IRAC Susceptibility Test Methods Series

Introduction & Overview

Version: 2

Introduction:

One of the most important factors governing the management of insecticide/acaricide use is the availability of sound baseline data on the susceptibility of the target pest to the toxicant. Baseline data can be defined as data obtained from a strain with no history of selection with the toxicant or toxicants with the same or related site of action showing cross resistance. Currently a wide range of bioassay and biochemical tests are employed to characterize the susceptibility of target pests to insecticides and acaricides. Unfortunately the results from different test methods may not be comparable since they measure different parameters which can lead to difficulties over the interpretation of monitoring data.

IRAC (Insecticide Resistance Action Committee), in fulfilling its aim of providing expert advice to CropLife International on all technical matters relating to insecticide and acaricide resistance, has addressed this issue with the aim of recommending a range of bioassay techniques to monitor insecticide and acaricide susceptibility for selected pest species of economic importance.

Objectives:

The specific objectives of the project were to:

(1) review the range of monitoring techniques currently available,
(2) select those most appropriate for future monitoring studies or develop new methods if appropriate to harmonize methodology,
(3) improve standards of susceptibility monitoring,
(4) allow a greater degree of comparison between studies.

Choice of methods:

Changes in insect/mite susceptibility to toxicants can take various forms, which often influences the sensitivity of given bioassay techniques in detecting these changes in the target population. Because tests may measure different parameters, a single test method is unlikely to provide a complete picture of the susceptibility of a given population. The methods presented here were chosen as being:

(1) reliable and reproducible under field usage allowing comparisons between tests;
(2) simple and easy to perform using a minimum of resources;
(3) consistent in distinguishing between susceptible and resistant phenotypes;
(4) relevant as far as possible to field performance of products;
(5) useful for a range of toxicant groups.

The tests should not be taken as the only acceptable method of measuring changes in susceptibility, but they are the methods most likely to satisfy these criteria.

For further information please contact the IRAC International Coordinator:
Alan Porter: aporter@intraspin.com
www.irac-online.org

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Function of the tests:

The tests can be used to characterize the susceptibility of a target pest population to one or more toxicants at a given instance, and to monitor changes in susceptibility through space and time.

Data generated from the tests can be used to aid in distinguishing between field control failure as a result of insecticide/acaricide resistance or other factors, to assist in the design of resistance management strategies and to gauge the success of these strategies over time.

Limitations of the tests:

The tests cannot normally be employed to predict the onset of insecticide/acaricide resistance nor as operational tools to guide immediate spray decisions. The tests are specific to particular life-history stages and can only detect changes in susceptibility expressed in that stage. They can only be used with confidence for toxicants which have been validated in the development of the methodology. As susceptibility testing often involves rearing the test sample for one or more generations in laboratory of glasshouse conditions, results from the tests may vary with the generation of pest tested, the sex/age/condition (including disease) of these organisms and the test holding conditions. These should be standardized as far as possible.

Sampling Procedures:

Data generated using the tests relates only to the test sample. Conclusions relating to the target population can only be made if the sample is truly representative of the population, thus sampling bias must be rigorously avoided. Consideration should be given to the crop or host plant sampled, the time and frequency of sampling, the crop-treatment history, the number, age, sex and life-history stage of organisms collected and the number, size and location of sampling areas. It must be ensured that test organisms are not the offspring of only one or a few females which can often be a problem with laboratory rearing.

Experimental design and analysis:

Meaningful documentation of changes in pest population susceptibility can only be made in comparison with appropriate baseline strains or populations. The choice of a susceptible baseline strain is critical and many laboratory strains are artificially susceptible compared with field populations.

Generally, the use of commercial formulation of the test compound is preferred to the use of technical material.

The choice between using a single discriminating dose or a range of doses depends on the objective of the test. If the objective is to detect a large change in suscepibility in a small portion of the population, then a single discriminating dose is more appropriate. This should

be selected as a dose which gives complete kill or high mortality of a susceptible population but zero or low mortality of a homogeneous resistant population.
If small changes in susceptibility are suspected or there is a range of resistance phenotypes already present in the population, the use of more than one dose is preferred. The choice of doses will depend on the range of resistance factors expressed. However, it is important to remember that if the population is heterogeneous in terms of susceptible and resistant phenotypes, the use of probit analysis is invalid.

Results should be recorded in terms of percentage mortality and corrected for mortality in the untreated using Abbott's formula. A standard form for use with all methods is appended.

Criteria for assessment should be carefully defined and strictly applied. Results from susceptibility tests will not always relate directly to field performance. This is because field performance is the result of a complex interaction of factors including environmental conditions, application equipment and pest pressure, in addition to the susceptibility of the population to be controlled. Results from the tests do, however, give an indication of the potential for field control failure due to a change in susceptibility of the pest.

Validation of the test methods:
The methods are initially presented as drafts and are granted an IRAC recommendation after a period of 2 years' field testing. The status of each method i.e. draft, proposed, approved is given under the "Details" section of each method The performance of each test against the criteria outlined above needs to be rigorously evaluated to ensure that the methods are satisfactory. To facilitate this process a standard data recording sheet parts I and II is available. Recipients are requested to return copies of completed recording sheets to the IRAC Coordinator, Alan Porter. Details are available by email at: aporter@intraspin.com

Modification of the test methods:

Minor changes, particularly with respect to the design of test containers, can generally be made without harming the validity of the method. However changes should not be made to quantified parts of the method, e.g. test holding temperature, duration of leaf dipping etc. Any changes should be clearly recorded with the test results.

IRAC Susceptibility Test Methods Series

Method No: 1

Version: 2

Details:

Method:	No:1	*Photograph Courtesy of: Scott Bauer, USDA ARS - Myzus persicae*
Status:	Approved	
Species:	*Myzus persicae*	
Species Stage	All	
Product Class:	organophosphates carbamates	
Comments: None		

Description:

Materials:
Petri dishes (9-cm diameter), plastic bags, cotton wool, untreated leaves, small forceps, fine pointed brush or cocktail stick, beakers or glass jars (ca. 100-ml capacity) for test liquids, 1-ml disposable plastic syringes for liquids or weighing balance for solids, hand lens or binocular microscope, maximum/minimum thermometer.

Methods:

(a) Sample apterous aphids by collecting infested leaves, selected at random from several plants. The leaves may be transported and held in plastic bags.
(b) Collect some non-infested, untreated, leaves or remove aphids from leaves using a small brush before treatment.
(c) Prepare appropriate test dilutions of formulations in water. The use of a wetter is not recommended.
(d) Agitate test liquids and then dip non-infested leaves for 5 secs, five leaves per treatment. Dip five control leaves in water only.
(e) Allow surface water to dry from leaves before placing them individually in Petri dishes and infesting each leaf with 20 adult aphids. The aphids can be transferred using a small pointed brush (with volatile insecticides it may be necessary to ventilate the Petri dishes by piercing the lids with a hot wire).
(f) Place a small piece of damp cotton wool around the petiole of each leaf.
(g) Store Petri dishes in an area where they are not exposed to direct sunlight or extremes of temperature. Record maximum and minimum temperatures.
(h) Using a hand lens or binocular microscope assess mortality after 24 h by checking each aphid's ability to show co-ordinated movement in response to a touch with a small brush or cocktail stick.
(i) Express results as percentage mortality and correct for untreated mortality using Abbott's formula. Untreated mortality should be recorded.

IRAC Susceptibility Test Methods Series

Method No: 1

Version: 2

Percautions & Notes:

None

References & Acknowledgements:

None

For further information please contact the IRAC International Coordinator:
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IRAC Susceptibility Test Methods Series

Method No: 2

Version: 2

Details:

Method:	No: 2	*Photograph Courtesy of: Andrea Battisti, Università di Padova Psylla spp*
Status:	Approved	
Species:	*Psylla spp*	
Species Stage	All	
Product Class:	organophosphates amitraz	
Comments: None		

Description:

Materials:
Jars/containers for holding shoots, beakers or glass jars (ca. 100-ml capacity) for test liquids, 1-ml disposable plastic syringes for liquids or weighing balance for solids, hand lens or binocular microscope, fine pointed brush or cocktail stick, maximum/minimum thermometer.

