CROP PROTECTION

Insecticide Resistance in Argentine Populations of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)

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Resistência a Inseticidas em Populações Argentinas de *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)

RESUMO - A traça-do-tomateiro, *Tuta absoluta* (Meyrick), é uma das pragas chaves no tomateiro na Argentina. O controle químico tem sido o principal método de controle empregado a partir da sua dispersão nos anos 70. Contudo, tem-se observado uma redução na eficácia de alguns dos inseticidas recomendados a partir da década de 80. O objetivo deste trabalho foi estudar a toxicidade de três inseticidas amplamente usados no controle químico de *T. absoluta* (abamectina, deltametrina e metamidofós) em larvas de uma população susceptível de laboratório (CASTELAR) e duas populações colectadas em casa de vegetação (ROSARIO e BELLA VISTA). Inseticidas foram diluídos em acetona e aplicados topicamente na região dorsal mediana do abdome de larvas no segundo dia do quarto estágio larval. Para cada inseticida estimou-se o LD_{50} e calculou-se o Nível de Resistência (NR = LD_{50} de cada população de casa de vegetação/LD_{50} população de laboratório). As populações de ROSARIO e BELLA VISTA mostraram os seguintes NRs: > 68.38 para deltametrina; 2.48 e 3.49 para abamectina, respetivamente; e 0.79 e 0.86 para metamidofós, respetivamente. A resistência a deltametrina observada em ROSARIO pode ser resultante da alta pressão seletiva exercida pelos piretróides nessa localidade. A resistência incipiente a abamectina detectada em BELLA VISTA pode ter sido causado pelo uso freqüente do inseticida nessa localidade ou pode estar associada à variação natural.

PALAVRAS-CHAVE: Traça-do-tomateiro, deltametrina, abamectina, metamidofós, resistência a inseticidas

ABSTRACT - The tomato leafminer, *Tuta absoluta* (Meyrick), is one of the key pests of tomato in Argentina. Since its dispersal in the 1970s, chemical control has been the main method of controlling it. However, reduced efficacy of some of the recommended insecticides has been observed since the 1980s. The aim of this work was to study the toxicity of three insecticides widely used in chemical control of *T. absoluta* (abamectin, deltamethrin and methamidophos) on larvae from a laboratory susceptible population (CASTELAR) and two greenhouse populations (ROSARIO and BELLA VISTA). Insecticides were dissolved in acetone and topically applied to the mid-dorsal abdominal region of two-day old 4th instar larvae. LD_{50} values were estimated and the Resistance Ratio (RR) for each insecticide was calculated (RR = LD_{50} value of each greenhouse population/LD_{50} value of the susceptible population). ROSARIO and BELLA VISTA populations showed the following RRs values: > 68.38 for deltamethrin; 2.48 and 3.49 for abamectin, respectively; and 0.79 and 0.86 for metamidophos, respectively. Deltamethrin resistance observed in ROSARIO could be due to the high selective pressure exerted by pyrethroids in this location. Deltamethrin resistance in BELLA VISTA is more difficult to explain, because pyrethroids were scarcely used in the greenhouse where the insects were sampled. The incipient abamectin resistance detected in the BELLA VISTA population could result from the frequent use of this insecticide in this location, although natural variation can not be discarded.

KEY WORDS: Tomato leafminer, deltamethrin, abamectin, metamidophos, insecticide resistance

Tomato crop is the second horticultural crop in Argentina, with a harvested area of 24,000 ha and an average yield of 30,833 kg/ha (FAO 1996). Seventy per cent of the production destination is for consumption in *natura* and the rest is industrialized (Gómez Riera 1992). Tomato produced under greenhouses as well as in outdoor areas for consumption in *natura* brings the highest gross financial return to farmers in the Litoral Region (Buenos Aires, Corrientes and Santa Fe provinces) (Stoppani & Rodríguez 1992).

The tomato leafminer, *Tuta absoluta* (Meyrick), is a neotropical oligophagous insect, which attacks solanaceous crops. Since the 1960s it has become one of the key pests of tomato crops in many South American countries (Souza *et al.* 1983, Larraín 1986, IAN 1994). A fruit importation from Chile may have introduced it to Mendoza province (Argentina) in April 1964 (Bahamondes & Mallea 1969); dissemination to other tomato production regions occurred through fruit commercialization (Benavent *et al.* 1978, Cáceres 1992, Riquelme 1993).

