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ORIGINAL PAPER

A four-year survey on insecticide resistance and likelihood of chemical control failure for tomato leaf miner *Tuta absoluta* in the European/Asian region

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Abstract *Tuta absoluta* is an invasive destructive pest that is currently posing a major threat for tomato production worldwide. Insecticides are a key component of typical pest management schemes. Resistance to diamides, the most recently introduced class of insecticides, was recently reported in Italy. Monitoring of insecticide efficacy is the basic tool for proactive evidence-based resistance management. Here, we report the findings of a 4-year survey performed at the Euro-Asian region. A total of 35 populations were collected between 2012 and 2016 from Greece, Italy, Spain, Israel and UK. The response of these populations was evaluated through laboratory bioassays with the main insecticides used for *T. absoluta* control: chlorantraniliprole, indoxacarb, emamectin benzoate and spinosad. Analysis of the results indicated six cases of low/moderate resistance to the emamectin benzoate (resistance ratio (RR) > 15-fold), a single case of resistance to spinosad (RR: 33-fold) and five cases of resistance to indoxacarb (RR: 13- to 91-fold). Likelihood of control failure was detected for indoxacarb, but reports of poor field performance were absent. Resistance to chlorantraniliprole, after 2015, was widespread in Italy and Greece with high RR (>64-fold) and significant likelihood of control failure in most cases. Chlorantraniliprole resistance was also detected in Israel (RR: 22,573-fold) but not in Spain and UK (RR < twofold). The absence of diamide resistance in tomato leaf miner populations in Spain is most likely linked to a recently established integrated pest management program including non-chemical measures and the rotational use of insecticides of different mode of action classes.

Keywords Chlorantraniliprole · Indoxacarb · Spinosad · Emamectin benzoate · Resistance · *Tuta absoluta* · Leaf miner · Borer · Tomato

Key message

- *Tuta absoluta* is a global tomato pest mainly control by insecticides.
- It is capable of developing insecticide resistance resulting in major crop losses.
- The evolution of resistance is dynamic and requires continues monitoring thus to successfully implement proactive resistance management.
- Within that frame, our work identified the first indications of resistance development to indoxacarb, spinosad and emamectin benzoate in the European/Asian region.
- Diamide resistance is expanding with the exception of Spain, a contrasting situation highlighting the benefits of IPM.

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Introduction

The tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is a major agricultural pest of tomato crops. The leaf miner was initially restricted to South America; however, in 2006, the pest invaded Europe most probably with a single introduction event from Central Chile (Urbaneja et al. 2007; Guillemaud et al. 2015) and rapidly became a global pest threatening tomato production in both greenhouse and field crops (Desneux et al. 2010, 2011; Tonnang et al. 2015; Campos et al. 2017). *Tuta absoluta* pest management relies largely on diverse strategies, involving both preventive and corrective control measures. Preventive measures include strict phytosanitary inspections of propagation material, use of infrastructures providing mechanical protection and close monitoring of the pest status with crop surveys and/or pheromone traps (Benvenga et al. 2007; Roditakis et al. 2010; Abbes et al. 2012; Guedes and Picanço 2012; Alili et al. 2014; Biondi et al. 2015; Ceparano et al. 2015). Corrective tomato leaf miner control measures include adult trapping, mating disruption and use of beneficial insects (predators and parasitoids), microbial and fungal pathogens, mineral oils as well as plant extracts (Michereff Filho et al. 2000; Brunherotto and Vendramim 2001; Urbaneja et al. 2009; Pires et al. 2010; González-Cabrera et al. 2011; Moreno et al. 2012; Biondi et al. 2013; Caparros Megido et al. 2013; Chailleux et al. 2013; Cocco et al. 2013; Zappalà et al. 2013; Abd El-Ghany et al. 2016; Aksoy and Kovanci 2016). Finally, one of the major components of any pest management scheme is the application of chemical insecticides which are extremely potent in controlling the pest when used in a timely manner (Siqueira et al. 2000b; Silva et al. 2011; Guedes and Picanço 2012; Roditakis et al. 2013a).

In Europe, a number of chemicals have been registered for *T. absoluta* control; however, the most extensively used insecticides belong to four distinct chemical classes addressing different modes of action, namely the diamides, the avermectins, the spinosyns and the oxadiazines (Sparks and Nauen 2015). Diamide insecticides belong to mode of action (MoA) Group 28 according to the Insecticide Resistance Action Committee (IRAC) MoA classification (IRAC 2016) and are activators of insect ryanodine receptors (RyR) located in the sarco- and endoplasmic reticulum in neuromuscular tissues (Lahm et al. 2005; Cordova et al. 2006; Ebbinghaus-Kintscher et al. 2007). Diamides interfere with release of Ca^{2+} from the internal stores of smooth and striated muscles, thus disturbing normal muscle function (Cordova et al. 2007; Lümmen 2013). Chlorantraniliprole is currently the only diamide insecticide registered for the control of *T. absoluta* in Europe, while in Israel two diamide insecticides are currently registered (chlorantraniliprole and cyantraniliprole). Avermectins are activators of the glutamate-gated chloride channels (GluCls) and cause neuronal and muscular system malfunctions (IRAC MoA Group 6) (Lasota and Dybas 1991; Fisher and Mrozik 1992; IRAC 2016). Registered chemicals from this group are the insecticides abamectin and the emamectin benzoate. Spinosyns (IRAC MoA Group 5) are nicotinic acetylcholine receptor (nAChR) allosteric activators. These insecticides cause a change in receptor conformation, leading to the opening of ion channels that is causing excitation of neurons in the central nervous system (Salgado 1998; Thompson et al. 2000). Spinosad is the only registered insecticide for tomato leaf miner control from this group. Oxadiazines (IRAC MoA Group 22A) are blockers of voltage-dependent sodium channels and different from pyrethroids and DDT (Silver et al. 2010; Wing et al. 2010; Jiang et al. 2015). Thus, oxadiazines form a different mode of action subgroup with only one representative in the IRAC classification, the insecticide indoxacarb. Indoxacarb is a pro-insecticide that requires metabolic activation by the targeted pest prior exhibiting insecticidal properties (Wing et al. 2010).

Overreliance on insecticides to tackle major pests, such as the tomato leaf miner, is not uncommon in current agriculture (Armes et al. 1996; Roditakis et al. 2009; Wang and Wu 2012). However, insecticide applications alone cannot be considered as a sustainable pest management solution (Deguine et al. 2008; Naranjo and Ellsworth 2009). The selection pressure enforced by the excessive use of chemicals on pest populations is favoring the survival of resistant genotypes driving the development of resistance and a substantial reduction in efficacy levels of insecticides (Perry et al. 1997). Insecticide resistance is one of the major problems in current pest control and associated with control failures in numerous cropping systems worldwide (Roditakis et al. 2009; Silva et al. 2011; Sparks and Nauen 2015). The phenomenon is gradually expanding, and to date more than 580 arthropod species have been reported to exhibit some form of resistance to chemical classes of insecticides, while resistance reports have been documented for at least 325 insecticides (Sparks and Nauen 2015). *Tuta absoluta* is no exception, since several cases of resistance have been documented to established as well as novel chemicals (Siqueira et al. 2000a, b, 2001; Lietti et al. 2005; Silva et al. 2011; Haddi et al. 2012; Gontijo et al. 2013; Campos et al. 2014b). With regard to the chemical classes evaluated in this study, high resistance levels have been reported for diamide insecticides in Italy and Brazil (Roditakis et al. 2015; Silva et al. 2016a). Spinosad resistance has been documented in Brazil and UK

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(Campos et al. 2014a; AHDB 2015), while no resistance to avermectins and oxadiazines has been reported to date.