Methods:

(a) Collect shoots infested with immature stages. The best time is when 1st and 2nd instar nymphs of the second generation are present which is late May (Italy) or early June (Central Europe). It is important to treat before much honeydew is produced.
(b) Place the shoots in water to keep them fresh.
(c) Using a good hand lens or a binocular microscope, count the number of live nymphs. Remove any adults.
(d) Prepare appropriate test dilutions of formulations in water. The use of a wetter is not recommended.
(e) Agitate test liquids and then dip shoots for 5 secs using five or six shoots at each rate. Dip equal number of control shoots in water only.
(f) Keep shoots in water in an area where they are not exposed to direct sunlight or extremes of temperature. Record maximum and minimum temperatures.
(g) After 24 h using a hand lens or binocular microscope, record number of surviving nymphs by checking their ability to show co-ordinated movement in response to a touch with a small brush or cocktail stick.
(h) Express results as percentage mortality and correct for untreated mortality using Abbott's formula. Untreated mortality should be recorded.

IRAC Susceptibility Test Methods Series

Method No: 2

Version: 2

Percautions & Notes:

None

References & Acknowledgements:

None

For further information please contact the IRAC International Coordinator:
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IRAC Susceptibility Test Methods Series

Method No: 3

Version: 2

Details:

Method:	No: 3	*Photograph Courtesy of: Whitney Cranshaw, Colorado State University Tetranychus spp*
Status:	Approved	
Species:	*Panonychus ulmi* *Tetranychus spp.*	
Species Stage	*P. ulmi* (summer eggs) *Tetranychus* (eggs)	
Product Class:	clofentezine hexythiazox tetradifon	
Comments: None		

Description:

Materials:
Petri dishes (9-cm diameter), filter paper to fit Petri dishes, cotton wool, *untreated* apple or plum leaves, small scissors, small forceps, fine pointed brush or cocktail stick, beakers or glass jars (ca. 100-ml capacity) for test liquids, 1-ml disposable plastic syringes for liquids for weighing balance for solids, hand lens (minimum 10 x) or binocular microscope, maximum/minimum thermometer.

Methods:

(a) Cut square sections about 1.5 x 1.5 cm from chemically untreated apple or plum leaves. Use young leaves, but not before they are fully expanded. Leaves must be in good condition. Use a minimum of four replicates (leaf sections) per treatment.

(b) Place these sections, upper surface uppermost, on a sheet of moist filter paper on moist cotton wool in open Petri dishes.

(c) Collect apple leaves with adult mites, and with the fine pointed brush or cocktail stick transfer 10 – 15 females onto each leaf section. Maintain at a minimum temperature of 20°C, minimum photoperiod 16 h and a high light intensity, but not in direct sunlight.

(d) After 24 h, check that the female mites have laid eggs. Aim for at least 20 eggs per leaf section. If there are not enough eggs, leave for a further 24 h. Do not leave longer than 48 h.

(e) When sufficient egg numbers have been obtained, remove the mites with the fine pointed brush or cocktail stick. Record the time when this is done.

(f) Prepare appropriate test dilutions of formulations in water. The use of a wetter is not recommended.

(g) Agitate test liquids and then dip the leaf sections for 5 secs. Dip equal number of control leaf sections in water only.

(h) Record the number of eggs per leaf section.

(i) Return leaf sections to Petri dishes and maintain in conditions described above. Record maximum and minimum temperatures. Moisten cotton wool daily.

(j) Using a hand lens or binocular microscope observe leaf sections daily until there has been complete (or nearly complete) hatch on the untreated (water only) leaf sections. Record numbers of un-hatched eggs on treated and untreated leaf sections.

(k) Express results as percentage mortality and correct for untreated mortality using Abbott's formula. Untreated mortality should be recorded.

Percautions & Notes:

If the lids are left off, the leaf sections may dry out and, unless the cotton wool can be moistened at least daily, the test may be invalidated by excessive control mortality. In such circumstances, the method may have to me modified to suit the local conditions, e.g. use lids with holes cut in them to reduce water loss without creating a condensation problem.

For *Tetranychus* spp. which live mainly on the lower leaf surface, the leaf sections may need to be placed lower surface uppermost. Leaves of kidney beans are particularly suitable.

References & Acknowledgements:

None

IRAC Susceptibility Test Methods Series

Method No: 4a

Version: 2

Details:

Method:	No 4a	
Status:	Approved	
Species:	*Panonychus ulmi* *Tetranychus spp.* *Panonychus citri*	
Species Stage	Adult	
Product Class:	bromopropylate, cyhexatin, dicofol, formetanate and propargite	*Photograph Courtesy of:* *Whitney Cranshaw, Colorado State University* *Tetranychus spp*
Comments:		

Description:

Materials:
Petri dishes (9-cm diameter), absorbent cotton wool, untreated leaves (for *Tetranychus* spp., leaves of kidney beans are particularly suitable; for *P. ulmi* use apple or plum leaves; for *P. citri* young leaves of sour orange, *Citrus aurantium*, or sweet orange, *Citrus sinensis*, may be used), scissors/guillotine for cutting cotton wool into strips for uniform dimensions, small forceps, fine sable brushes (to reduce risk of cross contamination, it is advisable to use separate brushes for manipulating mites on each set of treated leaves), beakers or glass jars (ca. 250-ml capacity) for test liquids, Agral or similar wetting agent, binocular microscope, equipment to prepare standard solutions, maximum/minimum thermometer.

Method:

(a) Collect adult mites from the field. A population of at least 10 adult mites per leaf is recommended.

(b) Collect a supply of untreated leaves. For *P. ulmi*, apple leaves may be used. However, use of leaves from related shrubs such as myrobalan (*Prunus cerasifera*) provide a more durable experimental substitute.

(c) Prepare test liquids. The use of a wetter is not recommended (see note).

(d) Agitate test liquids and dip individual leaves for 5 s. Allow surface liquid to dry from leaves before placing them in Petri dishes. Use a minimum of five replicates per treatment.

(e) Place a layer of cotton wool over the base of Petri dish. Add tap water to the point of saturation, but avoid build-up of standing water.

(f) Place treated leaves, top surface uppermost, on wet cotton wool base.

(g) Cut strips of cotton wool approximately 1 cm in width and soak in tap water. Lay cotton wool strips around the perimeter of each treated leaf, half over the leaf and half over the cotton wool bed. Place a small piece of damp cotton wool around the petiole of each leaf.

(h) Use a binocular microscope or hand lens to ensure that there are no gaps between the leaves and cotton wool strips. Remove any mites already on the leaves.

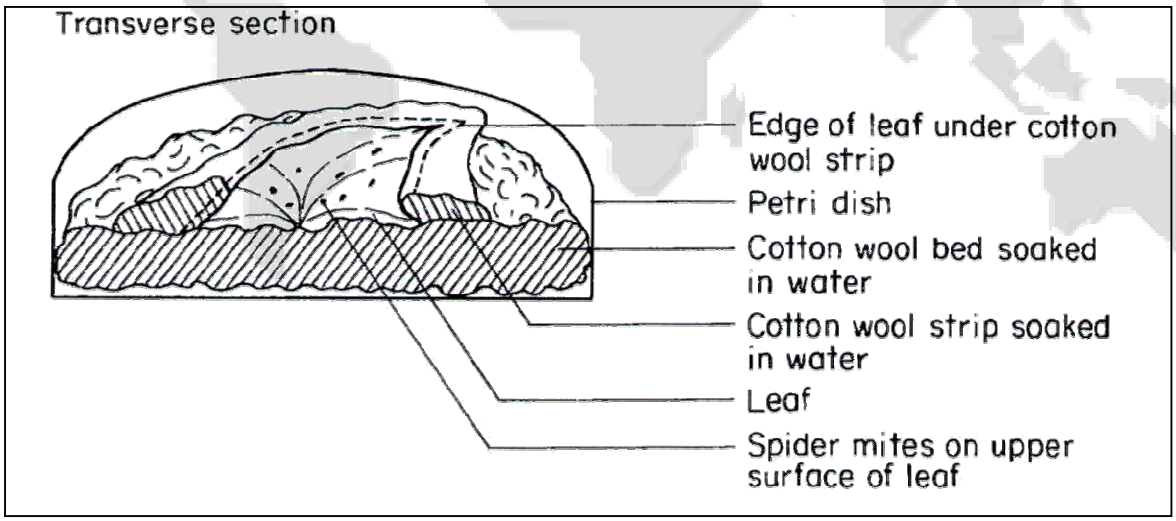
(i) Use a fine sable brush to place 10 adult female mites on the surface of each treated leaf.

(j) Maintain tests at 21 ± 3°C in a room where they are not exposed to direct sunlight and at 65-90% relative humidity. The Petri dishes must be left open.