Larvae can damage tomato plants during all growth stages, producing large galleries in their leaves, burrowing stalks, apical buds, green and ripe fruits (Cáceres 1992, IAN 1994). It can cause important yield losses in different production regions and under diverse production systems (Benavent *et al.* 1978, Cáceres 1992).

Since its introduction, chemical control has been the main method of control used against *T. absoluta* in all production regions in Argentina. Horticultural growers have tried to decrease its injure applying insecticides two times a week during a single cultivation period. Effective chemical control was difficult to achieve because of the mine-feeding behaviour of larvae, lack of a threshold action, and deficient spraying technology.

Initially, the only insecticides used against tomato leafminer in Argentina were organophosphates, which were gradually replaced by pyrethroids during the 1970s. In the early 1980s, cartap, alternated with pyrethroids, and thiocyclam were introduced with the former showing excellent efficacy at that moment. During the 1990s, insecticides with novel sites of action such as abamectin, acylurea insect growth regulators, spinosad, tebufenozide and chlorfenapyr were introduced (Galarza & Larroque 1984, Polack 1999, Cáceres 2000).

Reports of insecticide resistance development in populations of *T. absoluta* were scarce. A decrease in the control efficiency of organophosphorous insecticides was observed in Bolivia and Chile, which could be satisfactorily controlled by pyrethroids (Moore 1983, Larraín 1986). Recently, the existence of resistance to organophosphates and pyrethroids in Chile (Salazar & Araya 1997, 2001) and to abamectin, cartap, methamidophos and permethrin in Brasil (Siqueira *et al.* 2000, 2001) were reported.

The suspicion about the development of resistance in Argentine populations of *T. absoluta* has been present since the 1980s, when growers and agronomic advisers observed that some of the compounds recommended for its control were loosing its effectiveness in the field. Apparently, the loss in effectiveness has not occurred with the same intensity and to the same compounds in all tomato producing regions. Nevertheless, the susceptibility of different populations to distinct active ingredients has not yet been determined. Owing to a decreasing activity of some insecticides used against *T. absoluta* and the report of resistant populations in Chile and Brasil, it is necessary to detect and quantify the resistance to the main insecticides used for its control in Argentina.

The objective of this work was to evaluate the toxicity of abamectin, deltamethrin and methamidophos to a laboratory-susceptible and two greenhouse populations of *T. absoluta*, in order to establish if insecticide resistance has developed in the last ones. The three insecticides used in this study are currently registered and widely used by farmers as chemical control of *T. absoluta* and other tomato pests in Argentina (CASAFE 2001).

Material and Methods

Biological Material. A susceptible population of *T. absoluta* (CASTELAR population), reared since 1993 without exposure to insecticides, was provided by the Insectario de Investigaciones para Lucha Biológica, IMIZA, CNIA-INTA. (Castelar, Buenos Aires Province).

Greenhouse individuals of *T. absoluta* were collected from tomato crops cultivated in the Módulo Experimental de Nuevas Tecnologías (Rosario, Santa Fe Province) (ROSARIO population) and in the Experimental Station, INTA (Bella Vista, Corrientes Province) (BELLA VISTA population). Both populations came from experimental crops grown under greenhouses and exposed to intense chemical treatments to maintain them free of pests. The greenhouses are made of wood and covered by plastic. The sides of them are lifted, whenever required, for regulating the temperature inside it. Information about insecticides used in each site during the current cultivation period was obtained. Individuals from both the susceptible and the greenhouse populations were separately reared on greenhouse organically-grown tomato plants (*Lycopersicon esculentum* Mill) cv. Presto (Petoseed ®) in a controlled environment room at 25 ± 2°C and under a photoperiod of 14:10 L:D. The experimental work was done on two-day old 4^{th} larval stage.

Chemicals. The insecticides used in this study were abamectin 94% (Chemotecnica, Argentina), deltamethrin > 99% (Roussell-Uclaf, France) and methamidophos 68% (Bayer, Argentina). Acetone pro-analysis (Merck, Argentina) was used as solvent.

