IRAC Susceptibility Test Methods Series

Method No: 025

Version: 2 (March 2014)

Details:

Method:	IRAC No. 025	
Status:	Approved	
Species:	Pollen Beetle, *Meligethes spp*	
Species Stage:	Adults	
Product Class:	Organophosphates	
Comments:		

Description:

Materials:
Insect-proof containers, fine pointed brush, glass beakers for test liquids, syringes/pipettes for liquids or weighing balance for solids, acetone, 20ml glass vials with lids, vial roller (or hotdog roller), small funnel or glass vial aspirator and Erlenmeyer to transfer beetles to vials, paper towels, ventilated holding cage, 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. The insects should not be subjected to excessive temperature, humidity or starvation stress after collection. Physical handling of the beetles should be reduced to a minimum. Leave empty space inside the bag. Before use, make sure the bag is hermetic – Check that it hasn't been damaged in the field. Make sure it is closed properly. Place the bag in an appropriate rigid cardboard box, in which it will be wedged without being too tight.

(b) Avoid overheating (direct sunrays on the parcel, vehicle heat during transport) and stock the insects in cool temperatures before giving them to package Delivery Company.

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

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

(e) It is recommended to test the population on arrival of it in the laboratory. If not, insects are stored into a ventilated holding cage for one night maximum at 13-15°C and tested the day after.

(f) The standard test organophosphate is chlorpyrifos (ethyl). Other organophosphates can be used, but the vial application concentrations may need to be adjusted to take account of differences in inherent potency between different organophosphates.

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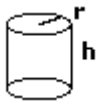
(g) The test containers are glass vials with an internal surface area of 20-80 cm^2. Newly purchased vials should be cleaned of potential residues from their manufacture by soaking overnight in soapy water, rinsing with acetone and air drying at room temperature for at least 4 hours before use.

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



Prepare accurate dilutions of the technical grade compound in acetone. For chlorpyrifos (ethyl) suitable test concentrations in µg per cm² glass surface have been determined as follows :
- 0,3 µg/cm² (16 % of the typical field application rate of 187,5 g ai/ha)
- Acetone only control

Note : Additional rates are required if a full dose response for the generation of accurate susceptibility data (LD50 values) are required.

(i) Glass vials should be filled with 500-1500 µl (depending on vial size, solution should cover base of vial when placed horizontally) of solution and rotated at room temperature (20°C - 25°C) until the acetone is completely evaporated. Two replicates of a minimum 1 concentrations and 1 control are required (i.e. 4 vials per test).

(j) Vials can be stored at -20°C for maximum 1 month. Don't not store and ship vials at room temperature.

(k) Place between ten and twenty adult beetles per vial (a funnel or glass vial aspirator, Erlenmeyer can be helpful in transferring the beetles to the vial). Close loosely (not tightly) the vial in order to let the chlorpyrifos vapor phase getting away from the vial. Store the vials at 20 ± 2ºC and avoiding exposure to an uneven light source or direct sunlight. Ensure that all vial is equally exposed to light (avoids beetles hiding in refuge of vial cap). Physical contact with the beetles should be kept to a minimum.

(l) The number of beetles severely affected (dead and moribund) is scored after a 24 hour period. The assessment is made by emptying the beetles from the glass vial onto the centre of a piece of paper with 8 cm circle drawn in the middle. The assessment should be made in bright light to stimulate beetle movement out the circle and the beetles that cannot exit the circle before a period of one minute should be considered severely affected.

(m) Express results as percentage affected. If greater than 20% of the beetles in the control treatment are severely affected, then the study should be considered as invalid for the purpose of resistance monitoring.

(n) At the date of this methodology, no resistance to OPs has been reported. Use the following "susceptibility rating scheme" to classify the susceptible level of populations.

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Rate	% affected	Classification	Code
0.3 µg/cm²	≤100 to 90	Susceptible	1
0.3 µg/cm²	<90	Potential to be tolerant. Re test using full dose response Report to IRAC	2

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:	
Recent insecticide applications in the sampled field:	
Product	
Application date	
Number of applications	

24 hour Assessment Sheet:

	Replicate 1			Replicate 2		
Application rate	**Affected**	**Alive**	**% affected**	**Dead**	**Alive**	**% affected**
0.3 µg/cm²						
Control						

For further information please contact the IRAC International Coordinator:
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Pollen beetle in ventilated holding cage