This study is a follow-up of the baseline susceptibility study on European *T. absoluta* populations (Roditakis et al. 2013a, b) that was conducted almost immediately after the invasion of the leaf miner in Greece and Italy (Viggiani et al. 2009; Roditakis et al. 2010). However, in the present study, the regions and the number of populations tested as well as the time frame of the survey were expanded, thus providing a more comprehensive overview than earlier studies on the efficacy levels of key insecticides for *T. absoluta* management in Europe. Based on our results, resistance to chlorantraniliprole is expanding as it was detected in a number of populations from Italy, Greece and Israel. The classes of oxadiazines, avermectin and spinosyns require attention since resistance cases were detected in European populations. Although the phenomenon is extremely limited, it has to be closely monitored.

Materials and methods

The populations for this survey were collected during a 5-year sampling period, from 2012 to 2016. In total, 35 distinct sites were sampled from 5 geographical regions of Europe and Minor Asia. More specifically, fifteen populations were collected from Greece, ten from Italy, seven from Spain, two from Israel and one from UK. A detailed record for each population is provided in Table 1. For each population, a code name was assigned that will be used for identification hereafter.

The samples originated from infested greenhouse tomato crops and in most cases growers had reported problems in the control of the tomato leaf miner. Sample collection protocol is fully described in Roditakis et al. (2015). Briefly, a number of *T. absoluta* infested tomato leaves from each site, bearing approx. 400–800 larvae, were collected in plastic bags. The samples were transferred to the laboratory within 48 h, and tomato leaves provided adequate nutrition during transfer. Upon arrival in the laboratory, larvae/leaves were transferred in insect-proof rearing cages and were provided with adequate number of insect-free potted tomato plants to resume development. *Tuta absoluta* rearing was conducted at 25 ± 1 °C, 65% RH and photoperiod at 16 h light:8 h dark. Tomato plants (*Solanum lycopersicum* L., var. Valuro) were used for the development of the populations. The plants were maintained pest-free in large insect-proof cages under semi-field conditions; no insecticides were used during the plant development phase (for details, see Roditakis et al. (2013a)).

Insecticides

Commercial formulations of the following insecticides were used: the diamide chlorantraniliprole (Altacor® 35WG, DuPont, France), the avermectin emamectin benzoate (Affirm® 095 SG, Syngenta, UK), the spinosyn spinosad (Laser® 480SC, Dow, USA) and the oxadiazine indoxacarb (Steward® 30WG, DuPont, France).

Bioassays

Approximately 100 moths were collected from the rearing cages and were allowed to oviposit on insect-free plants for 24–48 h. These plants were incubated separately until the larvae reached the second instar. Subsequently, the IRAC method 022 (www.irac-online.org) was adopted, with slight modifications, for the toxicological assays. The method protocol is a classical leaf dip assay that is fully described in Roditakis et al. (2013b). Briefly, tomato leaves, either cut in square pieces or entire leaflets, were immersed in serial insecticide concentrations containing Triton X-100 (0.2 g L^{-1}) as a nonionic wetting agent. Treated leaves were allowed to dry for 1–2 h at room temperature and subsequently placed adaxially on moist tissue paper in a multi-well Repli-dish. A single second instar larva was placed in each well; subsequently, all wells were sealed with transparent ventilated adhesive lids. Bioassays with Spanish populations were conducted following a slightly modified version of the leaf dip protocol which is described in details in Roditakis et al. (2013b). All bioassays were incubated in growth chambers at 25 ± 0.5 °C and 65 ± 5% relative humidity. Mortality was assessed after 3 days of exposure, after larvae were carefully removed from leaf galleries under magnifying glass. Larvae were considered dead if they were unable to move the length of their bodies after gentle prodding with a camel-hair brush.

Data analysis

Mortality data from dose–response bioassays were subjected to probit analysis based on Finney (1964) using PriProbit 3.4 (Sakuma 1998) or Polo Plus (LeOra software, USA). Both types of software test the linearity of dose–mortality response and provide the slope, the lethal concentrations (LC) and the 95% fiducial limits (FL) of the lethal concentration for each mortality line. Using the appropriate function, the relative potency ratio among responses was calculated. Responses were considered significantly different when the 95% confidence interval of relative potency ratio did not include the value 1. Percentage mortality values generated in bioassays were corrected using Abbott's formula (Abbot 1925).

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Table 1 Information on populations of *Tuta absoluta* used in the present study