Bioassay. Insecticides were topically applied to the mid-dorsal abdominal region of the larvae using a micro syringe provided with a dispenser. Each insect received 0.2 µl of a solution of insecticide in acetone. Control groups were topically treated with acetone alone. Five to seven doses of each compound and 15 to 20 larvae for each dose were used to estimate the Lethal Dose 50% (LD_{50}) values. Three to five independent replicates for each bioassay were done. Data of the different replicates were pooled when the confidence limits of their respective LD_{50} overlapped.

After treatment, the larvae were individually placed in 3 cm^3 plastic vials (13 x 35 mm) in a controlled environment room at 27 ± 2°C and under a photoperiod of 14:10 (L:D). Mortality was recorded 24h after treatment under stereoscopic microscope (10x). Larvae were considered as dead when they were not able to move back to ventral position after being placed on their dorsum.

The LD_{50} values were calculated using the Probit method (Lichtfield & Wilcoxon 1949). In all cases, differences between values were considered significant ($P < 0.05$) if the respective 95% confidence limits did not overlap.

Resistance Ratios (RRs) values were calculated by dividing the LD_{50} value of each greenhouse population by the LD_{50} value of the susceptible population. Confidence limits of RRs were calculated according to Robertson & Preisler (1992). RRs were considered significantly different from 1 ($P < 0.05$) when their 95% confidence limits did not include 1.

Results

Fig. 1 shows the dose-response relationship for abamectin and metamidophos in an insecticide susceptible (CASTELAR) and two greenhouse populations (BELLA VISTA and ROSARIO) of *T. absoluta*. The DL_{50} and RRs values are shown in Table 1. The LD_{50} value of deltamethrin was 0.35 µg/larva for the CASTELAR population. The LD_{50} values of deltamethrin could not be calculated for the BELLA VISTA and ROSARIO populations because the highest dose applied (24 mg/larva, which is near the solubility limit of the insecticide) caused only 31.9 and 18.3% of mortality, respectively. The RRs values were > 68.4 in both cases, indicating a high resistance to deltamethrin.

The LD_{50} values of methamidophos were 0.81, 0.70 and 0.65 µg/larva for the CASTELAR, BELLA VISTA and ROSARIO populations, respectively. No significant differences among those values were observed ($P > 0.05$). The RRs values were not significantly different from 1 ($P > 0.05$). Hence, no resistance to this insecticide was observed in the greenhouse populations.

The LD_{50} values of abamectin were 0.16, 0.57 and 0.41 ηg/larva for the CASTELAR, BELLA VISTA and ROSARIO populations, respectively. The BELLA VISTA LD_{50} value was significantly higher than the CASTELAR one ($P < 0.05$), but there was no significant difference between the ROSARIO and CASTELAR values ($P > 0.05$). BELLA VISTA and ROSARIO RRs were 3.49 and 2.48, respectively. Both values differed significantly from 1 ($P < 0.05$). These results indicated a slight resistance to abamectin in the BELLA VISTA population.

