

Session 3

**International Working Group & Country Group Review
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Methods WG

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Team Members

- **Tatjana Sikuljak, BASF (Interim Chair)**
- **Harald Köhler, Bayer Crop Science (Vice Chair)**
- **Jean-Luc Rison, DuPont**
- **Lixin Mao, BASF**
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- **Shuvash Bhattarai, Chemtura**
- **Chouaibou Mouhammadou, Vestergaard-Frandsen**
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Team Goals

Team Goals:

- To develop a single point of contact for researchers to gain information on how to conduct insecticide resistance bioassays.
- To provide IRAC approved methods in order to steer researchers to use these validated methods, so that data generated by independent researchers can be compared directly.

What are we doing to meet these goals:

- Development of a searchable database for finding both IRAC approved methods and those which are used by researchers but have not been approved by IRAC.
- Increasing diversity and rate of validation of IRAC approved methods, including public health pests, biotechnology methods, biochemical methods and molecular techniques.

- Number of approved methods: 19
- Methods posted as “under review”:
 - Armyworms and bollworms (larvae/diamides)
 - *Meligethes aeneus* (adults/neonicotinoids)
 - *Tuta absoluta* (L2/oxadiazins, diamides, spinosins)
 - *Myzus persicae* (nymphs/cyantraniliprole)
 - *Aphis gossypii* (apterous adults, nymphs/cyantraniliprole)
 - *Meligethes spp.* (adults/organophosphates)
 - *Musca domestica* (adults/imidacloprid) – about to be finalised
- Methods under review (diamides):
 - Colorado potato beetle
 - Rice stemborer
 - Rice leaffolder
 - Whiteflies

- **Total number of references: 148 !**

- **Reference covering a broad range of species:**
 - aphids
 - thrips
 - cutworms
 - stinkbugs
 - leafminers
 - scales
 - mealybugs
 - weevils
 - flea beetles
 - wireworms

- and public health pests:**
 - house fly
 - mosquitoes

Importance of Susceptibility Testing

One of the important factors governing the management of insecticide and acaricide use is the availability of sound baseline and monitoring data on the susceptibility of the target pest to the toxicant. Baseline data can be defined as data obtained from a strain with no selection history by 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 but unfortunately the results from different methods may not be comparable since they measure different parameters which can lead to difficulties over the interpretation of monitoring data.

IRAC has addressed this issue by establishing a Methods Working Group which evaluates and recommends a range of bioassay techniques for pest species of economic importance.

The goals of the team are:

- To establish a single contact point for researchers to gain information on how to conduct insecticide resistance bioassays
- To provide IRAC approved methods, so that data generated by independent researchers can be directly compared

To be able to continue providing additional methods we would like to encourage you to submit your testing methods to us.

Choice of Method & Limitations

Changes in insect and mite susceptibility to toxicants can take various forms, which often influences the suitability of given bioassay techniques. 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 IRAC recommended bioassays were chosen as being:

- Reliable and reproducible under field use allowing data comparisons
- Simple and easy to perform using a minimum of resources
- Consistent in distinguishing between susceptible and resistant phenotypes
- Relevant as far as possible to field performance of products
- Useful where possible for a range of toxicant groups

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 insect pest for one or more generations in laboratory or 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 testing conditions. These should be standardized as far as possible.

Sampling, Test Design & Analysis

Sampling Procedure

It is important that samples used in the test are 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 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 & Analysis

The choice of a susceptible baseline strain is critical in obtaining meaningful data as 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 susceptibility 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 χ^2 analysis (LC/LD) may be invalid if the model indicates a significant heterogeneity (Chi-square test).

Results should be recorded in terms of percentage mortality and corrected for mortality in the untreated control using Abbott's formula. A standard form is available on the IRAC website.

Results from susceptibility tests will not always relate directly to field performance due to 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 test do, however, give an indication of the potential for field control failure due to a change in susceptibility of the pest.