Country	Population	Location	Coordinates	Sampling	Crop
Reference	GR-Lab	Greece, Peloponnese		Aug-10	*GH T
	ES-Sus	Spain, Murcia, Aguilas		Jan-11	GH T
Greece	GR-ARV-12-1	Vianos, Arvi	34°59′N, 25°24′E	May-12	GH T
	GR-TYMP-12-2	Tympaki, Sivas	35°0′N, 24°48′E	Jun-12	GH T
	GR-TYMP-14-1	Tympaki, Klima	35°5′N, 24°45′E	Mar-14	GH T
	GR-IER-14-1	Ierapetra, Kentri	35°2′N, 25°44′E	Apr-14	GH T
	GR-IER-14-2	Ierapetra, Sopates	35°1′N, 25°38′E	Mar-14	GH T
	GR-IER-14-3	Ierapetra, Mpountoules	35°1′N, 25°43′E	May-14	GH T
	GR-IER-15-3	Ierapetra, Vainia	35°0′N, 25°46′E	May-15	GH T
	GR-IER-15-2	Ierapetra, Kalogeri	35°1′N, 25°39′E	May-15	GH T
	GR-PEL-15-1	Trifilia, Gargalianoi	37°0′N, 21°39′E	Jun-15	GH T
	GR-TYMP-16-1	Tympaki, Tympaki	35° 5′N, 24°45′E	Apr-16	GH T
	GR-TYMP-16-2	Tympaki, Vori	35° 4′N, 24°48′E	Apr-16	GH T
	GR-TYMP-16-3	Tympaki, Lagolio	35° 5′N, 24°47′E	Apr-16	GH T
	GR-DRAM-16-4	Drama, Kalos Agros	41° 6′N, 24°5′E	Jun-16	GH T
	GR-PREV-16-5	Preveza, Petritsia	38°59′N, 20°44′E	Jun-16	GH T
	GR-IER-16-6	Ierapetra, Kalithea	35° 1′N, 25°43′E	Jun-16	GH T
Italy	IT-PACH-14-1	Siracusa, Pachino	36°40′N, 15°5′E	May-14	GH T
	IT-PACH-14-2	Siracusa, Pachino	36°40′N, 15°5′E	May-14	GH T
	IT-GELA-14-1	Caltanissetta, Gela	37°1′N, 14°19′E	May-14	GH T
	IT-ACAT-14-1	Ragusa, Acate	36°59′N, 14°23′E	May-14	GH T
	IT-RAG-15-1	Ragusa, Punta Braccetto	36°49′N, 14°28′E	May-15	GH T
	IT-RAG-15-2	Ragusa, Scicli	36°45′N, 14°41′E	May-15	GH T
	IT-MAR-15-1	Ragusa, Marina di Acate	36°59′N, 14°23′E	Jun-15	GH T
	IT-MAR-15-2	Ragusa, Marina di Acate	36°59′N, 14°23′E	Jun-15	GH T
	IT-FOND-16-1	Latina, Salto Covino	41°18′N, 13°20′E	Aug-16	GH T
	IT-FOND-16-2	Latina, Sperlonga	41°16′N, 13°24′E	Aug-16	GH T
Spain	ES-MUR-12	Spain, Murcia, Mazarron	37°36′N, 1°17′W	Jul-12	GH T
	ES-MUR-14-1	Spain, Murcia, Cañada de Gallego	37°33′N, 1°25′W	May-10	GH T
	ES-MUR-14-2	Spain, Murcia, Puntas de Calnegre	37°31′N, 1°26′W	Nov-14	GH T
	ES-ALM-14	Spain, Almeria, La Mojonera	36°45′N, 2°41′W	Nov-14	GH T
	ES-MUR-15	Spain, Murcia, La Palma	37°41′N, 0°57′W	Aug-15	GH T
	ES-ALM-15	Spain, Almeria, La Cañada	36°50′N, 2°24′W	Jul-15	GH T
	ES-MUR-16	Spain, Murcia, Totana	37°46′N, 1°28′W	Jul-16	GH T
Israel	ISR-15-1	Negev, Kmehin	30°55′N, 34°25′E	Sep-15	GH T
	ISR-15-2	mixed locations sample (Tamra, Ibblin, Tubas)	N/A	Nov-15	GH T
UK	UK-16-1	North Yorkshire, Stokesley	54°27′N, 1°11′W	Sep-16	GH T

Collections were made from 2012 to 2016. All strains were collected from infested greenhouse tomato crops and were maintained thereafter on tomato plants under laboratory conditions for 1–2 generations

GH T greenhouse tomato, *FD T* field tomato

The likelihood of insecticide control failure is a well-established index of insecticide efficacy (ffrench-Constant and Roush 1990; Roditakis et al. 2013a; Guedes 2017) comparing the estimated % mortality at the label rate to an 80% threshold. The particular threshold was initially set following the Brazilian legislation (Silva et al. 2011). Currently two approaches estimating control failure likelihood have been used (Silva et al. 2011; Gontijo et al. 2013). Here, the protocol by Silva et al. (2011) was adopted. Briefly, the mortality achieved by the label rate would be considered significantly lower than 80% when the lower 95% fiducial limit of the LC_{80} was found higher than

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the recommended rate. The maximum recommended label rates (RLR) for the tested insecticides for Southern Europe were: emamectin benzoate, 14.2 mg L^{-1}, chlorantraniliprole 42 mg L^{-1}, spinosad 120 mg L^{-1}, indoxacarb 37.5 mg L^{-1}.

Results

The probit analysis results are presented in Tables 2, 3, 4 and 5. The responses of the populations to the tested insecticides were homogenous and fitted the Log-dose probit-mortality model. The assays for the populations from Italy, Greece, Israel and UK were conducted in Heraklion, and responses were compared to the GR-Lab reference strain, while the populations from Spain were tested in Cartagena and these responses were compared to the ES-Sus reference strain.

Diamide insecticide: chlorantraniliprole

The response of 35 populations in total was tested against the insecticide chlorantraniliprole (Table 2). The tested populations exhibited a wide range of slope coefficients; the highest slope was 3.26 observed in a Greek population collected in 2012 (GR-ARV-12-1), the lowest slopes (>0.9) were observed in Greek populations collected in 2016 at the Tympaki area (GR-TYMP-16-3, GR-TYMP-16-2, GR-TYMP-16-1). The majority of the populations (23) exhibited slopes between 1.00 and 2.10, suggesting a relatively homogenous response to the diamide insecticide at European level.

Earlier samples from Italy (Roditakis et al. 2013b) and Greece (Roditakis et al. 2013a) collected between 2010 and 2011 exhibited LC_{50} values comparable to the current reference strain and the baseline data published for chlorantraniliprole. The same was observed in 2012 for populations collected from Greece (Table 2). The first populations exhibiting resistance to diamides were collected in 2014 from Italy, representing the first cases of resistance at a global level (Roditakis et al. 2015). At the same period, the LC_{50} of Greek populations ranged between 0.38 and 2.45 mg L^{-1} indicating rather low resistance ratio (RR, calculated based on GR-Lab strain, LC_{50} 0.31 mg L^{-1}) up to eightfold. This small but statistically significant divergence from the baseline data was reported in Roditakis et al. (2015) as an alarming indication of incipient resistance development. Thereafter, in 2015–2016 sampling period, high resistance levels to chlorantraniliprole were detected in Italy, up to 2704-fold ($LC_{50:}$ 838 mg L^{-1}, IT-MAR-15-2), Greece up to 3200-fold (LC_{50}: >1000 mg L^{-1}, GR-DRAM-16-4) and Israel up to 22,570-fold ($LC_{50:}$ 6998 mg L^{-1}, ISR-15-2). In general, the LC_{50} values to chlorantraniliprole were high (>16 mg L^{-1}/RR > 54-fold) with just 2 exceptions (IT-RAG-15-1 and ISR-15-1) suggesting moderate to high levels of resistance for the populations collected in 2016–2015 from Italy, Greece and Israel. Taking into account that the particular samples were mostly collected from fields with reported control failures, it is suggested that the problematic pest control could be associated with resistance to diamides.

For the Spanish populations, the earliest sample was collected in 2012 (Table 2). The LC_{50} values ranged between 0.12 mg L^{-1} (ES-MUR-16) and 1.45 mg L^{-1} (ES-MUR-14-2). The resistance ratio (RR, calculated based on ES-Sus strain, LC_{50} 0.20 mg L^{-1}) was below twofold in general with one exception (eightfold) suggesting absence of diamide resistance. For the UK population (UK-16-1) collected in 2016, no resistance was detected since the estimated LC_{50} (0.17 mg L^{-1}) was comparable to the LC_{50} of the reference strain.

Oxadiazine insecticide: indoxacarb

The response of 30 populations was evaluated versus the insecticide indoxacarb (Table 3). The tested populations exhibited limited variability in the range of slopes; the lowest slope was 0.61 observed in a Greek population collected in 2016 (GR-PREV-16-5); and the highest slope was 2.17 observed in a Greek population collected in 2014 (GR-IER-14-2). Nevertheless, the majority of the populations (21) exhibited slopes between 1.00 and 1.86, suggesting homogenous responses to the oxadiazine insecticide in Europe.