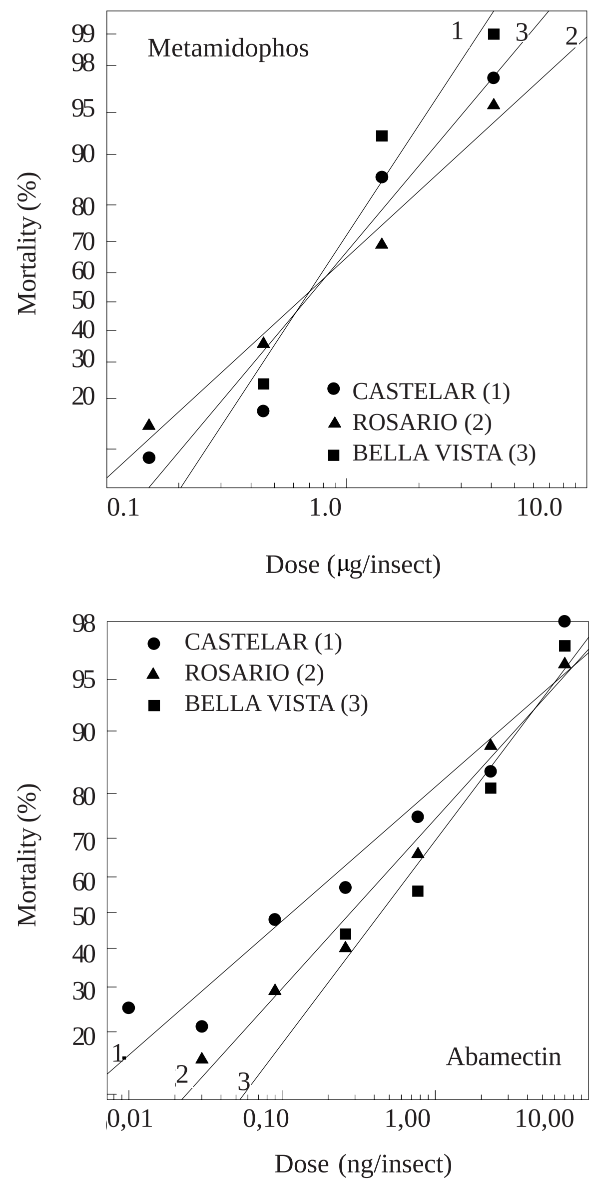

Figure 1. Dose-response regressions for metamidophos and abamectin topically applied on *T. absoluta* 4th larval stage from a laboratory (CASTELAR) and two greenhouse populations (ROSARIO and BELLA VISTA).

Discussion

There are very few studies of insecticide resistance in *T. absoluta*. Salazar & Araya (1997) reported resistance to deltamethrin, metamidophos, esfenvalerate, lambda-cyhalothrin and mevinphos in Chilean populations of this pest. Studying different larval stages from several localities, they found RRs values ranging from 2.2 to 8.2 for deltamethrin, from 1.6 to 3.9 for metamidophos, from 1.9 to 12.6 for esfenvalerate, from 1.8 to 11.5 for lambda-cyhalothrin, and from 1.9 to 5.5 for mevinphos. Later, Salazar & Araya (2001) found higher RRs values for the same insecticides in other populations of *T. absoluta*.

Resistance to abamectin, cartap, methamidophos and permethrin was reported in several Brazilian populations of *T. absoluta* (Siqueira *et al.* 2000). The RRs values varied from 5.2 to 9.4 for abamectin, from 2.2 to 21.9 for cartap, from 2.6 to 4.2 for metamidophos, and from 1.9 to 6.6 for permethrin. In other study, Siqueira *et al*. (2001) studied the toxicity of abamectin, with and without synergists, to six abamectin resistant populations of *T. absoluta*. A complex result was

Table 1. Susceptibility of Argentine populations of the tomato leafminer, *T. absoluta*, to insecticides.

Insecticide	Population	n	Slope ± SE	LD_{50} (95% CL) [1] µg a.i./larva	χ^2	Resistance Ratio[2] (95% CL)[1]
Deltamethrin	CASTELAR	479	0.88 ± 0.13	0.35 (0.22 – 0.55)	3.06 NS [3]	--
	ROSARIO	115	--	> 24.00	--	> 68.38
	BELLA VISTA	130	--	> 24.00	--	> 68.38
Methamidophos	CASTELAR	230	4.42 ± 0.45	0.81 (0.65 – 0.96)	2.12 NS	--
	ROSARIO	222	1.85 ± 0.44	0.65 (0.21 – 1.06)	1.06 NS	0.79 (0.40 – 1.60)
	BELLA VISTA	146	4.16 ± 0.45	0.70 (0.55 – 0.85)	0.71 NS	0.86 (0.65 – 1.5)
				ηg a.i./larva		
Abamectin	CASTELAR	348	0.97 ± 0.12	0.16 (0.09 – 0.27)	4.83 NS	--
	ROSARIO	324	1.35 ± 0.19	0.41 (0.23 – 0.62)	0.98 NS	2.48 [5] (1.22 – 5.02)
	BELLA VISTA	188	1.43 ± 0.29	0.57 [4] (0.28 – 0.92)	1.47 NS	3.49 [5] (1.64 – 7.42)

