



Insecticide Resistance Action Committee



The first cases of diamide-resistant *Tuta absoluta* (Meyrick)

Summary:

The Tomato leaf miner *Tuta absoluta* has quickly become the major lepidopteran pest in protected tomato crops throughout the Mediterranean basin since its first occurrence in 2006-2007. Following its introduction into the region, the severe *T. absoluta* outbreaks have been successfully controlled by use of effective insecticides. *T. absoluta* is known for having a high propensity to evolve insecticide resistance. This article reports on the sensitivity monitoring executed in Italy, Spain and Greece since 2009. The results of the 2014 bioassays revealed the first cases of *T. absoluta* resistance to the diamide insecticides (MoA Group 28) chlorantraniliprole and flubendiamide in South-East Sicily. Another case was detected in Crete in 2015. The resistant strains feature high resistance ratios vs. sensitive strains. The ongoing molecular and inheritance studies suggest target-site mutation as the likely resistance mechanism. On-farm interviews have highlighted consistent and widespread abuse of diamide-based products over the last 5-6 years, with users not respecting the label prescriptions and neglecting good agricultural practices including IPM. Integrated control strategies are discussed, aiming to prevent further directional selection of resistance alleles via the adoption of stringent IPM strategies inclusive of reasoned IRM/MoA alternation programmes.

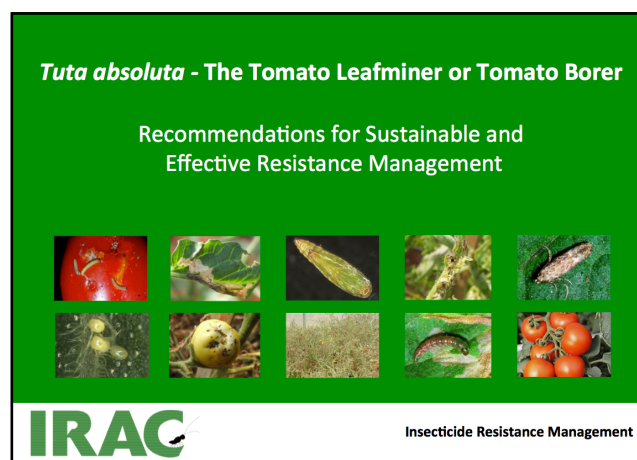


Figure 1. The front page of the IRAC *Tuta absoluta* booklet, in which it outlines that IRM (Insecticide Resistance Management) is functional to IPM and that the alternation of effective insecticidal MoA is essential to maintain insect susceptibility (1).

Baseline sensitivity bioassays:

When developing a new insecticidal mode of action for control of resistance-prone species a priority task is the determination of the baseline sensitivity, quantifying the insecticide toxicity to the field populations of the pest before commercial introduction. Having a reliable bioassay method is critical for both establishing the baseline sensitivity and the post commercialisation sensitivity monitoring surveys. The initial *T. absoluta* baseline sensitivity to diamide insecticides from Mediterranean countries and Latin America indicated a consistent and high *T. absoluta* sensitivity and the lack of cross-resistance to other insecticides. In 2009-2011 a collaborative research project validated the novel leaf-dip bioassay method for *T. absoluta* proposed by IRAC (2), and determined the baseline susceptibility of 23 pest populations collected from commercial greenhouses and fields in Spain, Italy and Greece. The baseline data indicated high sensitivity to chlorantraniliprole for all *T. absoluta* populations tested.

