IRAC Susceptibility Test Methods Series

Method No: 021

Version: 3.4

Details:

Method:	IRAC No. 021
Status:	Approved
Species:	Pollen Beetle (*Meligethes aeneus)*
Species Stage	Adults
Product Class:	Neonicotinoids

Comments:
The methodology "**A**dult-**V**ial-**T**est for neonicotinoids", using thiacloprid as reference, is based on the IRAC Method No. 011 for synthetic pyrethroids. It can be used for monitoring sensitivity of *Meligethes spp.* populations in oilseed rape to neonicotinoids. Since their introduction neonicotinoids are widely used in Europe for pollen beetle control. To support the resistance management approaches in oilseed rape it is obligatory to carry out a sensitivity monitoring.

Objectives:

Susceptibility Baseline: ☒
Resistance Monitoring: ☒

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 200 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. Physically handling of the beetles should be reduced to a minimum.

(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 laboratory; 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 (or equivalent) and left to recover overnight.

(e) The standard test neonicotinoid is thiacloprid (using as the commercially available formulation 'Biscaya®' (240g thiacloprid / litre in oil dispersion, preliminary trials revealed that technical material is not appropriate). Other neonicotinoids were not tested yet.

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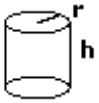
(f) The test containers are glass vials with an internal surface area of 20-80 cm^2. Newly purchased vials should be cleaned of potential residues from their manufacture by soaking overnight in soapy water, rinsing with acetone and air drying for at least 4 hours before use.

(g) 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 = $\pi r^2 + (2 \pi r)^* h$

(h) Prepare accurate dilutions of Biscaya® as follows:
Prepare the stock solution by solving Biscaya® in distilled water (2% of the total volume of solvent) ad acetone to 100% of solvent volume. All further dilutions will be made in pure acetone.
For thiacloprid suitable test concentrations in µg per cm^2 glass surface have been determined as follows:
1,44 µg/cm² (200% of the field recommended rate of 72 g ai/ha),
0,72 µg/cm² (100% field rate),
0.144 µg/cm² (20% field rate), and
acetone only for control,
Optionally a 4% and a 0.8% of the field rate can be useful to obtain good dose response curves. Rates over 200% aren't possible, because of the oil based formulation (the upper limit of 200% of oil-dispersed thiacloprid was determined by testing blank oil dispersion formulation without active ingredient).

(i) Glass vials should be filled with 500-1500 µl (depending on vial size, at the start of the coating process the interior walls of the vials should be completely covered by the test solution) of solution and rotated at room temperature until the acetone is completely evaporated. Because of the oil dispersion formulation and small amounts of water in the solutions, the vials have to rotate for a minimum of 2h and subjection to a further evaporation phase without rotation for a minimum of 2h (or overnight) is obligatory before locking and storing the vials. The prepared vials can be stored at room temperature (dark) for a minimum 8 weeks without a significant loss of thiacloprid.

(j) Two replicates of each concentration and control are required (i.e. 8 vials per test).

(k) 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).

(l) Prior to assessment of affected beetles (dead and moribund) after 24h vials are briefly shaken to differentiate alive and affected beetles more easily.

(m) Express results as percentage mortality, correcting for "untreated" (control) mortality using Abbott's formula. Untreated mortality should be quoted, if greater than 20%, the study should be considered as invalid.

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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 (active ingredient)	
Application date	
Number of applications	

For further information please contact the IRAC International Coordinator:
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Assessment Sheet:

Please find below mortality values ± SD one can expect at the different thiacloprid concentrations to be tested (values are based on 108 populations tested):

200% of field rate: **98% ± 3%**

100% of field rate: **93% ± 6%**

20% of field rate: **50% ± 10%**

24h-assessement

	Replicate 1			Replicate 2		
Application rate	Dead	Alive	%mortality	Dead	Alive	%mortality
200%						
100%						
20%						
Control						

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. This method was optimized for conducting Adult-Vial-Tests using an oil-dispersed formulation of thiacloprid. Other neonicotinoids and/or other formulations have to be verified before using in an Adult-Vial-Test.

References & Acknowledgements:

The thiacloprid Adult Vial Test including baseline and monitoring data is described in:

Zimmer and Nauen (2011) *Pest Management Science* **67**, 599-608

Figures 1 is courtesy of Syngenta
Figure 2 is courtesy of Bayer CropScience
Biscaya® is a registered trademark of Bayer CropScience

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