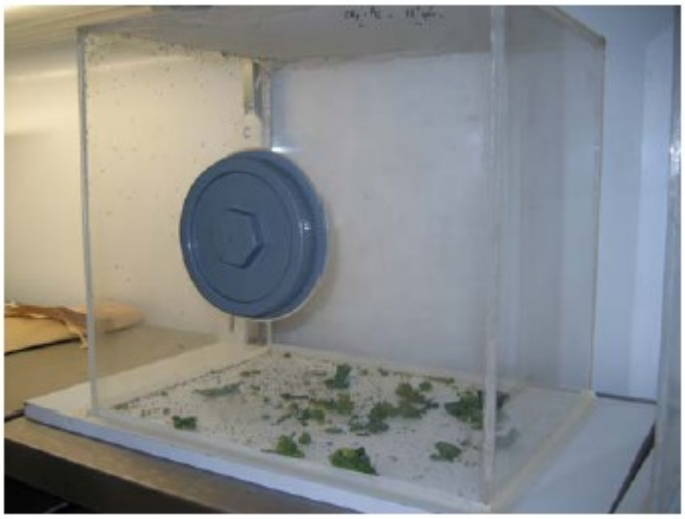
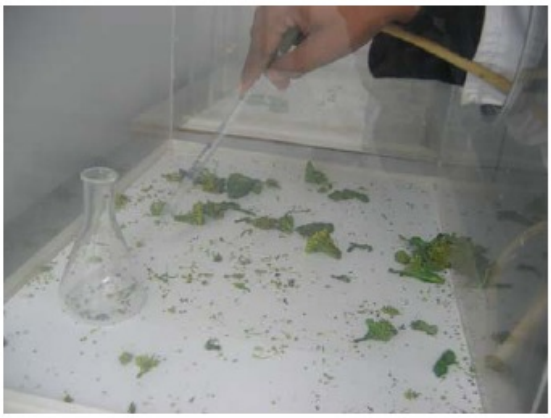


Photo : T.Martin (CIRAD)

Use of the glass vial aspirator to collect pollen beetle from the ventilated holding cage to the erlenmeyer



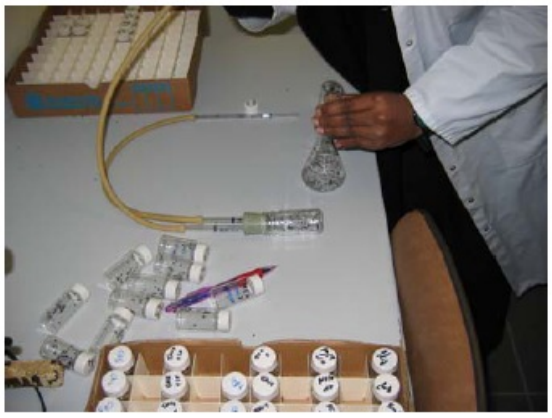


Photo : T.Martin (CIRAD)

Note: Any other adequate method can be used.

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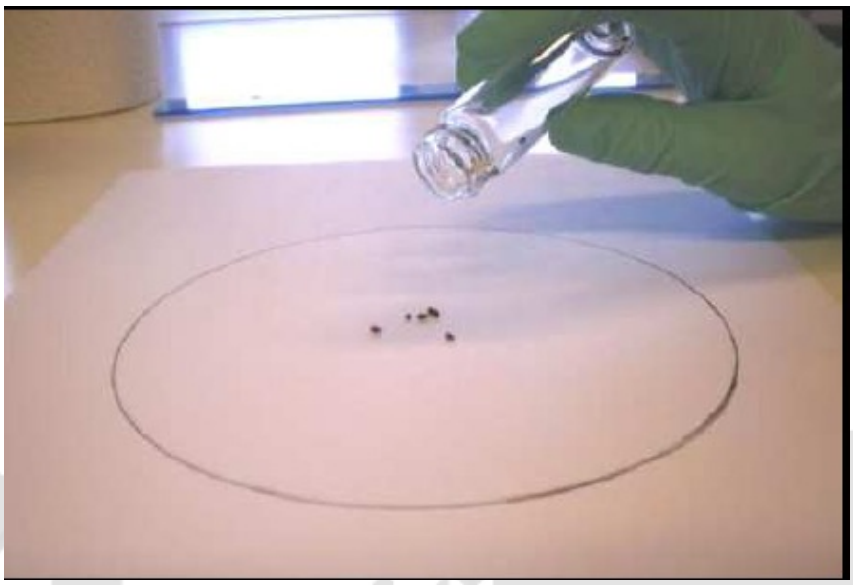
Assessment method of affected pollen beetle



References & Acknowledgements:

Reference to methodology: This method using chlorpyrifos (ethyl) as reference is adapted from the IRAC n°11 methodology used for pyrethroid. It was already been used in Europe for monitoring sensitivity of *Meligethes spp* populations in oilseed rape to OPs.
Thanks to all partners that have collaborated in designing and checking this methodology.

Pictures are courtesy of CIRAD (Centre de Coopération Internationale en Recherche Agronomique pour le Développement).

Literature references :

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