The first cases of diamide-resistant *Tuta absoluta (Meyrick)* and the alternation of the insecticidal modes of action as a key IPM practice for sustainable control.

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Abstract: This paper reports on the sensitivity monitoring executed by DuPont and collaborators in Italy, Spain, Greece since 2009 in order to detect possible sensitivity shifts of *T. absoluta* from baseline sensitivity to the diamide insecticide chlorantraniliprole[1] (MoA group 28). The results of the 2014 bioassays revealed the first cases of *T. absoluta* resistance to diamide insecticides in South-East Sicily. The resistant strains feature high resistance ratios *vs.* sensitive strains. Unpublished molecular and inheritance studies suggest target-site mutation as the likely resistance mechanism. On-farm interviews have highlighted consistent and widespread abuse of chlorantraniliprole-based products over the last 5-6 years, with growers not respecting product label directions for use and neglecting good agricultural practices including IPM. In this paper, potential 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.

Key words (and abbreviations): integrated pest management (IPM), insecticide resistance management (IRM), insecticide resistance action committee (IRAC), mode of action (MoA), ryanodine receptor (RyR), diamide insecticides, resistance ratio (RR), chlorantraniliprole, flubendiamide, *Tuta absoluta*.

Introduction

The Tomato leaf miner *Tuta absoluta* (Lepidoptera: Gelechiidae) 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 also by repeated use of effective insecticides. *T. absoluta* is known for having a high propensity to develop resistance to insecticides. The resistance reports are associated mainly to conventional chemistries such as organophosphates (OPs), pyrethroids and avermectins (Siqueira *et al.* 2000a, 2000b; Siqueira *et al.* 2001; Lietti *et al.* 2005; Silva *et al.* 2011; Haddi *et al.* 2012; Roditakis *et al.* 2013a). A recent study form Brazil confirmed field evolved resistance to spinosad (Campos *et al.* 2014a) while Roditakis *et al.* (2015) recently reported resistance to chlorantraniliprole in Italy and Greece.

Chlorantraniliprole (Rynaxypyr™[2]) was the first diamide insecticide to be extensively registered for use in greenhouse and field tomato across the Mediterranean countries featuring high efficacy on *T. absoluta,* extremely low mammalian toxicity and low toxicity to biological control agents (Bassi *et al.,* 2007, 2012). The first IRM accomplishment when a new active substance is being commercially developed for control of resistance-prone species like *T. absoluta* is the determination of the baseline sensitivity, quantifying the insecticide toxicity to the field populations

[1] MoA group 28 = ryanodine receptor modulator
[2] Registered trademark of E.I Du Pont de Nemours & Co. (Inc.)

of the pest before commercial introduction. Having a robust and easy to implement bioassay is critical for both establishing the baseline sensitivity as well as post commercialization sensitivity monitoring surveys. The initial *T. absoluta* baseline sensitivity to chlorantraniliprole from Mediterranean countries and Latin America indicated a consistent and high *T. absoluta* sensitivity and lack of cross-resistance to other insecticides. This paper reports on the first detection of diamide-resistant *T. absoluta* in Sicily in 2014 and outlines a sustainable approach with regards to the insecticide resistance management (IRM) of *T. absoluta.*

Material and methods

Baseline sensitivity bioassays

In 2009-2011 DuPont has coordinated a collaborative research aimed to validate the novel leaf-dip bioassay method for *T. absoluta* proposed by IRAC (IRAC, 2012), and to determine the product baseline susceptibility of 23 pest populations collected from commercial greenhouses and fields in Spain, Italy and Greece (Roditakis *et al.*, 2013a).

Figure 1. The collection sites for the *T. absoluta* populations tested as part of the baseline sensitivity program. In brackets the number of populations collected per site. The laboratory bioassays were performed by NAGREF in Heraklion (GR), the University of Cartagena (SP), and the University of Catania (IT).

Targeted sensitivity monitoring bioassays (2014)

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.

Results and discussion

The baseline bioassay results (Roditakis *et al.*, 2013a) indicated high sensitivity to chlorantraniliprole for all the *T. absoluta* populations tested. A relatively low variability was observed between the most and the least sensitive population tested (maximum a 6-fold difference in sensitivity) which can be attributed to natural variation. Comparing responses among groups, slightly different LC_{50} value ranges were obtained by the three different laboratories (respectively 0.10-0.56; 0.23-1.34; 0.04-0.24). Such variability between laboratories in sensitivity data is not rare in ring bioassays and could be explained by minor methodology differences (*e.g.* the tomato cv. adopted, the reading of moribund larvae). Therefore the IRAC method was deemed reliable, providing a useful tool in designing IRM strategies; however an independent bioassay repetition remains best practice to validate results whenever significant deviation from the baseline are being detected. The comparative results of the *T. absoluta* baseline study versus the targeted monitoring in South-East Sicily (2014) are presented in Table 1.

Table 1. Baseline sensitivity (2009-2011) vis-à-vis targeted sensitivity bioassays (2014) of *T. absoluta* populations sampled in South-East Sicily. Estimated LC_{50} 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.

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

The 2014 bioassay results indicate high or very high resistant ratios (RR) for both diamide insecticides. Relative to the sensitive reference strain from Crete (NAGREF Lab) resistance ratios were ranging from 265 to 2414. The complete set of bioassay results was published (Roditakis *et al.*, 2015) as the first confirmed case of *T. absoluta* resistance to diamide insecticides worldwide.