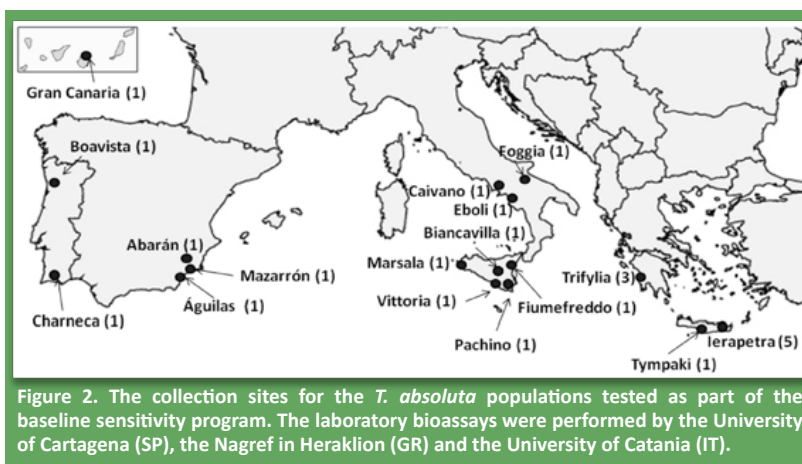


Figure 2. The collection sites for the *T. absoluta* populations tested as part of the baseline sensitivity program. The laboratory bioassays were performed by the University of Cartagena (SP), the Nagref in Heraklion (GR) and the University of Catania (IT).

Targeted sensitivity monitoring bioassays:

Triggered by reports of sub-optimal *T. absoluta* control in commercial tomato production, four *T. absoluta* populations were sampled from tomato greenhouses located in South-East Sicily (IT) in May 2014 and assayed at Nagref (GR) according to IRAC Method No. 022 to assess their sensitivity to chlorantraniliprole and flubendiamide. The comparative results of the *T. absoluta* baseline study versus the targeted monitoring in South-East Sicily (2014) are presented in Table 1.

Baseline (Sicily 2009-2011)					Field populations (Sicily 2014)								
Chlorantraniliprole					Chlorantraniliprole					Flubendiamide			
Population	Reps.	N.	LC ₅₀	CL 95%	Population	N	LC ₅₀	CL 95%	RR	N	LC ₅₀	CL 95%	RR
Pachino	2	384	0.58	0.21-1.21	NAGREF lab ref.	187	0.18	0.13-0.30	1	186	0.79	0.31-1.50	1
Vittoria F ₁	1	192	0.74	0.29-1.34	Pachino 1	189	47.6	31-77	265	127	993	384-1649	1257
Vittoria F ₂	1	224	0.78	0.44-1.28	Pachino 2	126	63.7	42-128	354	128	1376	792-2772	1742
Marsala	2	384	0.93	0.48-1.58	Acate (t2)	192	435	165-1193	2414	/	/	/	/
Fiumefreddo	2	384	1.34	0.49-2.86	Gela	191	225	135-343	1250	190	1019	500-2131	1289

Table 1. Results of baseline sensitivity (2009-2011) vis-à-vis targeted sensitivity bioassays (2014) of *T. absoluta* populations sampled in South-East Sicily. Estimated LC₅₀ based on log-dose probit-mortality data with chlorantraniliprole and flubendiamide. In all 2014 assays, the Nagref lab strain is the susceptible reference strain used. (3)

The 2014 bioassay results indicate high or very high resistant ratios (RR) for both diamide insecticides. Relative to the sensitive reference strain from Crete, resistance ratios were ranging from 265 to 2414. The complete set of bioassay results was published as the first confirmed case of *T. absoluta* resistance to diamide insecticides worldwide (4). In summer 2015, another case of diamide-resistance of *T. absoluta* was found in a Cretan greenhouse (unpublished data). The *T. absoluta* strains in question were kept under rearing for subsequent studies (e.g. cross-resistance to other MoA, fitness cost, mechanism of resistance). No indications for cross-resistance were found to other insecticides/MoA groups such as MoA Group 5 (e.g. spinosad), MoA Group 6 (e.g. emamectine benzoate), or MoA Group 22A (indoxacarb). Molecular studies are ongoing and suggest the involvement of target-site resistance to one of the resistant *T. absoluta* populations. Troczka *et al.* (5) have demonstrated that diamide-resistance in *P. xylostella* was associated with a mutation (G4946E) in the C-terminal membrane-spanning domain of the RyR (Ryanodine receptor). The functional implications of this target-site mutation associated with diamide resistance in diamondback moth were recently described by Steinbach *et al.* (6)

One of the key questions now in Sicily is the extent of the resistance phenomenon in greenhouse tomato crops and the level of impact this may have on *T. absoluta* control management in the area. It is of critical importance that the guidelines on rational use of diamides in pest management schemes for *T. absoluta* are strictly followed. Large-plot GEP trials conducted in 2015 in commercial greenhouse tomato crops in Sicily, South Spain and Greece have confirmed the benefit of applying integrated control strategies including IRM best practices. Implementing straight forward MoA alternation programs like the one highlighted in Fig. 3 are providing effective *T. absoluta* control in Sicily even under high pest pressure.