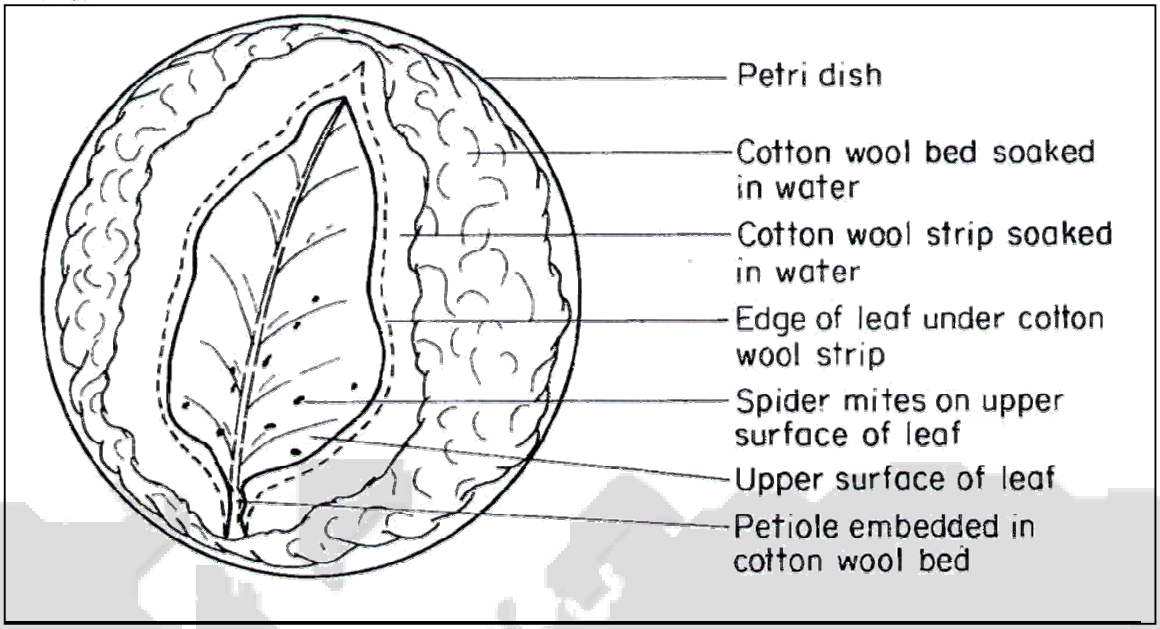
(k) Using a hand lens or binocular microscope, assess mortality after 72 or 96 h depending on speed of action of the test compound. Use a fine sable brush to stimulate individual mites. If they are incapable of walking, record them as dead.

(l) Express results as percentage mortality and correct for untreated mortality using Abbott's formula. Untreated mortality should be recorded.

Diagram:



Plan



Percautions & Notes:

Notes

For *Tetranychus spp*. which live mainly on the lower leaf surface, the leaves may need to be placed lower surface uppermost.

Since formulated compounds are used, addition of wetter is considered unnecessary, particularity in tests where a high discriminating dose is used for determining resistance. However, in special cases, e.g. where low doses are to be used for determinations of LD_{50} values and/or with citrus leaves, addition of a wetter may be necessary to achieve leaf wetting. In this case, the type and concentration of wetter should be noted and quoted with results. Escaped adults should be disregarded from calculation of percentage mortality.

References & Acknowledgements:

None

IRAC Susceptibility Test Methods Series

Method No: 4b

Version: 2

Details:

Method:	No 4b	*Photograph Courtesy of: Cornell University IPM Program*
Status:	Approved	
Species:	*Panonychus ulmi*	
Species Stage	Adult	
Product Class:	Suitable for METI acaricides fenazaquin, pyridaben & tebufenpyrad	
Comments:		

Description:

Materials:
Plastic Petri dishes (4-5-cm diameter), glass bottles/beakers (5-cm internal diameter, 10-cm deep) for dipping test liquids, 100-ml glass beakers for dilution of formulations, 1-ml disposable plastic syringes for liquids, fine sable brushes (to reduce risk of cross contamination, it is advisable to use separate brushes for manipulating mites for each treatment), hand lens (minimum 10x) or binocular microscope, maximum/minimum thermometer.

Method
(a) Collect adult mites from the field. A population density of at least 10 adult mites per leaf is recommended. If it is not possible to collect sufficient adult mites at any one time, numbers may be increased by maintaining temporary cultures on untreated related shrubs such as myrobalan or cherry plum (*Prunus cerasifera*).

(b) Prepare 100ppm and 10ppm solutions of test liquids. The use of a wetter is not recommended. The lower concentration is a discriminating dose with an expected mortality of greater than 98%, and the higher concentration provides a measure of the intensity of resistant types present. Survivors at 100ppm are considered to be resistant to 10 times the discriminating dose.

(c) Dip Petri dishes and lids in test solutions for 30 seconds, drain for 10 seconds, and allow to dry for at least 30 minutes before use. Use a minimum of five replicates per treatment. Dishes dipped in water plus emulsifier should be used as controls.

(d) Place 20 adult mites in the base of each dish using a fine sable brush commencing with the lowest concentration. Cover each dish with a lid ensuring mites cannot escape, place in a holding tray and cover with opaque tissue paper.

(e) Maintain at a temperature of 20-25°C, under low light intensity or dark conditions, avoiding exposure to direct sunlight.

(f) Using a hand lens or binocular microscope, assess mortality after 3 hours (fenazaquin, tebufenpyrad) or 4 hours (pyribaden) according to the speed of action of the compound. Use a sable brush to stimulate individual mites, recording those that fail to show any signs of movement as 'dead'.

(g) Express results as percentage mortality and correct for untreated mortality using Abbott's formula. Control mortality should be recorded.

Percautions & Notes:

The test is designed for use with disposable plastic Petri dishes. Similar-sized re-useable glass dishes may also be used. Contaminated dishes are cleaned by soaking overnight in a dilute Decon solution.

Ideally, fresh solutions should be prepared for each bioassay using a premixing procedure that ensures formulation integrity. If material is limited, stock solutions can be stored in a refrigerator for a short period/few days and fresh solutions prepared on a daily basis.

References & Acknowledgements:

None

IRAC Susceptibility Test Methods Series

Method No: 4c

Version: 2

Details:

Method:	No 4c	
Status:	Approved	
Species:	*Panonychus ulmi*	
Species Stage	Adult	
Product Class:	*METI acaricides (fenpyroximate)*	*Photograph Courtesy of: Cornell University IPM Program*
Comments:		

Description:

Materials:
Perspex holding cells, rubber bands, 100-ml glass beakers for diluting formulations, 1-ml disposable plastic syringes for liquids, fine sable brushes (to reduce risk of cross contamination it is advisable to use separate brushes for manipulating mites for each treatment), hand lens (minimum 10x) or binocular microscope, maximum/minimum thermometer.

Method:
(a) Collect adult mites from the field. A population density of at least 10 adult mites per leaf is recommended. If it is not possible to collect sufficient mites at any one time, numbers may be increased by maintaining temporary cultures on untreated related shrubs such as myrobalan or cherry plum (*Prunus cerasifera)*.

(b) Collect a supply of untreated myrobalan or *Prunus cerasifera* leaves.

(c) Prepare 100ppm and 10ppm solutions of test solutions. The use of a wetter is not recommended. The lower concentration is a discriminating dose with an expected mortality of greater than 98%, and the higher concentration provides a measure of the intensity of resistant types present. Survivors at 100ppm are considered to be resistant to 10 times the discriminating dose.

(d) Dip individual leaves for 5 seconds in 100-ml of the required concentration of acaricide and allow leaves to dry before use. Each leaf is then sandwiched in a Perspex holding cell (fig. 1) so that the upper leaf surface is exposed internally and the petiole extends outside the cell into a 3-cm scintillation vial containing water. Use a minimum of five replicates per treatment. Leaves dipped in water plus emulsifier should be used as controls.

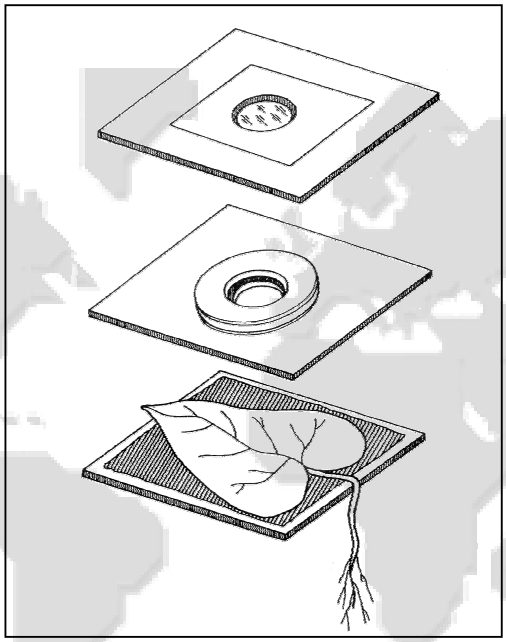
(e) Use a fine sable brush to place 10 adult female mites onto the leaf surface and close the holding cell using rubber bands. Batches of fully-assembled cells are stored together on wet cotton wool in a plastic seed tray.