The Greek populations collected between 2012 and 2014 exhibited LC_{50} values ranging between 0.65 mg L^{-1} (GR-ARV-12-1) and 8.37 mg L^{-1} (GR-IER-14-1). The resistance ratio was always found below tenfold, suggesting absence of resistance to indoxacarb. In the 2015–2016 sampling period, the LC_{50} values ranged between 3.38 mg L^{-1} (GR-DRAM-16-4) and 88.0 mg L^{-1} (GR-PREV-15-5). In 5 out of the 8 populations tested, the resistance ratio was higher than tenfold, indicating a major shift in the response to indoxacarb compared to the 2012–2014 sampling period. In the case of populations GR-TYP-16-2 and GR-PREV-16-5, in particular, the resistance ratio was estimated at 69- and 91-fold, respectively, indicating moderate to high levels of resistance to indoxacarb. Moderate levels of resistance were also detected in one of the Israeli populations (ISR-15-2) that exhibited an LC_{50} value of 35.3 mg L^{-1} to indoxacarb (RR: 36-fold).

Minimal variability in the responses of the Italian and the Spanish populations to indoxacarb was observed throughout the sampling period (Table 3). For Italy, the lowest LC_{50} to

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Table 2 Log-dose probit-mortality data in foliar bioassays (72 h) for the insecticide chlorantraniliprole against second instar larvae of different *Tuta absoluta* populations

Year	Strain	N	LC_{50}	FL 95%		RR	LC_{80}	FL 95%	Slope	s.e.	X^2	*df*
	GR-Lab	192	0.31	0.22–0.45	a		1.08	0.71–1.93	1.58	0.16	6.4	4
	ES-Sus	330	0.2	0.14–0.23	A		0.43	0.33–0.61	2.30	0.23	35.9	25
Italy												
2014	IT-PACH-14-1*	189	47.6	30.8–77.1	c	154[a]	243	136–611	1.19	0.17	8.0	4
	IT-PACH-14-2*	126	63.7	42.1–128	cd	205	204	108–1123	1.66	0.42	2.4	1
	IT-ACAT-14-1*	191	225	135–343	ef	726	762	493–1369	1.58	0.24	3.0	3
	IT-GELA-14-1*	192	435	165–1193	f	1402	3022	1124–79,653	0.99	0.30	4.4	3
2015	IT-RAG-15-1	191	5.12	1.73–9.63	b	17	38.4	21.5–88.2	0.96	0.26	1.1	3
	IT-RAG-15-2	192	88.9	55.3–145	cde	287	345	200–913	1.42	0.52	1.2	3
	IT-MAR-15-1	191	634	323–1013	f	2044	2057	1297–3728	1.64	0.94	1.8	3
	IT-MAR-15-2	191	838	457–1344	f	2704	3399	2085–6891	1.38	0.76	3.7	3
2016	IT-FOND-16-1	256	151	84.6–236	def	486	602	380–1143	1.39	0.57	4.1	5
	IT-FOND-16-2	223	288	151–468	f	929	1226	742–2611	1.34	0.67	0.9	4
Greece												
2012	GR-TYMP-12-2*	190	0.14	0.09–1.98	ab	0.5[a]	0.36	0.25–0.56	2.12	0.32	1.3	3
	GR-ARV-12-1*	191	0.17	0.12–0.23	a	1	0.31	0.23–0.49	3.26	0.69	0.3	3
2014	GR-TYMP-14-1*	189	0.38	0.17–0.57	a	1	1.1	0.73–2.0	1.81	0.42	1.6	3
	GR-IER-14-2*	189	1.34	0.77–2.33	b	4	7.8	4.0–27.7	1.10	0.21	4.4	3
	GR-IER-14-3*	242	1.91	0.97–3.25	b	6	8.8	4.8–28.9	1.27	0.28	0.9	4
	GR-IER-14-1*	159	2.45	1.24–17.0	bc	8	17.6	5.0–2476	0.98	0.31	0.1	2
2015	GR-IER-15-2	145	16.7	8.71–42.2	cd	54	78.9	33.8–858	1.24	0.40	0.1	2
	GR-PEL-15-1	191	178	107–264	e	574	460	308–10,476	2.04	0.95	1.3	3
	GR-IER-15-3	192	>1000			>3200						
2016	GR-TYMP-16-3	224	19.9	3.9–53.5	cd	64	500	183–2759	0.60	0.28	4.7	4
	GR-TYMP-16-2	223	77.3	30.3–229	de	249	4767	1072–103,120	0.47	0.18	6.0	5
	GR-PREV-16-5	222	219	133–336	e	707	857	545–1590	1.42	0.53	1.1	4
	GR-IER-16-6	192	315	180–508	e	1015	983	594–2629	1.70	1.00	2.8	3
	GR-TYMP-16-1	224	440	188–1522	e	1418	3867	1224–152,920	0.89	0.66	1.2	4
	GR-DRAM-16-4	254	>1000			>3200						
Spain												
2012	ES-MUR-12	240	0.14	0.09–0.26	A	1[b]	0.42	0.23–1.61	1.71	0.28	21.5	13
2014	ES-MUR-14-1	240	0.15	0.12–0.20	A	1	0.41	0.30–0.64	1.99	0.28	14.2	16
	ES-MUR-14-2	240	1.45	1.08–2.06	C	8	4.93	3.15–11.04	1.58	0.26	13.1	16
	ES-ALM-14	240	0.44	0.29–0.69	B	2	0.99	0.69–2.00	2.37	0.38	25.7	16
2015	ES-MUR-15	120	0.13	0.06–0.22	A	1	0.35	0.21–1.02	1.94	0.53	2.4	4
	ES-ALM-15	120	0.14	0.07–0.23	A	1	0.34	0.21–0.85	2.08	0.52	2.3	4
2016	ES-MUR-16	120	0.12	0.02–0.22	A	1	0.55	0.30–2.63	1.25	0.41	4.4	7
Other regions												
2015	ISR-15-1	192	1.74	0.36–2.76	a	6	4.48	2.85–11.21	2.05	0.42	3.4	3
	ISR-15-2	190	6998	4510–9847	b	22,573	17,438	12,197–31,228	2.12	1.67	0.1	1
2016	UK-16-1	256	0.17	0.10–0.24	a	1	0.37	0.27–0.61	2.50	0.36	2.6	5

The populations have been grouped by country and sampling period (collection year). Within each sampling period, the results are in ascending order

N number of larvae tested, *FL* fiducial limits, *RR* resistance ratio, LC_{50} *in mg* L^{-1}, Chi-square testing linearity of dose–mortality response: * data from Roditakis et al. (2015), [a] resistance ratio (RR) calculated is based on strain GR-Lab, [b] resistance ratio (RR) calculated is based on strain ES-Sus. Different letters indicate significant differences in the responses ($P < 0.05$, see text for details) within a country. Capital letters indicate comparisons with the respective reference strain

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Table 3 Log-dose probit-mortality data in foliar bioassays (72 h) for the insecticide indoxacarb against second instar larvae of different *Tuta absoluta* populations