[1]95% CL = 95% confidence limits; [2]Resistance ratio = LD_{50} greenhouse population/LD_{50} laboratory population; [3]NS = No significant; [4]Significantly different from the laboratory-susceptible population (CASTELAR) (P < 0.05); [5]Significantly different from 1 (P < 0.05).

obtained: piperonyl butoxide (an inhibitor of the mixed function microsomal oxidases activity —MFMO—) suppressed the abamectin resistance completely in only one population and partially in the rest; triphenylphosphate (an inhibitor of the esterase activity) suppressed completely the abamectin resistance in four populations; diethyl maleate (an inhibitor of the glutathion S-transferase activity) suppressed partially the resistance in nearly all populations. These results suggested to the authors that the resistance to abamectin in *T. absoluta* populations may be oligo or even polyfactorial, and that several genes should be involved.

In this work, the existence of insecticide resistance in Argentine populations of *T. absoluta* has been experimentally demonstrated for the first time. Deltamethrin resistance was observed in the two populations studied (ROSARIO and BELLA VISTA), and a weak resistance to abamectin was observed just in one of them (BELLA VISTA).

Deltamethrin is an insecticide widely used in the Horticultural Belt of Rosario city (where the ROSARIO population was collected). We found a high level of resistance (> 68.4) to deltamethrin in individuals from the ROSARIO population. In 75% of cases, pyrethroids (deltamethrin or lambda-cyhalothrin) were sprayed before our sampling (Table 2). The deltamethrin resistance observed could be due to the high selective pressure exerted by pyrethroids on this population.

Abamectin was introduced for tomato leafminer control in the Horticultural Belt of Rosario city in the 1990s to overcome the decrease of cartap efficacy. The ROSARIO population received no application of abamectin, neither before the insects collection nor in recent previous years (Table 2). We found no significant differences between the LD_{50} values of this insecticide evaluated on the ROSARIO and CASTELAR populations.

Metamidophos is a broad spectrum systemic insecticide used by the farmers in the Horticultural Belt of Rosario city only during the vegetative growth stage of tomato because of its pre-harvest interval of 10 days. The lack of resistance to metamidophos in the ROSARIO population could be due to the scarce use of this compound in the sampling site (Table 2).

Deltamethrin was ineffective to control the tomato leafminer in the Experimental Station of Bella Vista city as resulted from efficacy assays carried out in the period 1981 to 1987 (Cáceres 1992), when this pest began to compromise tomato production in this location. However, deltamethrin is used by farmers to control several tomato and pepper pest species (9.5% of the total number of sprays per cultivation cycle) (S. Cáceres, personal communication). The total number of sprays received by the BELLA VISTA population before

Table 2. Insecticides applied to control the ROSARIO population of *T. absoluta* before the insects were collected for this study. Módulo Experimental de Nuevas Tecnologías, Rosario, Santa Fe.

Months (year 2000)	Insecticide	Number of applications	Commercial name
June to October	Deltamethrin	7	Decis 5
July to November	Lamdba-cyhalotrin	5	Karate
July	Methamidophos	1	Tamaron
August	Cartap	1	Padam 50 SP
September and October	Imidacloprid	2	Confidor

the insect collection was 11, with seven applications of abamectin (63.6%), three of imidacloprid (27.3%) and one of deltamethrin (9%) (Table 3). During the two previous years, deltamethrin was not used in the greenhouse where this population was collected, but it was applied in other greenhouses of the same Experimental Station.