(f) Maintain at a temperature of 20-25°C, under low light intensity or dark conditions, avoiding exposure to direct sunlight.

(g) Using a hand lens or binocular microscope, assess mortality after (24, 48) 72 hours. Use a sable brush to stimulate individual mites, recording those that fail to show any signs of movement as 'dead'.

(h) Express results as percentage mortality and correct for untreated mortality using Abbott's formula. Control mortality should be recorded.

Diagram:



Percautions & Notes:

Ideally, fresh solutions should be prepared for each bioassay using a premixing procedure that avoids excessive dilution and maintains formulation integrity. If material is limited, stock solutions can be stored in a refrigerator for a short period/few days and fresh solutions prepared on a daily basis.

References & Acknowledgements:

None

IRAC Susceptibility Test Methods Series

Method No: 5

Version: 2

Details:

Method:	No 5	*Nilaparvata lugens*
Status:	Approved	
Species:	*Nilaparvata lugens* *Nephotettix cincticeps*	
Species Stage	Adult	
Product Class:	Suitable for all insecticides	
Comments:		

Description:

Materials:
Transparent plastic or glass tubes, or suitable glass jars. Cut plastic stoppers (see figure), fine, soft spring tweezers, containers for solution preparation. 30-50 ml plastic syringes, 100-1000-µl micro-pipettes for liquids or microbalance for solids, Extravon (Invadin) or a similar non-ionic wetting agent, untreated rice seedlings 10-12 days old (BPH susceptible cultivar grown in seedling box), paper towel, maximum/minimum thermometer.

Method:

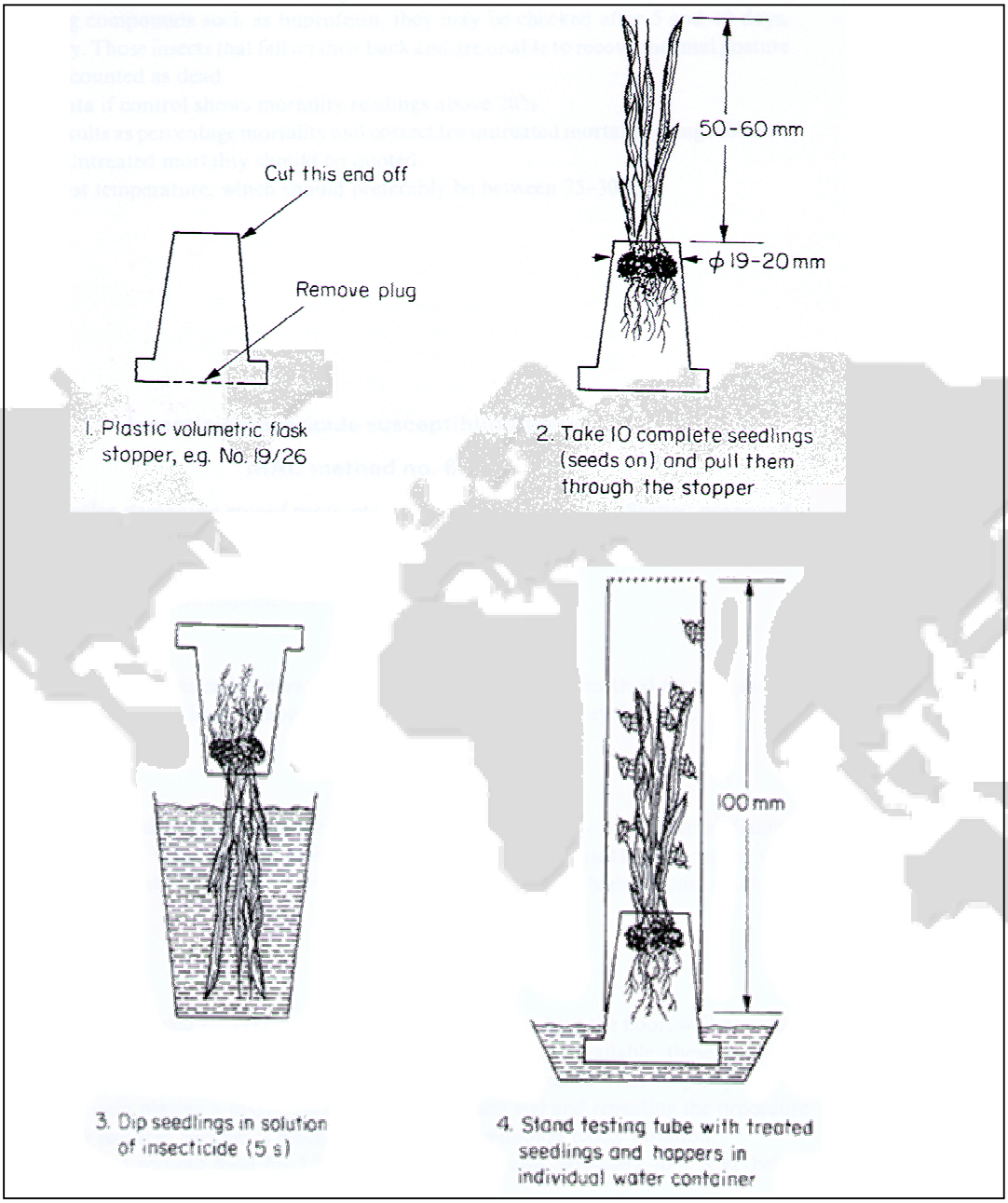
(a) Make test solutions in water containing 0.03 % w/v Extravon (or similar wetter) using formulated insecticides. At least five to six concentrations are required. The highest concentration should be based on the use recommendation in g ai ha^{-1} converted into p.p.m. (e.g. 500 g ai ha^{-1}—500 p.p.m.; 200 g ai ha^{-1}—200 p.p.m.). Use a 0.4-fold dilution or if necessary, other dilution factors (0.5- or 0.8-fold) to obtain two different mortalities above and below 50 %, respectively. Use water-Extravon alone for untreated control.
(b) Prepare treatment tubes as shown in Fig.
(c) Dip seedlings completely for 5 s in the test solutions and leave them to dry in air for 10-15 min depending on the ambient relative humidity.
(d) Field populations of hoppers may be collected by hand or by suction and kept in holding cages containing potted rice plants. Insects should be collected at random from several points in a field and from a few fields in an area then pooled together as parent stock.
(e) Collect adult females of hoppers (for insect growth regulators, use 3rd-4th instar nymphs) from the holding cages by suction and transfer them to the testing tubes.
(f) Depending on availability, use either short-wing (or long-wing) form in any one test, but do not mix the two forms in the same test. Use 10-15 insects per tube.
(g) Each dose should be replicated at least three times, i.e. three tubes per concentration.
(h) Mortalities should be checked at 24 h and 48 h for organophosphates or carbamates. For slow-acting compounds such as buprofezin, they may be checked after 5 and 10 days, respectively. Those insects that fall on their back and are unable to recover normal posture should be counted as dead.
(i) Discard data if control shows mortality readings above 20 %.
(j) Express results as percentage mortality and correct for untreated mortality using Abbott's formula. Untreated mortality should be recorded.
(k) Indicate test temperature, which should preferably be between 25-30°C.

IRAC Susceptibility Test Methods Series

Method No: 5
Version: 2

Diagram:

Insecticide/acaricide susceptibility tests



References & Acknowledgements:

None

IRAC Susceptibility Test Methods Series

Method No: 6

Version: 2

Details:

Method:	No: 6	*Photograph Courtesy of: Clemson University USDA Cooperative Extension Slide Series - Tribolium castaneum*
Status:	Approved	
Species:	beetles damaging stored products	
Species Stage	All	
Product Class:	malathion, pirimiphos-methyl, fenitrothion	
Comments: None		

Description:

Method:

(a) Beetles are collected by: (1) sieving the infested commodity, (2) brushing the floor, wall or surface of bags, (3) using a pooter/aspirator. If sufficient insects are available, these should be separated from the commodity prior to testing and placed in vials in groups of 40, progressively placing a maximum of 10 insects in each vial and repeating the procedure until the required number is reached. This helps to minimize batch variation.