Year	Strain	N	LC$_{50}$	FL 95%		RR	LC$_{80}$	FL 95%	Slope	s.e.	X^2	*df*
	GR-Lab	192	0.97	0.66–1.41	a		3.65	2.36–6.84	1.46	0.11	0.6	4
	ES-Sus	150	0.66	0.39–1.13	A		2.44	1.37–7.52	1.48	0.25	11.4	10
Italy												
2014	IT-PACH-14-2	191	0.87	0.41–1.45	a	1[a]	3.33	2.02–6.23	1.45	0.25	2.0	3
	IT-ACAT-14-1	172	2.63	1.32–4.22	ab	3	8.07	5.05–15.4	1.73	0.34	0.5	3
	IT-PACH-14-1	158	5.21	2.80–8.35	bc	5	16.8	10.4–35.6	1.65	0.32	0.6	2
	IT-GELA-14-1	191	9.21	5.01–15.43	c	9	33.6	19.3–103	1.49	0.34	0.6	3
2015	IT-RAG-15-2	192	4.89	3.12–7.20	bc	5	17.6	11.7–30.9	1.51	0.22	3.4	3
	IT-RAG-15-1	192	12.8	3.09–29.9	bc	13	205	80.4–1504	0.69	0.29	3.8	3
2016	IT-FOND-16-1	256	2.52	1.53–3.91	b	3	11.6	7.35–20.94	1.26	0.15	6.1	5
	IT-FOND-16-2	254	8.25	4.25–13.89	c	9	42.9	24.68–99.6	1.17	0.27	4.1	5
Greece												
2012	GR-ARV-12-1	192	0.65	0.41–0.91	a	1[a]			1.56	0.26	1.1	3
	GR-TYMP-12-2	192	3.58	1.51–6.03	b	4	11.9	7.19–21.6	1.61	0.32	3.1	3
2014	GR-TYMP-14-1	190	1.73	0.71–9.00	ab	2	26.9	6.13–2321	0.70	0.19	2.7	3
	GR-IER-14-3	192	6.42	3.87–10.9	b	7	32.5	17.5–93.1	1.19	0.20	0.5	3
	GR-IER-14-2	160	6.75	4.90–9.82	b	7	16.5	11.3–29.2	2.17	0.43	4.0	2
	GR-IER-14-1	172	8.37	3.56–15.9	bc	9	37.3	19.0–190	1.29	0.35	0.3	3
2015	GR-IER-15-2	188	8.56	5.06–13.1	bc	9	25.1	16.0–54.7	1.80	0.42	2.1	3
	GR-PEL-15-1	191	12.1	5.47–30.8	bcde	12	86.8	33.0–938	0.98	0.29	0.4	3
	GR-IER-15-3	192	19.9	11.0–31.0	cde	21	66.2	42.6–118.2	1.61	0.45	4.3	3
2016	GR-DRAM-16-4	228	3.48	0.87–8.20	ab	4	9.85	4.36–52.0	1.86	0.41	12.7	5
	GR-TYMP-16-1	221	6.52	0.22–24.51	abcd	7	45.2	12.9–4196	1.00	0.37	12.6	4
	GR-TYMP-16-3	192	15.0	6.20–24.4	bcd	15	69.1	44.8–126	1.26	0.43	0.4	3
	GR-TYMP-16-2	224	66.9	28.8–125	ef	69	614	296.6–2429	0.87	0.39	7.2	4
	GR-PREV-16-5	192	88.0	17.3–204	def	91	2036	768–21,195	0.61	0.40	2.8	3
Spain												
2012	ES-MUR-12	240	0.80	0.44–1.24	A	1[b]	2.29	1.45–6.88	1.85	0.42	19.5	16
2014	ES-MUR-14-1	270	0.22	0.11–0.39	A	0.3	1.61	0.86–4.29	0.98	0.13	26.7	19
	ES-MUR-14-2	120	0.42	0.06–1.60	A	1	2.61	0.81–132.39	1.06	0.25	10.7	7
	ES-ALM-14	270	0.67	0.36–1.18	A	1	5.98	2.94–20.86	0.89	0.16	13.8	19
2015	ES-MUR-15	120	0.38	0.12–1.77	A	1	2.24	0.69–80.27	1.11	0.2	13.9	7
Other regions												
2015	ISR-15-1	192	0.77	0.34–1.33	a	1[a]	3.94	2.33–8.07	1.19	0.15	2.8	3
	ISR-15-2	192	35.3	15.8–61.7	b	36	172	99.2–367	1.22	0.44	1.9	3
2016	UK-16-1	192	0.26	0.16–0.37	a	0.3	0.75	0.51–1.26	1.82	0.19	1.6	3

See footnote of Table 2 for details

indoxacarb was 0.87 mg L^{-1} reported in 2014 (IT-PACH-14-2) and the highest was 12.8 mg L^{-1} reported in 2015 (IT-RAG-15-1). The resistance ratio (RR, calculated based on GR-Lab, LC$_{50}$ 0.97 mg L^{-1}), in all sampling periods, was always found below ninefold, with just one exception (13-fold, IT-RAG-15-1), suggesting that the populations collected from Italy were not considered resistant to indoxacarb. For the Spanish populations, the LC$_{50}$ values ranged between 0.22 mg L^{-1} (ES-MUR-14-1) and 0.80 mg L^{-1} (ES-MUR-12), exhibiting typical responses of susceptible populations to the oxadiazine insecticide in all cases (responses compared to ES-Sus strain, LC$_{50}$: 0.66 mg L^{-1}). The UK population was also found susceptible to indoxacarb (LC$_{50}$: 0.26 mg L^{-1}, RR: 1).

Avermectin insecticide: emamectin benzoate

The response of 27 populations in total was evaluated vs. the insecticide emamectin benzoate (Table 4). The tested populations exhibited limited variability in the range of

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Table 4 Log-dose probit-mortality data in foliar bioassays (72 h) for the insecticide emamectin benzoate against second instar larvae of different *Tuta absoluta* populations

