IRAC Susceptibility Test Methods Series

Method No: 027

Version: 2.0

Method:	IRAC No. 027	 *Meligethes aeneus* adult *Photograph Courtesy of:DuPont Crop Protection*
Status:	Approved	
Species:	Pollen Beetle (*Meligethes aeneus)*	
Species Stage	Adults	
Product Class:	Oxadiazine (indoxacarb)	
Comments: This method, validated by Dr. Thieme (BTL Bio-Test Labor GmbH Sagerheide), is an adaptation of the vial test method (IRAC #11) currently used for monitoring sensitivity of *Meligethes aeneus* populations to pyrethroids.		

Objectives:

Susceptibility Baseline: ☒
Resistance Monitoring: ☒

Description:

Materials:

Insect-proof containers, fine pointed brush, glass beakers for test liquids, syringes/pipettes for liquids or weighing balance for solids, acetone, 20 to 40ml glass vials with lids, vial roller (or hotdog roller), small funnel to transfer beetles to vials, paper towels, ventilated holding cage, maximum/minimum thermometer.

Method:

(a) Collect approximately 300 adult beetles at different locations across an infested field. Store beetles in an aerated plastic container. Place some dry paper towel at the bottom of the container, and add some oilseed rape leaves plus two or three inflorescences as food source (Figures 1a, 1b). 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 may be useful for tracking samples and interpreting susceptibility results later on.

(c) Ship the containers as quickly as possible to the test laboratory. The transportation method should avoid excessive temperature, humidity or starvation stress.

(d) It is recommended that on arrival to the laboratory, the beetles be released into a ventilated holding cage and allowed to recover overnight.

(e) The standard test oxadiazine is formulated indoxacarb. No other active substance from this chemical family has been tested so far.

(f) The test containers are glass vials with an inner surface area of 20-40 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.

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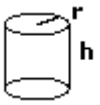
(g) Determine the surface area of the glass vials by:
h = height of the vial
r = radius of the bottom
Surface Area = area of bottom + area of the side
Surface Area = $\pi r^2 + (2 \pi r) * h$



(h) Prepare accurate dilutions of the formulated compound with 5% water + 95% acetone. In order to generate accurate estimates of the susceptibility of pollen beetles, a set of five to six concentrations of indoxacarb should be tested. The suitable test concentrations in ng ai per cm^2 inner glass surface have been determined as follows:

255 ng/cm² (100% of the European field application rate of 25.5 g ai/ha),
90.5 ng/cm²
63.75 ng/cm²
31.88 ng/cm²
9.4 ng/cm²
1.99 ng/cm²
Water + acetone only as Control

If it is not possible to test the full range of concentrations, 2 rates + the untreated control are then recommended as follows:

255 ng/cm² (100% of the European field application rate of 25.5 g ai/ha),
63.75 ng/cm² (25% of the European field application rate of 25.5 g ai/ha).
Water + acetone only as Control

(i) Glass vials should be filled with 500-1500 µl (depending on vial size) of solution and rotated at room temperature until the water/acetone mixture is completely evaporated, at least for 4 hours.

(j) Treated vials can be kept for 28 days after production, stored in a fridge (5 ± 2°C), or for 14 days stored at room temperature (20 ± 2°C).

(k) A minimum of three replicates of each concentration and control are required.

(l) Place a minimum of ten adult beetles per vial (a funnel can be helpful in transferring the beetles to the vial). It is recommended to avoid any hand contact with the beetles during the transfer. Transferring insects in a cool environment (cold chamber at 10°C) will slow down movement of the insects and may facilitate this operation. Then cap and store the vials upright at 20 ± 2ºC in a dark ct-room at 20 ± 2°C.

(m) After twenty-four hours, count affected (including dead and moribund) and alive beetles. Beetles, which cannot make co-ordinated movement over a period of 60 seconds, should be considered dead or moribund.

(n) Express results as percentage mortalities. If control mortality is greater than 20% the study should be considered as invalid for the purposes of resistance monitoring.

(o) No case of resistance or clear reduction of pollen beetle susceptibility has been detected since the beginning of susceptibility monitoring with indoxacarb, though it is not possible to propose a 'susceptibility rating scheme' at this stage. However, hereafter follows a few examples of results that should be expected:

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Table 1. Examples of adult pollen beetle percent mortality provided by the indoxacarb vial test after 24 hour exposure

Location	Rate (ng/cm^2)					
	1.99	9.4	31.88	**63.75**	90.5	**255**
AU-Gerhaus	7.7	89.7	100.0	**100.0**	100.0	**100.0**
DE-Freising	5.0	71.8	100.0	**100.0**	100.0	**100.0**
DE-Erbach	0.0	2.5	75.0	**100.0**	100.0	**100.0**
FR-Nambsheim 1	15.0	41.5	97.5	**100.0**	100.0	**100.0**
FR-Nambsheim 2	15.0	40.0	75.0	**97.5**	100.0	**100.0**
FR-St Loup de Varennes	16.3	85.4	100.0	**100.0**	100.0	**100.0**
HU-Latokep	2.4	70.0	100.0	**100.0**	100.0	**100.0**
HU-Sorkifalud	17.1	90.5	100.0	**100.0**	100.0	**100.0**
PL-Lipno	0.0	35.0	78.0	**100.0**	100.0	**100.0**
SU-VästraKlagstorp	0.0	37.5	92.5	**97.6**	100.0	**100.0**
UK-Boxworth	9.8	60.0	87.5	**100.0**	100.0	**100.0**

Testing only the two Discriminant Concentrations (DC) of indoxacarb (63.75 and 255 ng/cm²), one could expect the following response:
Expected mortality at DC rates: > 90%

Fig. 1a: Collecting pollen beetles in an oilseed rape field

Photograph Courtesy of: DuPont Crop Protection

Fig. 1b: Preparing pollen beetles before shipment to the laboratory for testing

Photograph Courtesy of: DuPont Crop Protection

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Sample Information 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 (active ingredient)	
Application date	
Number of applications	

24 hour Assessment Sheet:

	Replicate 1			Replicate 2			Replicate 3		
Application Rate	Affected	Alive	% affected	Affected	Alive	% affected	Affected	Alive	% affected
1.99 ng/cm^2									
9.40 ng/cm^2									
31.88 ng/cm^2									
63.75 ng/cm^2									
90.50 ng/cm^2									
255 ng/cm^2									
Control									

For further information please contact the IRAC International Coordinator:
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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. Two different formulations of indoxacarb have been tested so far: a solid WG formulation containing 30% indoxacarb and a liquid EC formulation containing 150 g/L of indoxacarb. Tested side by side, there was no significant difference in the response provided by these 2 formulations, which means both of them could be used for the evaluation of pollen beetle's susceptibility to indoxacarb.
3. The doses indicated for testing are expressed in ng/cm^2 of active substance.

References & Acknowledgements:

This method is an adaptation of the vial test method (IRAC #11) currently used for monitoring sensitivity of *Meligethes aeneus* populations to synthetic pyrethroids. This method has been validated by other researchers like JKI (Julius Kühn-Institut) and LfULG (Sächsisches Landesamt für Umwelt, Landwirtschaft und Geologie). Thanks to Dr T. Thieme for developing and validating the method in BTL laboratory (BTL Bio-Test Labor GmbH Sagerheide).