(b) Hold insects without food for 1 h at 25-27°C and at approximately 70 % R.H. before assigning batches randomly to treatments.

(c) Select appropriate pre-treated filter papers according to the species under investigation and place on a firm clean flat surface (e.g. glass). Place plastic rings centrally on top of papers. See Table 1 for proposed discriminating doses. Where insects to be tested include *Sitophilus* spp. the inside surface of rings must be painted with a thin film of Fluon to prevent escape. It may also be desirable to treat the base of the rings to minimize insecticide contamination.

Table 1: Provisional discriminating doses of insecticide for application to filter papers used in discriminating dose tests*

Insecticide	*Sitophilus oryzae/zeamais*+	*Tribolium castaneum*+
Pirimiphos-methyl	2.0Ψ	0.7
Fenitrothion	0.8	0.5
Malathion	1.5	0.5

* An evaluation of deltamethrin and permethrin is under way and recommended discriminating doses for the pyrethroids will be available in the final version.
Ψ % insecticide applied in primary oil solvent.
\+ Exposure period employed: *Sitophilus* spp.-6 h., *T. castaneum* –5h.

(d) Place insects on test papers inside rings, and hold insects under the conditions described above, avoiding direct sunlight. Covers may be necessary to retain some species which tend to fly at 25°C.

(e) After the recommended exposure period (according to species), examine insects and record number knocked down (defined as inability to stand and walk) or dead. A gentle push with forceps is usually sufficient to categorize individuals.

(f) From the results of observations in a test, insects are classed as fully susceptible (i.e. not resistant), where complete knockdown occurs, partially resistant when there is less than complete knockdown, or wholly resistant when no knockdown takes place. Where partial or no knockdown takes place, further investigation may be necessary using a graded range of insecticide concentrations in order to determine the degree of resistance. Advice concerning these tests should be sought from local government specialists but, failing this, advice may be obtained from the Natural Resources Institute (NRI), Chatham Maritime, Chatham, Kent, England, ME4 4TB, UK).

Percautions & Notes:

- Enquiries about the availability of insecticide-impregnated filter papers should be addressed to NRI. Only freshly treated papers should be used. Papers more than 6 months old should be discarded and new samples obtained from NRI.
- If there are insufficient insects available, or further tests are desirable, they may be bred in the laboratory and tested in the following F1 generation. The medium in which the sample beetles were originally collected may contain sufficient eggs for progeny. Details of the life cycles and expected times of emergence of key species are given in FAO method no. 15.
- It is highly desirable that all data concerning the infestation from which the insects were collected should be recorded, including details of the infested food, type of warehouse, location and previous insecticide usage.
- Since plastic tends to absorb insecticide more readily than does glass, it is essential, that plastic rings are decontaminated thoroughly after each test by overnight soaking in several changes of water and detergent.
- Care should be taken not to extrapolate the results of these findings to other populations or locations in the area, unless there is substantial supporting data.

References & Acknowledgements:

The method employed basically follows that recommended by FAO (Method No. 15) and has been adapted for use by those without (or with only limited) laboratory facilities.

IRAC Susceptibility Test Methods Series

Method No: 7

Version: 2

Details:

Method:	No: 7	*Heliothis larvae* *Photograph Courtesy of: Syngenta*
Status:	Approved	
Species:	Leaf-eating lepidoptera (including *Heliothis, Helicoverpa*) and Coleoptera on cotton, vegetable and field crops	
Species Stage	Larvae	
Product Class:	organophosphates, carbamates, pyrethroids, organochlorines and insect growth regulators	
Comments: None		

Description:

Materials:
Insect-proof containers, scissors, forceps, 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, paper towels, maximum/minimum thermometer.

Method:

(a) Collecta representative sample of insects from a field. These may be larvae suitable for immediate testing, or eggs/L1 larvae for rearing to the appropriate stage or material from which an F1 population for testing can be reared. The insects should not be subjected to temperature, humidity or starvation stress after collection.

(b) Collect sufficient non-infested, untreated leaves. Whole leaves are preferred or, for some crops, the distal portions. Do not allow leaves to wilt.

(c) Prepare accurate dilutions of the test compound from identified commercial product. For initial studies, five widely spaced rates are recommended. 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.

(d) Dip leaves individually in the test liquid for 5 s with gentle agitation and place to surface-dry on paper towelling. Do not allow to wilt. Dip the same number of leaves per treatment, and treat sufficient leaf material to avoid starvation stress in the “untreated” during the test. Commence dipping the “untreated” first and work up through the test dilutions.

(e) Place the treated surface-dry leaves in the labelled test containers, which must be suitable for holding enough leaf material in good condition for up to 3 days.

(f) Add equal numbers of neonate larvae (*Heliothis/Helicoverpa*) or recently molted L2 larvae to each container, so that a minimum total of 40 larvae are used per treatment, divided between at least four replicate containers. It only one leaf surface is accessible to the larvae, ensure that this is the correct one for the species involved. If cannibalism is a problem (e.g. *Heliothis* and *Helicoverpa* spp.), reduce the number or larvae per container, but increase the replication.

(g) 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 25°C is preferred.

(h) In the case of rapidly acting compounds, a final assessment of larval mortalities[2] is made after 48 h. For slowly acting compounds (e.g. benzoylureas, insect growth regulators etc.) a first assessment is made at 72 h, when the leaves are changed for fresh leaves treated, as before, with the appropriate insecticide dilution. The containers are held for a further period before the final assessment, either for 72 h or until larvae in the "untreated" (control) have moulted again.

(i) Express results as percentage mortalities, correcting for "untreated" (control) mortalities using Abbott's formula. Untreated mortality should be recorded.

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. At each assessment, larvae are classed as either: (a) unaffected, giving a normal response (such as taking a coordinated step) when gently stimulated by touch, or (b) dead or affected, the latter giving an abnormal response to stimulation or showing abnormal growth which should be described. For some benzoylureas, colour changes are reliable indicators of effectiveness and should be recorded. Thus, % response (‚mortality') will include both dead and affected.

References & Acknowledgements:

None

IRAC Susceptibility Test Methods Series

Method No: 8

Version: 2

Details:

Method:	No 8	*Photograph Courtesy of: Central Science Laboratory, Harpenden Archives, British Crown*
Status:	Approved	
Species:	Bemisia tabaci	
Species Stage	Adult	
Product Class:	amitraz	
Comments: None		

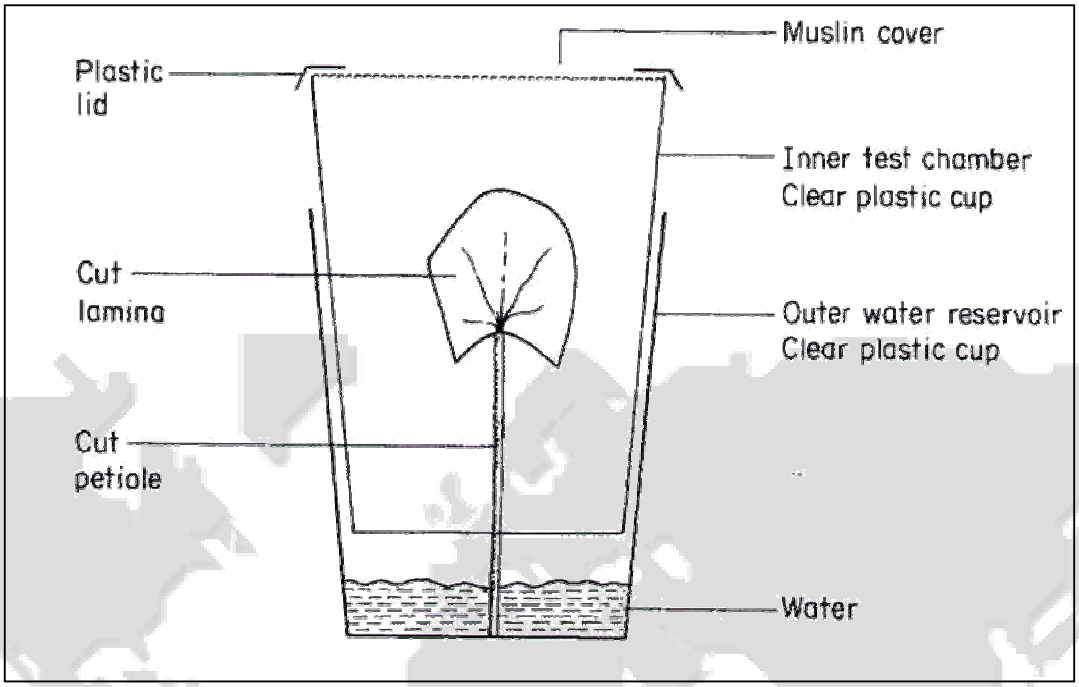
Description:

Materials:
Clean plastic cups with push-on plastic lids, plasticine, nylon muslin with very small mesh (approximately 10.000 apertures cm^{-2}) sharp knife or scalpel, scissors, glue, nail or other similar metal rod, untreated leaves.

