); agar powder; small forceps; camel hair brushes; beakers or glass jars (ca. 100-ml capacity) for test liquids; pipette for liquid or weighing balance for solid products, maximum/minimum thermometer.

Methods:

- a) Collect adults by using a sweep net or a stem-tap sample along the rows of a grove selected for sampling. Insects can also be collected directly using an aspirator if the population density is low. Nymphs (4th instar) can be collected by cutting off leaf flush. Transport insects or plant material in an ice cooler to the laboratory to prevent exposure to temperature extremes. Adults should be released on citrus plants until use. Flush with nymphs should be maintained by placing stems in water until use.
- b) Prepare agar by mixing 1.5 % w/w agar powder in distilled water, heat until boiling. Allow the solution to cool while constantly mixing. After cooling for approximately 10 minutes, pour 3-5 ml agar solution into Petri-dishes to form a solid bed (3-4 mm thick). NOTE: Different brands of agar powder may require different concentration than 1.5% to form a solid bed, be sure to determine an appropriate concentration before starting the study.
- c) Collect clean and untreated citrus leaves for cutting leaf-disks (6 cm diameter).
- d) Prepare appropriate number of test dilutions of products in water with 0.2% mineral oil (for better coverage). For dose-response calculation (e.g. LC₅₀ or LC₉₀), at least 5-6 concentrations covering a range from low to high mortality (including a control) are required.
- e) Agitate test liquids and then dip leaf-disks individually for 30 s. Replicate each treatment at least three times. Use a solvent only blank for the 'untreated' control.
- f) Allow surface water on the leaf disks to dry before placing them individually in the Petri dishes (the

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layer of agar will keep the leaf disc moist for the duration of the study).

- g) Infest each dish with 20-25 adults or nymphs (but not a mix of the two) using a camel hair brush or an aspirator, and then seal the Petri dishes with parafilm. Ice packs or carbon dioxide can be used to slow down the adult activity to facilitate the transfer of adults on to the treated leaf-disks in Petri dishes.
- h) Store the Petri dishes at room temperature away from direct sunlight and extreme temperatures. Record maximum and minimum temperatures.
- i) Assess mortality after 24 h. Insects found flaccid, dried, light colored and unable to move or right themselves when probed with a camel hair brush are considered dead.
- j) Express results as percentage mortality, correcting for 'untreated' (control) mortality using Abbott's formula. The mortality data is subjected a probit or logit dose response analysis to provide LC_{50} or LC_{90} .

Precautions & Notes:

- a) 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.
- b) Commercially formulated insecticide products may contain varied concentrations active ingredient(s). Ensure insecticide dilutions are based on active ingredient content (g a.i.). Technical grade products also can be used with acetone or other organic solvents instead of water. Mixture products (contain equal to or more than two a.i.s) should not be used to determine the susceptibility of insect populations to the single insecticide.
- c) Wherever possible, bioassays to measure the variation in insecticide susceptibility should be run in parallel with a bioassay to measure the susceptibility of a known susceptible standard population of the target insect.

References & Acknowledgements:

Tiwari, S., R. S. Mann, M. E. Rogers, and L. L. Stelinski. Insecticide resistance in field populations of Asian citrus psyllid in Florida. *Pest Manag. Sci.* 67:1258-1268 (2011).

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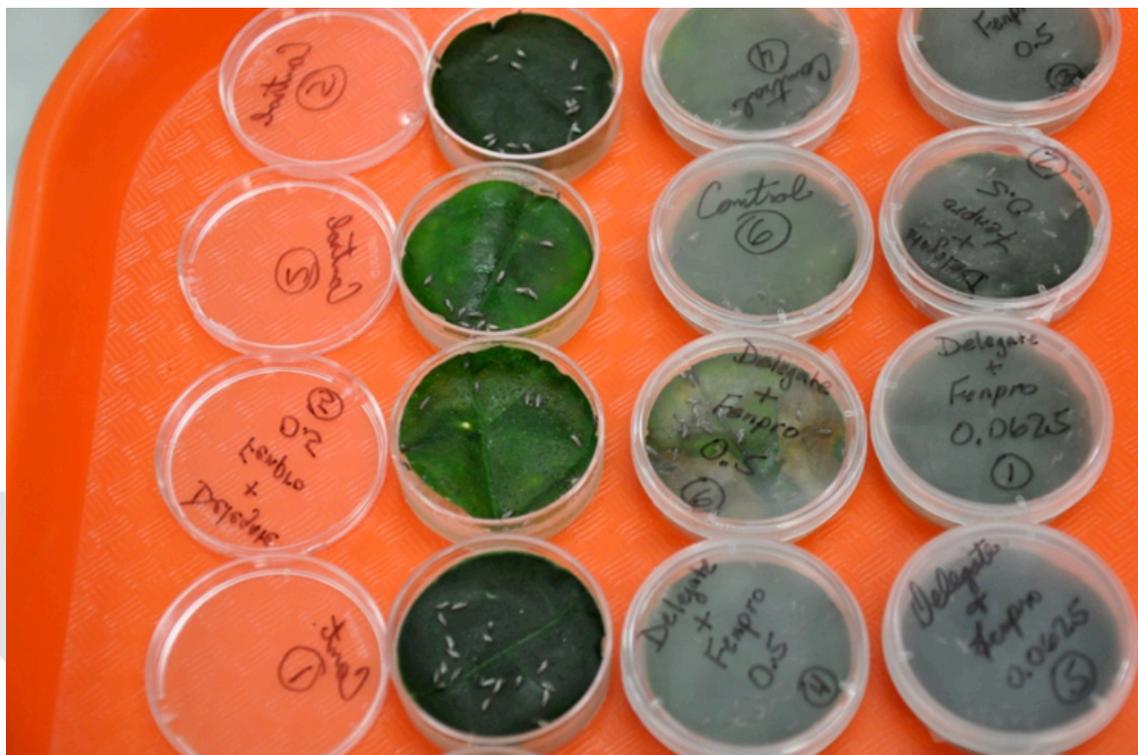


Photo provided by Tiwari, S.

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