IRAC Methods & eMethods Database



IRAC Recommended Methods

No	Species	Stage	No	Species	Stage
001	<i>Spodoptera littoralis</i>	A	013	<i>Spodoptera littoralis</i>	A
002	<i>Spodoptera littoralis</i>	A	014	<i>Spodoptera littoralis</i>	L
003	<i>Spodoptera littoralis</i>	L	015	<i>Spodoptera littoralis</i>	A
004	<i>Spodoptera littoralis</i>	A	016	<i>Spodoptera littoralis</i>	L
005	<i>Spodoptera littoralis</i>	A	017	<i>Spodoptera littoralis</i>	L
006	<i>Spodoptera littoralis</i>	A	018	<i>Spodoptera littoralis</i>	L
007	<i>Spodoptera littoralis</i>	L	019	<i>Spodoptera littoralis</i>	A
008	<i>Spodoptera littoralis</i>	A	020	<i>Spodoptera littoralis</i>	L
009	<i>Spodoptera littoralis</i>	L	021	<i>Spodoptera littoralis</i>	A
010	<i>Spodoptera littoralis</i>	A	022	<i>Spodoptera littoralis</i>	L
011	<i>Spodoptera littoralis</i>	A	023	<i>Spodoptera littoralis</i>	N
012	<i>Spodoptera littoralis</i>	A	024	<i>Spodoptera littoralis</i>	N

Life Stages: A - Adult, L - Larvae, N - Nymph, E - Egg

IRAC eMethods

IRAC eMethods is a database, searchable by species and life stage, of IRAC recommended methods and those described by researchers but not evaluated or approved by IRAC. Example belows of search by **Species**:

Species	Method	Status
<i>Musca domestica</i>	adult (IRAC Method #1)	IRAC approved
<i>Psylla goss</i>	- all stages (IRAC Method #2)	IRAC approved
<i>Pemphigus ulmi</i> & <i>Tetraneura spp.</i>	- adults (IRAC Method #3)	IRAC approved
<i>Blattella germanica</i> & <i>Periplaneta cockroach</i>	- adults (IRAC Method #4)	IRAC approved
<i>Spodoptera littoralis</i>	- all stages (IRAC Method #5)	IRAC approved
<i>Acrostiphium succulentum</i>	- adults (IRAC Method #6)	IRAC approved
<i>Trialeurodes vaporariorum</i> & <i>Trialeurodes spp.</i>	- adult (IRAC Method #7)	IRAC approved
<i>B. tabaci</i> & <i>T. vaporariorum</i>	- nymph & egg (IRAC Method #8)	IRAC approved
<i>Cydia pomonella</i>	- larvae	IRAC approved
<i>Mytila didactyla</i>	- larvae	IRAC approved
<i>aphid</i>	- adult or nymph	IRAC approved



Tuta absoluta Method Video (Draft)

Insecticide Resistance Action Committee



Insecticide Resistance Action Committee

Goals & SMART Objectives 2011/2012

Goals	Objectives	Timeline
Develop single point of contact for insecticide and acaricide resistance monitoring methods	<ul style="list-style-type: none"> • Populate e-methods tool with a range of methods used to measure insecticide susceptibility against key agricultural, horticultural and public health pests. • Methods sourced from literature, companies and external contacts. • Populate e-methods with additional references 	Q3, Q4 2011/ Q1, Q2 2012
Develop single point of contact for insecticide and acaricide resistance monitoring methods	<ul style="list-style-type: none"> • Once established with new methodologies, promote new e-methods tool through e-connection and design of a poster to be used at industry events. • Finish a procedural video for Tuta method and initiate new procedural video for a different method 	Q2, 2011 Q4 2011 Q4 2011/Q1 2012
To provide IRAC approved methods in order to steer researchers to use these validated methods, so that data generated by independent researchers can be compared directly	<ul style="list-style-type: none"> • Deliver minimum of two new crop IRAC approved methods • Work with PH Team on possibility of approving or developing a mosquito methodology. Commission appropriate internal or external studies and validate as required to finalise methods for inclusion in the IRAC methods series. • Liaise with Biotech Team to deliver one Biotech method. 	Q2, Q3, Q4 2010/ Q4 2011 Q4 2012