Method:

(a) Turn a plastic lid upside down and cut a circular hole (approximately 4 cm diameter) with a scalpel.
(b) Cut a roughly circular piece if muslin (approximately 5.5 cm diameter) and glue to the inside of the prepared plastic lid.
(c) Heat the nail (or metal rod) and pierce a hole in the bottom of the plastic cup.
(d) Select uncontaminated cotton leaves and with scissors cut the petiole to a length of approximately 4 cm. Cut the lamina to give an approximately square area (2 x 2 cm). Prepare a minimum of four replicates per treatment.
(e) Prepare test liquids. The use of a wetter is not recommended. Agitate test liquids and then dip prepared leaves for 5 s holding the leaf by the petiole.
(f) Leave the leaves to dry in the open air (approximately 5 min).
(g) Pass the petiole of a test leaf through the hole in the plastic cup until it protrudes by approximately 1 cm.
(h) Fix the petiole in position by pressing a small ball of plasticine around the petiole where it enters the cut hole.
(i) Take the cup to an infested cotton plant and with the lid on the upper surface of an infested leaf move the cup upwards onto the underleaf surface. Move the lid and cup together sideways off the leaf to trap the adult whiteflies. Repeat procedure until approximately 50 adults per cup have been collected. Place a small amount of water in a second plastic cup and stand the test cup inside this so that it is supported by the protruding petiole.
(j) Record number of adults per cup. The whiteflies will settle on the cut leaf.
(k) Hold containers, in constant conditions wherever possible, for 48 h. Extremes of temperature should be avoided, 25°C is preferred. Containers must not be exposed to direct sunlight.
(l) Record living and dead whiteflies after 48 h.
(m) Express results as percentage mortality and correct for untreated mortality using Abbott's formula. Untreated mortality should be recorded.

Diagram:



Percautions & Notes:

- The test chambers must be very dry as whiteflies die if they get caught up in water.
- Some of the insects may become stuck to the surface of the test chamber because of the static charge present.
- If the compound under test has repellent properties, the whiteflies will not settle on the test leaf and this may affect mortality.

References & Acknowledgements:

None

IRAC Susceptibility Test Methods Series

Method No: 9

Version: 2

Details:

Method:	No 9	*Leucoptera scitella mines* *Photograph Courtesy of:* Syngenta
Status:	Approved	
Species:	Leucoptera scitella *Lithocolletis blancardella*	
Species Stage	Eggs Larvae	
Product Class:	IGRs e.g. diflubenzuron, novaflumuron, teflubenzuron and flufenoxuron	
Comments: None		

Description:

Materials:
Clean plastic cups with push-on plastic lids, plasticine, nylon muslin with very small mesh (approximately 10.000 apertures cm^{-2}) sharp knife or scalpel, scissors, glue, nail or other similar metal rod, untreated leaves.

Method

(a) At the start of adult flight, select terminals on upper half of tree. Select five unmined leaves from each terminal (1 replicate). Cut off terminal above selected leaves and remove several leaves below the five unmined leaves. Attach treatment label where leaves have been removed.

(b) Prepare 1 litre of each test liquid containing 0.2 % wetter (such as Agral). Agitate test liquids and dip selected leaves for 2 s, replicating each treatment 30 times. Dip equal numbers of terminals in water (untreated control).

(c) Assess *Leucoptera scitella* after 3 weeks by recording total number of mines and numbers of mines of greater than 3 mm diameter. Assume mines of less than 3 mm diameter are ‘dead‘. Calculate percent mortality for each treatment.

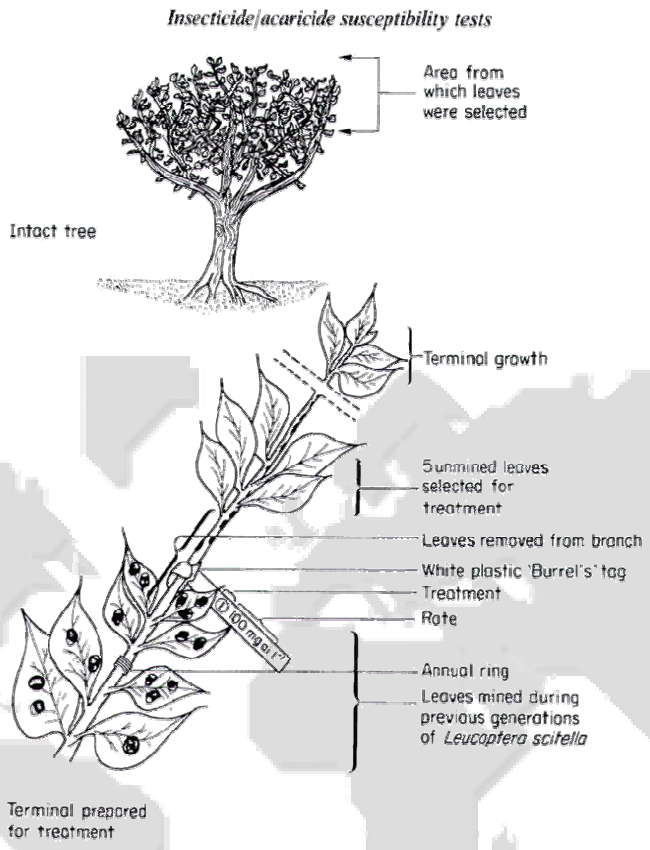
(d) Assess *Lithocolletis blancardella* after three weeks, by recording total numbers of mines and numbers of mines where damaged leaf tissue is clearly visible from both upper and lower leaf surfaces. Assume mines where leaf damage is only visible from the lower surface are ‘dead‘. Calculate percent mortality for each treatment.

IRAC Susceptibility Test Methods Series

Method No: 9

Version: 2

Diagram:



Percautions & Notes:

For the insecticides listed, concentrations of around 50 mg a.i. l^{-1} are suggested for discrimination between resistant and susceptible types. This method is not suitable for systemic insecticides.

References & Acknowledgements:

None

IRAC Susceptibility Test Methods Series

Method No: 10a

Version: 2

Details:

Method:	No 10a	*Photograph Courtesy of: Jack T. Reed, Mississippi State University*
Status:	Approved	
Species:	*Frankliniella occidentalis*	
Species Stage	adults	
Product Class:	spinosad, acephate, acrinathrin, chlorfenapyr, chlorpyrifos-methyl, deltamethrin, endosulfan, fenpropathrin, formetanate, methiocarb	
Comments: None		

Description:

Materials:
Plastic 30-ml Medi cups and lids or similar vials with closure, pods of French, haricot or green beans, aspirator/pooter, 200-ml glass beakers, six 250-ml measuring cylinders and pipette for dilution of formulations, wetter (Tween 20 or equivalent), Vaseline or liquid paraffin, mesh, fine sable brush, hand lens (minimum 10x) or binocular microscope, max./minimum thermometer.

Sampling:
Collect adult thrips from the test locality by sampling flowers and shaking them into a clean plastic bag to dislodge the thrips. Maintain thrips in a cool place (e.g. cooler box) to reduce activity. Adult thrips may be collected and transferred from the bag using an aspirator. Use only healthy live thrips for bioassay.

Method:
a) Cut bean pods into 20mm length sections and seal the cut ends with Vaseline or liquid paraffin. This prevents thrips from hiding in the cracks of the cut ends and the bean sections from drying out.

b) Prepare test solutions by dilution of formulations in distilled water containing 0.005% Triton X-100, Tween 20 or equivalent emulsifier to act as a supplementary wetting agent at very low insecticide concentrations. Formulations should be pre-mixed at a high concentration first to ensure the creation of a stable formulation before dilution to the recommended test concentrations. Adequate wetting of plant surfaces may be achieved by adding one drop of wetting agent to each 100ml of test solution.

(c) Starting with the control (water/wetter only) progress from low to high concentrations. Pour 150-ml of test solution into a 200-ml beaker, immerse the bean sections for 30s, constantly agitating the solution to ensure adequate contact.