Year	Strain	N	LC_{50}	FL 95%		RR	LC_{80}	FL 95%	Slope	s.e.	X^2	*df*
	GR-Lab	192	0.05	0.036–0.07	a		0.18	0.12–0.31	1.58	0.28	5.5	4
	ES-Sus	150	0.01	0.010–0.016	A		0.02	0.019–0.035	3.16	0.60	9.8	10
Italy												
2014	IT-ACAT-14-1	179	0.22	0.09–0.38	b	4[a]	0.90	0.53–1.79	1.38	0.25	2.4	3
	IT-PACH-14-2	191	0.27	0.15–0.4	b	5	0.67	0.45–1.14	2.15	0.42	0.6	3
	IT-PACH-14-1	192	0.85	0.42–1.37	c	17	2.95	1.82–5.7	1.55	0.28	1.1	3
	IT-GELA-14-1	190	1.74	0.89–3.22	c	35	10.4	5.2–36.56	1.08	0.21	0.4	3
2015	IT-RAG-15-1	191	0.25	0.12–0.39	b	5	0.91	0.58–1.68	1.49	0.16	5.2	3
	IT-RAG-15-2	191	0.75	0.36–1.25	bc	15	2.70	1.64–5.31	1.52	0.17	3.3	3
2016	IT-FOND-16-1	224	0.81	0.44–1.27	c	16	3.36	2.16–5.99	1.36	0.14	1.9	4
	IT-FOND-16-2	223	0.82	0.47–1.25	c	16	2.63	1.73–4.75	1.67	0.15	2.2	4
Greece												
2014	GR-TYMP-14-1	191	0.11	0.07–0.16	ab	2[a]	0.3	0.23–0.53	1.89	0.31	2.0	3
	GR-IER-14-3	191	0.16	0.09–0.26	b	3	0.8	0.47–1.54	1.27	0.20	1.8	3
	GR-IER-14-1	192	0.31	0.11–0.53	bc	6	1.2	0.73–2.75	1.40	0.32	5.4	3
	GR-IER-14-2	192	0.38	0.16–0.63	bc	8	1.3	0.79–2.35	1.59	0.31	4.2	3
2015	GR-IER-15-3	192	0.07	0.008–0.17	ab	1	0.76	0.36–1.96	0.81	0.13	3.3	3
	GR-IER-15-2	172	0.56	0.30–0.89	c	11	1.53	0.97–2.78	1.94	0.19	1.8	3
	GR-PEL-15-1	192	0.57	0.27–0.94	c	11	2.03	1.26–3.79	1.54	0.16	4.9	3
2016	GR-TYMP-16-2	191	0.17	0.10–0.27	b	3	0.72	0.46–1.38	1.39	0.18	3.1	3
	GR-DRAM-16-4	192	0.17	0.10–0.24	b	3	0.46	0.32–0.76	1.92	0.23	4.4	3
	GR-TYMP-16-1	160	0.21	0.09–0.37	bc	4	1.23	0.70–3.44	1.11	0.16	1.9	2
	GR-TYMP-16-3	191	0.25	0.16–0.37	bc	5	0.82	0.55–1.43	1.67	0.18	3.5	3
	GR-PREV-16-5	217	0.60	0.36–0.88	c	12	1.67	1.14–2.77	1.88	0.16	4.1	4
	GR-IER-16-6	192	0.80	0.48–1.35	c	16	3.33	1.88–9.21	1.36	0.14	0.9	3
Spain												
2014	ES-MUR-14-2	120	0.02	0.002–0.064	A	2[b]	0.11	0.04–1.16	1.16	0.29	8.6	7
	ES-ALM-14	120	0.04	0.018–0.107	B	3	0.23	0.09–9.53	1.12	0.38	2.3	7
2015	ES-MUR-15	150	0.01	0.0001–0.0551	A	1	0.19	0.04–11.90	0.68	0.14	23.0	10
Other regions												
2015	ISR-15-1	192	0.17	0.10–0.25	b	3[a]	0.56	0.37–0.98	1.61	0.19	4.6	3
	ISR-15-2	191	0.51	0.30–0.76	c	10	1.36	0.92–2.24	1.99	0.17	1.7	3
2016	UK-16-1	192	0.05	0.03–0.07	a	1	0.11	0.08–0.18	2.42	0.55	2.9	3

See footnote of Table 2 for details

slopes; the lowest slope (0.68) was observed in a Spanish population collected in 2015 (ES-MUR-15); and the highest slope (2.42) was observed in the UK population collected in 2016 (UK-16-1). The majority of the populations (23) exhibited slopes between 1.00 and 2.00, suggesting homogenous responses to the avermectin insecticide. The differentiation in the responses for populations collected in Greece and Italy was generally low throughout the sampling period. The LC_{50} ranged between 0.07 mg L^{-1} (GR-IER-15-3) and 1.74 mg L^{-1} (IT-GELA-14-1). The resistance factors ranged between susceptibility levels (twofold and fourfold for Greece and Italy, respectively) and low resistance levels (15- to 16-fold) with one exception (35-fold, IT-GELA-14-1). During the sampling period, six populations in total were detected with low to moderate resistance levels. High susceptibility levels were observed for the Spanish populations. The LC_{50} ranged between 0.01 mg L^{-1} (ES-MUR-15) and 0.04 mg L^{-1} (ES-ALM-14), and resistance factor ranged between onefold to threefold, respectively. The populations from Israel exhibited LC_{50} values below 0.52 mg L^{-1} (tenfold resistance ratio) and the population from UK exhibited a response similar to the reference strain (LC_{50}: 0.05 mg L^{-1}).

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Table 5 Log-dose probit-mortality data in foliar bioassays (72 h) for the insecticide spinosad against second instar larvae of different *Tuta absoluta* populations

Year	Strain	*N*	LC_{50}	FL 95%		RR	LC_{80}	FL 95%	Slope	s.e.	X^2	*df*
	GR-Lab	192	0.27	0.15–0.40	a		0.76	0.51–1.29	1.86	0.19	7.0	3
	ES-Sus	180	0.29	0.14–0.50	A		0.6	0.39–1.32	2.92	0.53	23.0	13
Italy												
2014	IT-PACH-14-2	188	0.11	0.06–0.16	a	0.4[a]	0.3	0.2–0.5	1.90	0.33	0.6	3
	IT-PACH-14-1	190	0.23	0.12–0.34	a	1	0.6	0.39–0.95	2.18	0.46	1.7	3
	IT-ACAT-14-1	192	0.31	0.21–0.41	a	1	0.5	0.4–0.82	3.69	0.84	1.5	3
	IT-GELA-14-1	192	0.45	0.24–0.61	a	2	0.8	0.61–1.35	3.21	0.88	2.6	3
2015	IT-RAG-15-1	159	0.13	0.05–0.24	a	0	0.5	0.28–0.96	1.48	0.21	3.4	2
	IT-RAG-15-2	191	0.41	0.26–0.58	a	2	0.93	0.66–1.51	2.38	0.21	2.2	3
2016	IT-FOND-16-1	192	0.19	0.14–0.26	a	1	0.43	0.31–0.67	2.42	0.28	1.4	3
	IT-FOND-16-2	191	0.22	0.15–0.28	a	1	0.35	0.27–0.54	4.18	0.65	0.3	3
Greece												
2014	GR-IER-14-3	159	0.09	0.04–0.15	a	0.3[a]	0.3	0.19–0.66	1.55	0.32	2.0	2
	GR-IER-14-1	192	0.12	0.079–0.17	a	0.4	0.2	0.16–0.36	3.41	0.86	0.1	3
	GR-IER-14-2	189	0.17	0.046–0.3	a	1	0.5	0.3–0.91	1.85	0.51	3.0	3
	GR-TYMP-14-1	189	0.23	0.12–0.35	a	1	0.7	0.48–1.33	1.64	0.29	0.5	3
2015	GR-PEL-15-1	192	0.28	0.18–0.40	a	1	0.79	0.54–1.35	1.89	0.19	1.5	2
	GR-IER-15-3	116	0.32	0.21–0.43	a	1	0.55	0.41–0.9	3.68	0.42	0.0	1
	GR-IER-15-2	190	0.59	0.37–0.82	a	2	1.21	0.87–1.89	2.72	0.19	5.9	3
2016	GR-TYMP-16-3	190	0.16	0.035–0.23	a	1	0.33	0.22–0.64	2.62	0.54	2.7	3
	GR-TYMP-16-2	191	0.18	0.12–0.24	a	1	0.30	0.23–0.46	4.01	0.66	3.0	3
	GR-DRAM-16-4	191	0.21	0.08–0.28	a	1	0.33	0.25–0.64	4.46	0.93	3.7	3
	GR-IER-16-6	191	0.26	0.17–0.36	a	1	0.49	0.36–0.77	3.12	0.36	0.1	3
	GR-TYMP-16-1	192	0.29	0.18–0.40	a	1	0.56	0.41–0.89	3.04	0.33	2.5	3
Spain												
2012	ES-MUR-12	120	0.16	0.09-0.33	A	1[b]	0.59	0.30–4.43	1.49	0.42	5.2	7
2014	ES-MUR-14-2	270	0.04	0.02–0.07	B	0.1	0.21	0.12–0.55	1.15	0.19	19.6	16
	ES-ALM-14	120	0.01	0.003–0.03	B	0.03	0.04	0.02–0.34	1.75	0.42	4.3	4
2016	ES-MUR-16	240	0.22	0.14–0.48	A	1	1.26	0.55–10.34	1.12	0.27	15.3	16
Other regions												
2015	ISR-15-1	175	0.17	0.13–0.23	a	1[a]	0.39	0.28–0.62	2.38	0.29	2.3	4
	ISR-15-2	185	0.20	0.12–0.28	a	1	0.46	0.32–0.75	2.29	0.27	4.2	3
2016	UK-16-1	224	9.02	4.27–29.9	b	33	206	52–3739	0.62	0.11	0.4	5