The populations 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 5 (*e.g.* spinosad), MoA 6 (*e.g.* emamectine benzoate), or MoA 22A (indoxacarb). Concerning the resistance mechanism, unpublished molecular studies have detected the target-site mutation G4946E (a glycine to glutamic acid substitution) in one of the resistant *T. absoluta* populations. Troczka *et al.* (2012) have demonstrated that diamide-resistance in *P. xylostella* was associated with the same mutation (G4946E) in the C-terminal membrane-spanning domain of the RyR (Ryanodine receptor).

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. In 2015, DuPont has been conducting trials in commercial greenhouse tomato crops in Sicily, South Spain and Greece evaluating the benefit of applying integrated control strategies including IRM best practices. Implementing straight forward MoA alternation programs like the one highlighted in fig. 3 have provided effective *T. absoluta* control in Sicily even under high pest pressure. (DuPont unpublished results, GEP trial data). It is therefore of critical importance that the guidelines on rational use of diamides in pest management schemes for *T. absoluta* are strictly followed. Only IRM and IPM compliant *T. absoluta* control strategies will provide protection against further expansion of insecticide resistance in *T. absoluta* populations in the Mediterranean region.

It is also important to mention the unprecedented efforts done proactively by the crop protection industry (IRAC, http://www.irac-online.org/) and the whole scientific community to prevent the onset of diamide resistance via strict label writing and educational activity (Teixeira *et al.* 2013).

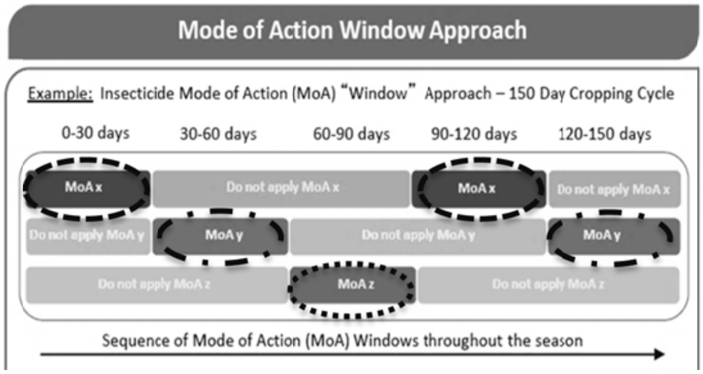

Figure 2. The simple yet robust MoA alternation scheme proposed by IRAC in their *T. absoluta* booklet.

Diamide insecticides in several regions such as Sicily are still considered the basis for tomato leafminer control due to their high efficacy and compatibility with the use of beneficial arthropods (parasitoids, predators and pollinators) (Dinter *et al.* 2008a, 2008b; Brugger *et al.* 2010; Biondi *et al.* 2012; Larson *et al.* 2014). This diamide dependency can be clearly illustrated by the high per cent usage of diamides in the sampled commercial greenhouses (between 50 and 90% of insecticide sprays). Despite the label prescription of maximum two applications per crop/year with chlorantraniliprole-based products in the European countries, on-farm interviews have highlighted consistent abuse of chlorantraniliprole-based products over the past 5-6 years. 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. 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.

The integration of chemical control with the use of beneficial arthropods, such as predators and parasitoids (Zappalà et al. 2012; Chailleux et al. 2013; Mollá et al. 2014) and suitable cultural, physical and other non chemical methods should be further investigated and implemented. 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. All possible tools need to be exploited to suppress further spread of diamide resistance in a devastating resistance-prone pest such as *T. absoluta*. The mode of action rotation for insecticide use is one of the key elements, but should be part of a wider IPM programme.

Studies in diamide resistant *P. xylostella* have shown that reversal of resistance may occur in absence of selection pressure (Wang et al. 2013; Ribeiro et al. 2014). On the other hand, other *P. xylostella* strains have shown long term stability of diamide resistance suggesting an absence of fitness cost (Steinbach et al. 2014). In extreme cases, late detection of resistance may lead to fixation of the alleles leading to complete resistance of the pest in an area, thus eliminating susceptible phenotypes and preventing potential future resistance reversal. The latter phenomenon has been demonstrated for pyrethroid resistance in *T. absoluta and Bemisia tabaci*: few / no susceptible insects to pyrethroids have been identified after extensive surveys (Tsagkarakou et al. 2009; Haddi et al. 2012).

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 chlorantraniliprole-based products and the associated population reduction effects on *T. absoluta*. Chlorantraniliprole fits well within IPM programmes. This paper is demonstrating that the abuse of a single insecticidal mode of action (MoA) in commercial agriculture can lead to insect resistance in as little of 5-6 years from commercial introduction. Among the factors that can favor resistance to any insecticidal MoA, the intensity of usage is the main parameter that is having an overriding influence. Besides non chemical means, nowadays a sufficient number of effective MoA is still available to farmers for IRM proof and effective *T. absoluta* control. Any effort should be made for breaking the buildup of resistance alleles to diamide insecticides (and to any other effective MoA group) and to prevent the selection of resistant homozygous populations of *T. absoluta*, via the stringent adoption of consistent IPM strategies and IRM (MoA alternation). 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.

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