(d) After immersion, place the dipped bean sections in a warm dry area on a raised mesh surface to dry. When dry, place the treated bean sections in labelled Medi cup vials, one per vial. Use a minimum of five replicates per treatment.

(e) Tap approximately 20 adult thrips into each vial and seal the lid.

(f) Store the vials in a warm humid area (25°C/70%RH) for 24h, avoiding exposure to direct sunlight for the requisite period.

(g) Using a hand lens or binocular microscope assess mortality after 24h. Use a paintbrush to stimulate individual thrips, recording those that are incapacitated or fail to show any signs of movement as 'dead'. Record the total number of thrips and the number of dead thrips per vial and enter onto the data sheet.

(h) Express the results as percentage mortality and correct for untreated mortality using Abbott's formula. Untreated mortality and other relevant test information should be quoted.

Percautions & Notes:

Ideally, fresh solutions should be prepared for each bioassay using a premixing procedure that ensures formulation integrity. If material is limited, stock solutions can be stored in a refrigerator for a period of a few days and fresh test solutions prepared on a daily basis.

References & Acknowledgements:

None

IRAC Susceptibility Test Methods Series

Method No: 10b

Version: 2

Details:

Method:	No 10b	*Photograph Courtesy of: Jack T. Reed, Mississippi State University*
Status:	Approved	
Species:	*Frankliniella occidentalis*	
Species Stage	Larvae	
Product Class:	for insect growth regulators e.g. lufenuron	
Comments: None		

Description:

Materials:
Plastic 30-ml Medi cups and lids or similar vials with closure, pods of French, haricot or green beans, aspirator/pooter, 200-ml glass beakers, six 250-ml measuring cylinders and pipette for dilution of formulations, wetter (Tween 20 or equivalent), Vaseline or liquid paraffin, mesh, foreceps, fine sable brush, hand lens (minimum 10x) or binocular microscope, max./minimum thermometer.

Sampling:
Collect adult thrips from the test locality by sampling flowers and shaking them into a clean plastic bag to dislodge the thrips. Maintain thrips in a cool place (e.g. cooler box) to reduce activity. Adult thrips may be collected and transferred from the bag using an aspirator. Use only healthy live thrips for bioassay purposes.

Method:
a) Cut bean pods into 20mm length sections and seal the cut ends with Vaseline or liquid paraffin. This prevents thrips from hiding in the cracks of the cut ends and the bean sections from drying out.

b) Thrips larvae of the required age for bioassay are obtained by breeding. To obtain eggs, transfer ca. 20 adult female thrips to a Medi cup supplied with a freshly cut pod section for a period of 8h or overnight, after which time the thrips should be removed. Incubate eggs at 25°C, minimum photoperiod of 16h and moderately high light intensity (not direct sunlight), until the desired developmental stage is reached.

b) Prepare test solutions by dilution of formulations in distilled water containing 0.005% Triton X-100, Tween 20 or equivalent emulsifier to act as a supplementary wetting agent at very low insecticide concentrations. Formulations should be pre-mixed at a high concentration first to ensure the creation of a stable formulation before dilution to the recommended test concentrations. Adequate wetting of plant surfaces may be achieved by adding one drop of wetting agent to each 100ml of test solution.

(c) Starting with the control (water/wetter only) progress from low to high concentrations. Pour 150-ml of test solution into a 200-ml beaker and dip the bean sections using forceps for ca. 30s ensuring complete immersion.

(d) After immersion, place the dipped bean sections in a warm dry area on a raised mesh surface to dry. When dry, place the treated bean sections in labelled Medi cup vials, one per vial. Use a minimum of five replicates per treatment.

(g) 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 incubate at 25°C and record IGR effects at regular intervals up to 7 days.

(i) Express results as percentage mortality and correct for untreated mortality using Abbott's formula. Untreated mortality and other relevant information should be recorded.

Percautions & Notes:

Ideally, fresh solutions should be prepared for each bioassay using a premixing procedure that ensures formulation integrity. If material is limited, stock solutions can be stored in a refrigerator for a period of a few days and fresh test solutions prepared on a daily basis.

References & Acknowledgements:

None

IRAC Susceptibility Test Methods Series

Method No: 11

Version: 1

Details:

Method:	No: 11
Status:	Approved
Species:	Pollen Beetle, *Meligethes aeneus*
Species Stage:	Adults
Product Class:	Synthetic pyrethroids

Comments: The method was developed as a result of discussions within the German Expert Committee on Pesticide Resistance – Insecticides (ECPR-I), and is a modification of a monitoring method formerly used by Bayer Crop Science and Syngenta. It is currently being widely used in Western Europe for monitoring sensitivity of *Meligethes aeneus* populations in oilseed rape to synthetic pyrethroids.

Description:

Materials:
Insect-proof containers, fine pointed brush, beakers for test liquids, syringes/pipettes for liquids or weighing balance for solids, acetone, syringes/pipettes for making dilutions, 20ml glass vials, vial roller (or hotdog roller), small funnel to transfer beetles to vials, binocular microscope or hand lens, paper towels, maximum/minimum thermometer.

Method:

(a) Collect approximately 500 adult beetles at different locations across the infested field. Store beetles in an aerated plastic container. Place some dry paper towel at the bottom of the container, and add some oil seed rape leaves plus two or three rape inflorescences as food source (Figure 1). The insects should not be subjected to excessive temperature, humidity or starvation stress after collection.

(b) Use the attached recording sheet for sampling details and other information that maybe useful for tracking samples and interpreting susceptibility results later on.

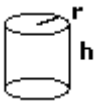
(c) Ship the containers as quickly as possible to the test lab. Make sure that the beetles can be tested directly after arrival of the container. Containers with beetles can be stored in a refrigerator overnight, though prolonged storage is undesirable.

(d) The standard test synthetic pyrethroid is lambda-cyhalothrin (technical available from

Fluka). Other synthetic pyrethroids can be used, but the vial application concentrations may need to be adjusted to take account of differences in inherent potency between different pyrethroids.

(e) The test containers are glass vials with an internal surface area of 20-80 cm^2. Newly purchased vials should be cleaned of potential residues from their manufacture by soaking overnight in soapy water, rinsing with acetone and air drying for at least 4 hours before use.

(f) Determine the surface area of the glass vials by:



(h is the height of the vial, r is the radius of the bottom)
Surface Area = Area of bottom + Area of the side
Surface Area = $\pi r^2 + (2 \pi r) * h$

(g) Prepare accurate dilutions of the technical grade compound in acetone. For lambda-cyhalothrin suitable test concentrations in µg per cm^2 glass surface have been determined as follows:
0.075 µg/cm² (100% of the European field application rate of 7.5 g ai/ha),
0.015 µg/cm² (20% rate),
0.003 µg/cm² (4% rate),
acetone only Control,
Optionally a 500 % rate can be useful to obtain good dose response curves.
Freshly prepared test solutions can be stored sealed and cooled for approximately 8 hours.

(h) Glass vials should be filled with 500-1500 µl (depending on vial size) of solution and rotated at room temperature until the acetone is completely evaporated. At the start of the coating process the interior walls of the vials should be completely covered by the test solution.

(i) Two replicates of each concentration and control are required (i.e. 10 vials per test).

(j) Place ten beetles per vial (a funnel can be helpful in transferring the beetles to the vial), cap and store upright at 20 ± 2ºC (Figure 2).

(k) After five hours (optionally recordings at additional intervals of 1h and 24h may be useful), shake the vials briefly and count dead, moribund and alive beetles.

(l) Express results as percentage mortalities, correcting for "untreated" (control) mortalities using Abbott's formula. Untreated mortality should be recorded.