See footnote of Table 2 for details

Spinosyn insecticide: spinosad

The response of 27 populations in total was tested against the insecticide spinosad (Table 5). The tested populations exhibited high variability in the range of slopes. The lowest slope (0.62) was observed in the UK population collected in 2016 (UK-16-1), and the highest slope (4.46) was observed in Greece in a strain collected in 2016 (GR-DRAM-16-4). The majority of the populations (16) exhibited very steep slopes, higher than 2. The responses of the Italian, the Greek and the Israeli populations were similar. The LC_{50} ranged between 0.09 mg L^{-1} (GR-IER-14-3) and 0.59 mg L^{-1} (GR-IER-15-2). The resistance factor ranged between 0.3-fold and twofold, indicating high susceptibility levels for all regions. High susceptibility was also observed for the Spanish populations. The LC_{50} ranged between 0.01 mg L^{-1} (ES- ALM-14) and 0.22 mg L^{-1} (ES-MUR-16), and the estimated RR indicated the absence of spinosad resistance in Spain. The estimated LC_{50} for the UK population (UK-16-1) was 9.02 mg L^{-1} resulting in a 33-fold resistance ratio (calculated based on GR-Lab strain, LC_{50} 0.05 mg L^{-1}), the highest detected in this data set. This resistance factor value indicates moderate resistance level to spinosad for the population from North Yorkshire.

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Likelihood of chemical control failure analysis

Studies estimating the likelihood of chemical control failure were performed. The RLR for insecticide chlorantraniliprole (42 mg L^{-1}) was found significantly lower than the LC_{80} in eighteen cases. Likelihood of control failure was reported in highly resistant populations collected after 2014 from Italy, Greece and Israel. In general, a resistance ratio (RR) over 60-fold was linked to significant likelihood of chemical control failure. Almost all of these eighteen populations were collected from fields with reported control failures, indicating that the metrics used in this study for predicting the likelihood of control failure were valid. For insecticide indoxacarb, significant potential of control failure was identified in four cases from Greece (GR-ER-15-3, GR-TYMP-16-3, GR-TYMP-16-2 and GR-PREV-16-5), one from Israel (ISR-15-2) and one from Italy (IT-RAG-15-1). These six cases were associated with indoxacarb resistance levels higher than 13-fold. The control failure likelihood for indoxacarb was found in combination with control failure likelihood for chlorantraniliprole (with one exception), suggesting exceptional cases of heavily treated fields. In accordance with the above findings, lack of performance of indoxacarb from commercial applications has not been reported to date. Finally, the likelihood of chemical control failure was estimated for insecticides emamectin benzoate and spinosad. Despite the detection of moderately resistance populations, potential control failure cases were not identified for these insecticides.

Discussion

Determination of baseline toxicity to insecticides and subsequent monitoring of the susceptibility levels is a key component of current insecticide resistance management strategies for any major pest (Zhao et al. 2006; Ishtiaq et al. 2011; Ellsworth et al. 2013; Gao et al. 2013). This approach is essential, particularly for novel insecticides, since alternative resistance monitoring tools, such as molecular markers or biochemical diagnostic tests, are usually not available. Susceptibility monitoring allows the early detection of potential cases of insecticide resistance and provides the opportunity to implement evidence-based strategies to inhibit or delay resistance development (Slater et al. 2017; Zimmer et al. 2017).

Tuta absoluta is a good example on how susceptibility monitoring can provide the basis for proactive resistance management. The tomato leaf miner exhibited its capacity to develop resistance to wide range of pesticides in early 2000, several years prior global invasion was initiated (Siqueira et al. 2000a, b; Lietti et al. 2005). Having this knowledge, baseline toxicity to major chemical classes, using European *T. absoluta* populations was established immediately after the invasion of the Old World (Roditakis et al. 2013a, b). Three years later, these baselines were used to demonstrate the first case of resistance development to diamide insecticides, a major component of *T. absoluta* management schemes (Roditakis et al. 2015). Consistent susceptibility monitoring for *T. absoluta* was also implemented in other regions of the world, such as Brazil, providing the respective benefits to the local farmers (Campos et al. 2014a; Silva et al. 2015, 2016a, b). Hereby, our group pursued and expanded this essential susceptibility monitoring processes, and the output of this work is presented in this study.

The insecticide emamectin benzoate is an extremely potent pest control tool for *T. absoluta* since no cases of control failure were detected throughout the monitoring period for all regions tested and resistance levels where generally low. However, in year 2016, in three cases (Italy and Greece) resistance ratio reached 16-fold, suggesting detection of low resistant levels and indicating a potential shift in the responses of *T. absoluta* populations to emamectin benzoate. To date, reports for resistance to avermectins for *T. absoluta* are practically absent. In 2000, Siqueira et al. (2000b) reported up to 9.4-fold resistance levels to abamectin in populations from Brazil; however, no other cases have been detected since. In these strains, efficacy of abamectin was synergized by piperonyl butoxide and triphenyl phosphate suggesting potential involvement of detoxification enzymes in abamectin resistance (Siqueira et al. 2001). Enhanced metabolic detoxification has been reported in abamectin-resistant pests, as well as several mutations in GluCls, the target site of avermectins (Kane et al. 2000; Rugg et al. 2005; Kwon et al. 2010; Pu et al. 2010; Dermauw et al. 2012; Liu et al. 2014; Riga et al. 2014; Wang et al. 2016a).