In the BELLA VISTA population, a high deltamethrin resistance (RR > 68.4) was observed, although this population received a relatively low number of pyrethroids applications. This result is more difficult to explain. Deltamethrin was used in other greenhouses in the Horticultural Belt of Bella Vista, so resistance could be due to migration. An incipient abamectin resistance was observed in BELLA VISTA (see below), so an alternative explanation is that deltamethrin resistance is due to cross-resistance after abamectin selective pressure. Pyrethroids and abamectin have different sites of action, however, a common metabolic detoxification mechanism could confer resistance to both insecticide groups. For instance, abamectin resistance in the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), largely resulted from increased MFMO activity (Clark *et al.* 1994). The involvement of MFMO in the resistance to deltamethrin was also confirmed in *Cidia pomonella* (L.) after *in vitro* metabolism studies (Sauphanor *et al.* 1997). Abro *et al.* (1988) reported a cypermethrin resistant population of *Plutella xylostella* (L.) which was also resistant to abamectin, indicating that cross-resistance between pyretroids and the latter is possible. The simultaneous application of piperonyl butoxide was followed by partial reversion of the resistance to deltamethrin in *C. pomonella* (Sauphanor *et al.* 1997). Piperonyl butoxide also synergized abamectin in resistant individuals of *P. xylostella* (Abro *et al.* 1988) and, as discussed above, *T. absoluta* (Siqueira *et al.* 2001). These results suggest that MFMO activity could be a mechanism of resistance to both pyrethroids and abamectin.

Abamectin, in combination with mineral oil, was introduced for chemical control of the tomato leafminer in Bella Vista in 1994 and it is still effective in the field (Cáceres 2000). Abamectin has been used in the greenhouses of the Experimental Station of Bella Vista before collecting the BELLA VISTA population and during the last two years, and a weak resistance to this compound (RR = 3.5) was observed. It is possible that resistance is arising due to the selective pressure exerted by the application of abamectin. Other possibility is that the values observed are due to natural variation (this can not be confirmed because in Argentina there is not a baseline of insecticide toxicity to *T. absoluta*).

Table 3. Insecticides applied to control the BELLA VISTA population of *T. absoluta* before the insects were collected for this study. Experimental Station, INTA, Bella Vista, Corrientes.

Month (year 2000)	Insecticide	Number of applications	Commercial name
April	Deltamethrin	1	Decis 5
May to September	Abamectin	7	Vertimec
July to September	Imidacloprid	3	Confidor

Methamidophos was not used for chemical control of the tomato leafminer in the Bella Vista Experimental Station, mainly due to the relatively low efficacy of this compound in comparison with abamectin (Cáceres 1992). Nevertheless, metamidophos is used by growers to control several tomato and pepper pest species (26.2% of the total number of sprays per cultivation cycle on tomato and pepper) (S. Cáceres, personal communication). The lack of usage in the sampling site would explain that no resistance was observed for this compound in the BELLA VISTA population (Table 3).

In the Litoral Region of Argentina, protected tomato production offers a favorable environment for the development of *T. absoluta* from May to August, providing it with food and shelter. Moreover, since populations develop faster in greenhouses than outdoors, the population level and the consequent damage abruptly increase since early October, which leads to early use of insecticides under protected cultivation. The tomato leafminer has several biological traits that favor resistance development. It has many generations per year (more than five), which overlap in protected crops, it has a relatively high fecundity (an average of 40 to 55 eggs per female) and it survives in the soil at the pupa stage (Botto 2000). The larvae and pupae are transported form one region to another in infested containers and fruits, allowing the mixing of individuals subjected to different chemical regimens.

Although a rotation of different active ingredients was done during each cultivation cycle in the two populations studied, it was not aimed to manage resistance. The lack of insecticide resistance management tactics favors resistance development. The occurrence of resistant individuals under greenhouses constitutes a potential danger. Taking into account that the sides of the greenhouses are lifted whenever required, for regulating the temperature inside them, the resistant individuals might migrate outdoors in spring, colonizing outdoor tomato crops. When insecticides are applied, the resistant individuals have a biological advantage in contrast with susceptible ones. This would contribute to the development of resistance in outdoor tomato crops.

Resistance development is a consequence of insecticide applications on an insect population and it must be managed. Resistance management should be a component of integrated pest management, which seeks to minimize pesticide usage through the application of alternative tactics such as cultural control and conservation of natural control through selective insecticides. Monitoring the susceptibility of different populations exposed to distinct active ingredients is essential. The data obtained by means of a monitoring plan should be used to design resistance managing strategies, for example, the alternation of insecticides with different modes of action and detoxification, or the diminishing of conventional insecticide use by employing alternative control tools. Several pests, such as thrips and white flies, develop on tomato crops and are sometimes controlled by the same compounds used against the tomato leafminer. For this reason, insecticide resistance management of *T. absoluta* should be considered in the context of key-pest arthropod complexes of crops.

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