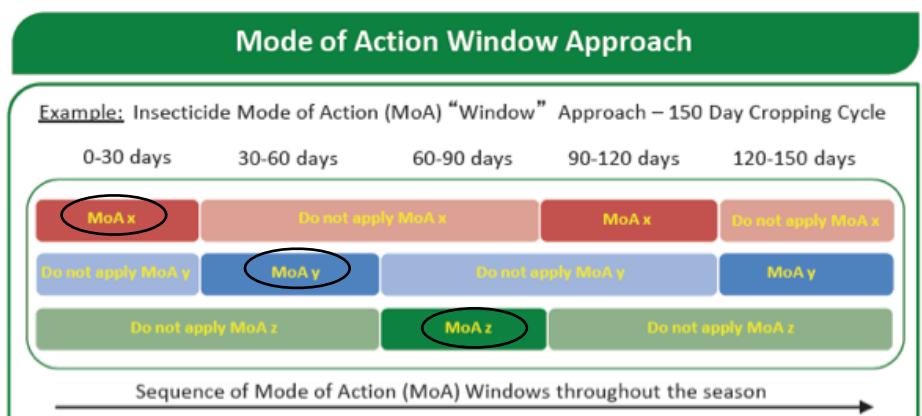


Figure 3. The simple yet robust MoA alternation scheme proposed in the IRAC *T. absoluta* booklet (1)

Development of high resistance levels to diamides might badly affect the current insect control management practices and potentially have a negative impact on tomato production in the region. Only IRM and IPM compliant control strategies will provide protection against further evolution of resistance in *T. absoluta* populations in the Mediterranean region. However reliance on insecticides alone will not provide the flexibility and sustainability required for a rational insect resistance management scheme, as part of an integrated pest management scenario. A reduction of the (ab)use of insecticides and in parallel adoption of IPM principles will be mandatory in order to mitigate directional selection of resistance in *T. absoluta* populations. The mode of action rotation for insecticide use is one of the key elements, but should be part of a wider IPM programme.

Conclusions:

Tuta absoluta was first found in the Mediterranean basin in 2006-2007. Since 2009 tomato growers have benefited from the high control levels provided by diamide-based products and the associated population reduction effects on *T. absoluta*. Diamide insecticides fit well within IPM programmes. This article shows that reliance on a single insecticidal mode of action (MoA) in commercial agriculture can lead to insect resistance in as little as 5-6 years from commercial introduction. Among the factors that can favour resistance to any insecticidal MoA, the intensity of usage is the main parameter that is having an overriding influence. Besides non-chemical means, currently a sufficient number of effective MoA is still available to farmers for IRM proof and effective *T. absoluta* control. Timely and effective efforts towards the adoption of IRM and IPM based pest control by growers will be vital in securing commercial tomato production in the Mediterranean region.

References:

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5. Troczka B, Zimmer CT, Elias J, Bass C, Davies TGE, Field LM, Schorn C, Williamson MS, Slater R, Nauen R (2012) Resistance to diamide insecticides in diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae) is associated with a mutation in the membrane-spanning domain of the ryanodine receptor. *Insect Biochemistry and Molecular Biology* **42**, 873-880
6. Steinbach D, Gutbrod O, Lümmer P, Matthiesen S, Schorn C, Nauen R (2015) Geographic spread, genetics and functional characteristics of ryanodine receptor based target-site resistance to diamide insecticides in diamondback moth, *Plutella xylostella*. *Insect Biochemistry and Molecular Biology* **63**, 14-22)

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