Spinosad exhibited minimal divergence from the previously defined baseline toxicity (Roditakis et al. 2013a) throughout the sampling period and for all regions in the Mediterranean. Our findings suggest that spinosad remains an extremely potent insecticide for *T. absoluta* control. A single population collected from UK exhibited a 33-fold resistance to spinosyns. Although control failure likelihood was not significant, the farmer reported problematic performance of spinosad (R. Jacobson and E. Roditakis, personal.com.). High levels (478-fold) of field evolved resistance to this class of insecticide have been previously reported in UK (AHDB 2015). Resistance levels identified in this study are substantially lower that those estimated in the 2015 report. Another case of spinosad control failure in Europe was recently reported in Portugal (Berger et al. 2016). High levels of spinosad resistance for *T. absoluta* have also been reported in populations from S. America

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(Reyes et al. 2012; Campos et al. 2014b). Spinosad resistance in *T. absoluta* has been associated with metabolic resistance (increased cytochrome P450 activity) target site mutations (G275E mutation in the nicotinic acetylcholine receptor α6 subunit) and an exon skipping event of the nAChR α6 subunit (Reyes et al. 2012; Berger et al. 2016; Silva et al. 2016c). The mechanisms involved in the spinosad-resistant UK population are currently unknown.

Indoxacarb is one of the basic pest management tools for *T. absoluta* consistently used in early/mid period of the cropping season. Resistance to indoxacarb has not been documented to date; however, the natural variability detected in the early baseline studies was relatively high (up to 12-fold) when compared to the baselines for other chemicals (Roditakis et al. 2013a, b). This variability indicated diverse responses from the European *T. absoluta* populations to this class of insecticides, which was not observed in a respective study conducted in Brazil (Silva et al. 2016b). In this study, indoxacarb exhibited high efficacy levels in all cases, with the exception of one population from Italy and four populations from Greece collected in 2015–2016 sampling period where resistance higher than 13-fold was detected with significant control failure likelihood. Indoxacarb resistance mechanism was recently investigated in laboratory selected strains (Roditakis et al. 2017a). It was demonstrated that the presence of F1845Y and V1848I mutations on segment 6 of Domain IV of sodium channel was strongly associated with the indoxacarb-resistant phenotype, while only partial involvement of detoxification enzymes could be detected. These mutations have been previously reported in indoxacarb-resistant *P. xylostella* (Wang et al. 2016b). Our finding indicated for the first time the presence of indoxacarb-resistant populations in Europe. Just four cases were detected; however, it is of critical importance to utilize these alarming indications and act proactively in order to maintain the high efficacy of the product.

Diamides are extremely potent chemical insecticides for lepidopteran control. They were recently introduced to the market with great success and currently represent approx. 8% of the insecticide market share (Jeanguenat 2013; Sparks and Nauen 2015). Due to their IPM compatible profile, diamides are an extremely versatile tool in *T. absoluta* control. However, due to their extensive use (often off-label), high resistance levels were reported in populations from Italy (Roditakis et al. 2015), and soon after, diamide resistance was reported in Brazil (Silva et al. 2016a). The results of our study indicated that diamide resistance remains well established in Italy and now expanded in Greece and Israel. The mechanisms involved in diamide resistance in *T. absoluta* were recently investigated by Roditakis et al. (2017b) in a range of strains from around the world. The presence of mutations in the transmembrane domain of the ryanodine receptor (RyR) conferring resistance to diamide insecticides was detected. Two of the mutations were novel (G4903V and I4746T) and two corresponded to previously described mutations in *P. xylostella* (G4946E and I4790M) (Troczka et al. 2012; Gong et al. 2014; Guo et al. 2014; Steinbach et al. 2015). Detoxification enzymes may play a role in diamide resistance; however, strong evidence with regard to the role of metabolic mechanisms in this respect is still lacking (Campos et al. 2015; Nauen and Steinbach 2016; Roditakis et al. 2017b). Although the diamide resistance problem is increasing, it is important to note that population sampling is commonly biased, as it is occasionally conducted in crops where pest control performance was problematic. Therefore, based on our field experience, susceptible populations still exist in Italy and Greece that are successfully controlled by diamides. However, in the case that diamide resistance is confirmed, rotation of MoAs as an IRM strategy may not have a notable, immediate effect on diamide resistance levels. The reason is that stability of diamide resistance to lepidopteran pest is extensive (Steinbach et al. 2015; Roditakis et al. 2016) while reversal of the phenomenon may be an extremely complex and long process since fitness cost to diamide resistance is limited or absent in *T. absoluta* (Elias and Stain 2016).

Despite the wide spread of diamide resistance in Europe and S. America, there are still regions, where diamide resistance has not been documented, as identified by this study. Spain, for example, is a region with greenhouse tomato production, pest management issues and climatic conditions comparable to the other Mediterranean countries. Nonetheless, Spanish populations were found susceptible to diamides. A proactive IRM strategy was designed very early after *T. absoluta* introduction in Spain. Industry, researchers and officials, such as Plant Protection Services, were collaborating as well as IRAC Spain to develop an IRM strategy based on rotation of MoAs but also promoting the adoption of non-chemical control methods, such as traps, insect-proof netting and biological control (Bielza et al. 2016) as part of a truly integrated *T. absoluta* management strategy. A huge effort was made by IRAC Spain to communicate this IRM strategy, through meetings and conferences aimed at growers and advisors, as well as through massive divulgation to cooperatives, distribution, officials, etc. Within this IPM approach, Spanish growers rely extensively on the predatory mirid bug *Nesidiocoris tenuis* (Reuter), which is released even in nurseries (preplanting) to allow early establishment and successful control of the tomato leaf miner (Calvo et al. 2012).

It is evident that, by following the basic IPM approaches and by implementing rational IRM tactics, the extreme selection pressure imposed by the frequent insecticide application is suppressed; thus, the driving force of

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insecticide resistance development diminishes. Consequently, the efficacy of the plant protection products can be maintained for prolonged periods as it is profoundly demonstrated in the Mediterranean by the case of Spain. It is important to note that pyrethroids should be avoided for control of *T. absoluta*. It has been demonstrated that pyrethroids are ineffective control agents for this particular pest (Roditakis et al. 2013a; Silva et al. 2015), and reliance on this chemical group may contribute to pest control failures (Silva et al. 2011; Gontijo et al. 2013). It is also noted that within an IRM MoA rotation scheme, alternation of indoxacarb with metaflumizone (an insecticide that belongs to the closely related MoA Group 22B) is not recommended due to indications of cross-resistance in *P. xylostella* (Wang et al. 2016b) and *T. absoluta* (Roditakis, unpublished data). Finally, it is essential to use efficient registered products, at the recommended rates and not exceeding the maximum number of applications as indicated on the product's labels. Sustainable tomato production in Europe is possible in spite of the threat of tomato leaf miner; however, the tomato production industry will have to comply with a major shift toward IPM in current pest management tactics.

Author contributions

ER conceived the idea and wrote the manuscript. JLR, RN and PB contributed in the experimental design and manuscript writing. ER, EV, MOHL and AT analyzed the data. EV, LGV and MRMA performed the bioassays.

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Compliance with ethical standards

Conflict of interest We declare that there is no conflict of interest.

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