Figure 1. Collecting pollen beetles in an oilseed rape field

Figure 2. Example of treated glass vials containing pollen beetles

Recording Sheet:

Sampling details	
Susceptibility problem previously apparent:	Yes/no
Date of beetle collection:	
Address:	
Name of collector	
Street	
Postal code	
City	
Region	
Geographical position (GPS), if available:	
Crop:	
Average number of insecticide applications in the region:	
Insecticide applications in the sampled field:	
Product	
Application date	
Number of applications	

Assessment Sheet:

5h-assessement

	Replicate 1			Replicate 2		
Application rate	Dead	Alive	%mortality	Dead	Alive	%mortality
500%						
100%						
20%						
5%						
Control						

Optional : 1h-assessment

	Replicate 1			Replicate 2		
Application rate	Dead	Alive	%mortality	Dead	Alive	%mortality
500%						
100%						
20%						
5%						
Control						

Optional : 24h-assessment

	Replicate 1			Replicate 2		
Application rate	Dead	Alive	%mortality	Dead	Alive	%mortality
500%						
100%						
20%						
5%						
Control						

Example data from a pyrethroid susceptible population

5h-assessment

	Replicate 1			Replicate 2		
Application rate	Dead	Alive	%mortality	Dead	Alive	%mortality
500%	12	0	100	11	0	100
100%	16	0	100	10	0	100
20%	5	5	50	7	3	70
5%	5	8	38	6	5	38
Control	0	11	0	0	10	0

Optional : 1h-assessment

	Replicate 1			Replicate 2		
Application rate	Dead	Alive	%mortality	Dead	Alive	%mortality
500%	12	0	100	11	0	100
100%	16	0	100	10	0	100
20%	4	6	40	7	3	70
5%	5	8	38	5	6	45
Control	0	11	0	0	10	0

Optional : 24h-assessment

	Replicate 1			Replicate 2		
Application rate	Dead	Alive	%mortality	Dead	Alive	%mortality
500%	12	0	100	11	0	100
100%	16	0	100	10	0	100
20%	6	4	60	7	3	70
5%	5	8	38	6	5	55
Control	0	11	0	2	8	20

Example data from a pyrethroid resistant population

5h-assessment

	Replicate 1			Replicate 2		
Application rate	Dead	Alive	%mortality	Dead	Alive	%mortality
500%	10	2	83	12	1	92
100%	5	6	45	9	3	75
20%	2	10	17	1	18	5
5%	2	19	10	0	11	0
Control	0	12	0	0	12	0

Optional : 1h-assessment

	Replicate 1			Replicate 2		
Application rate	Dead	Alive	%mortality	Dead	Alive	%mortality
500%	10	2	83	11	2	85
100%	3	8	27	3	9	25
20%	1	11	8	1	18	5
5%	2	19	10	0	11	0
Control	0	12	0	0	12	0

Optional : 24h-assessment

	Replicate 1			Replicate 2		
Application rate	Dead	Alive	%mortality	Dead	Alive	%mortality
500%	12	0	100	13	0	100
100%	6	5	55	10	2	83
20%	3	9	25	2	17	11
5%	3	18	14	0	11	0
Control	0	12	0	2	10	17

Precautions & Notes:

1. Where glass equipment is used it must be adequately cleaned with an appropriate organic solvent before re-use to prevent cross-contamination.
2. Different batches of technical grade insecticide may vary in concentration of active ingredient (usually between 85-99% a.i.). Purity needs to be taken into account when preparing the test solutions.

References & Acknowledgements:

Thanks to Juergen Langwald, BASF AG, for translating the method text from German to English and to Russell Slater, Syngenta, for comments on the method. Figures 1 & 2 are courtesy of Syngenta.

IRAC Susceptibility Test Methods Series

Method No: 12a
Version: 1

Details:

Method: No: 12a

Status: Approved

Species: Whiteflies, *Trialeurodes vaporariorum* and *Bemisia tabaci*

Species Stage: Adults

Product Class: Neonicotinoids, pymetrozine, pyrethroids, organophosphates and other whitefly adulticides

Photograph Courtesy of: Central Science Laboratory, Harpenden Archives, British Crown

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.

Description:

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

Method:
(a) Ventilate the Petri-dish lids with small holes with a diameter too small for the whiteflies to escape 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 necessitate using a different concentration than 1%, so experiment first to determine the required agar to water ratio for the required level of gelling.

(c) Use the metal tube to cut leaf-discs from clean, untreated host plant leaves.
(d) Immerse the leaf discs into serial dilutions of 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 three replicates per concentration, including the control.
(f) Once the agar has cooled and set, lay a 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 anaesthetize the whiteflies using carbon dioxide to facilitate transfer. Place the ventilated lid onto the Petri-dish base and ensure a close-fitting 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 leaf disc is abaxial (lower) surface down and the adults oriented normally.
(i) Hold the dishes under natural light or in a light cycling incubator at 20 ± 2ºC.
(j) Assess mortality at 48 hours after treatment using a binocular microscope or hand lens.
(k) Express results as percentage mortalities, correcting for "untreated" (control) mortalities using Abbott's formula. Untreated mortality should be recorded.

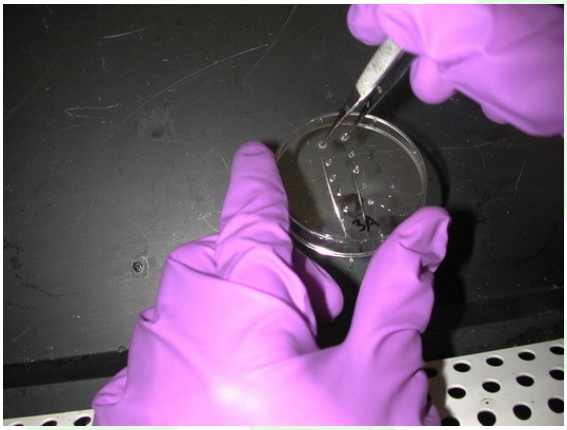


Figure 1. Preparing Petri dishes with ventilated lids

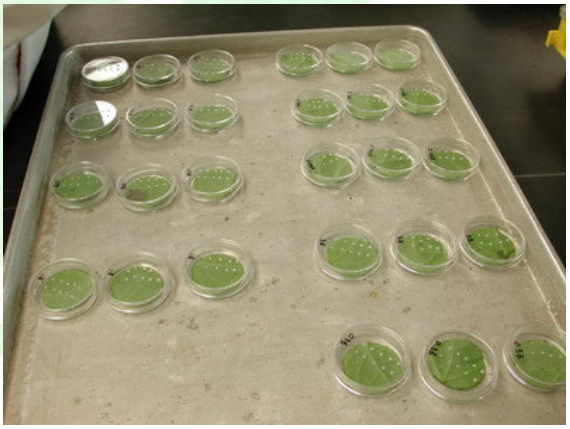


Figure 2. Bioassay in progress showing Petri dishes containing treated leaf disks and whiteflies

Precautions & Notes:

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

Example Data:

Results from field collected *Bemisia tabaci*
Totals of three replicates

Insecticide	Concentration (ppm)	No. Alive	No. Dead	% mortality	Corrected % mortality*
Control	0	62	2	3.1	
Imidacloprid	0.06	53	3	5.4	2.4
	0.1	57	8	12.3	9.5
	0.6	40	19	32.2	30.0
	3	7	60	89.6	89.3
Pymetrozine	0.3	45	6	11.8	9.0
	1	40	16	28.6	26.3
	3	31	20	39.2	37.3
	10	13	33	71.7	70.8

*Abbott's formula: Corrected % mortality = (% alive control - % alive treated) x 100% / (% alive control)

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.

IRAC Susceptibility Test Methods Series

Version: 2

Annex 1

IRAC INSECTICIDE/ACARICIDE SUSCEPTIBILITY TESTS
DATA RECORDING SHEET; PART I

General information

IRAC test method no. .....

Investigator.....

Address.....
.....

Details of insects/mites collected

Species/stage.....

Crop/cultivar (where applicable).....

Country/location/date.....

Brief history of recent insecticide/acaricide use. .....
……

Test details

Treatment date/assessment date .....

Insect/mite stage used.....

Holding conditions (min/max °C, humidity).....….....

Type of container.....

Plant material (crop, cultivar, size etc.).....

Test insecticide/acaricide

Active ingredient formulation.....

Origin/batch no./date.....

Additional wetter if used: specify.....…

Remarks

.....

For further information please contact the IRAC International Coordinator:
Alan Porter: aporter@intraspin.com
www.irac-online.org

IRAC Susceptibility Test Methods Series

Version: 2

Annex 1

IRAC INSECTICIDE/ACARICIDE SUSCEPTIBILITY TESTS - DATA RECORDING SHEET; PART II*

		Replicate 1		Replicate 2		Replicate 3		Replicate 4		Replicate 5		Replicate 6Ψ		Overall total			
Compound	Dose (% a.i.)	Dead/ moribund	Total	Dead/ moribund	Total	Dead/ moribund	Total	Dead/ moribund	Total	Dead/ moribund	Total	Dead/ moribund	Total	Dead/ moribund	Total	% Mortality	% corrected Mortality+
Intreated control																	

* Must be accompanied by Part I

Ψ If more than 6 replicates use second line

\+ Corrected mortality = $\frac{P}{100} \frac{C}{C} \times 100$ where P = % mortality in treatment and C = % mortality in controls

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