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Pyrethroid resistance in Italian populations of the mite *Varroa destructor*: a focus on the Lombardy region

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Abstract

Varroa destructor Anderson et Trueman (Acari Varroidae) is a major pest of honey bees and synthetic acaricide treatments remain the most effective tool to contain its infestations. In 1991, pyrethroid resistance was first reported in Lombardy, and is now spread worldwide. Recently, three different mutations (L925V/I/M) occurring in the voltage-gated sodium channel have been associated with tau-fluvalinate resistance. Furthermore, in the literature, indirect evidence from laboratory bioassays have indicated that high levels of esterases may be involved in mites resistant to tau-fluvalinate. This study provides an update on the actual spread of target-site resistance to tau-fluvalinate in *V. destructor* samples collected in the Lombardy region. TaqMan assays showed that mutation L925V is present in this area, however only low frequencies of this resistant allele were detected. The majority of resistant mites were found in the homozygous form (11%), and only a small fraction possessed the heterozygous genotype (2%). Additionally, a protocol was set up to detect esterase activity directly in single mites. Slight variability was observed among different populations collected in Lombardy. Additional studies are needed to confirm the involvement of esterases in resistance to pyrethroids in *V. destructor* and whether this can be correlated to changes in enzyme activity.

Key words: pyrethroid resistance, tau-fluvalinate, L925V, esterases.

Introduction

Varroa destructor Anderson et Trueman (Acari Varroidae) is an ectoparasitic mite of *Apis mellifera* L. (Hymenoptera Apidae) and has become one of the major factors responsible for bee colony losses (Nazzi *et al.*, 2012). The mite represents an important biotic stress for bees, causing not only damage by direct feeding on larvae and adults, but also transmitting a wide range of bee viruses (Rosenkranz *et al.*, 2010). Beehive management is an important key for the control of this pest: without management an infested colony will die in 2-3 years (Wang *et al.*, 2002) or even less (Maggi *et al.*, 2016). Beekeepers rely on different approaches to prevent the proliferation of the mites, but the most effective are still chemical treatments. The pyrethroid tau-fluvalinate has been the most commonly used synthetic acaricide for both efficacy and ease of use, together with its relatively low toxicity towards bees (Rosenkranz *et al.*, 2010). For many years, tau-fluvalinate has been successfully used to contain *V. destructor* infestations below critical levels; however, since the 1990s reports of treatment failures have been documented in Europe and research on resistance has continued (González-Cabrera *et al.*, 2018).

The first detection of resistance (1991) was in Lombardy, in North-West Italy (Lodesani *et al.*, 1995; Milani, 1995; Martin, 2004). In 1995, resistant mites were collected in Sicily, Switzerland, Slovenia and Southern France. Subsequently, tau-fluvalinate resistance spread throughout Europe, reaching the UK (Thompson *et al.*, 2002) and Spain (Gracia-Salinas *et al.*, 2006). Pyrethroid-resistant mites were also detected outside Europe: in the USA, the first report (1997) was in South Dakota (Baxter *et al.*, 1998) and a few years later nationwide (Martin, 2004). There were additional reports from Argentina (Fernandez and Omar, 1997) and Israel (Mozes-Koch *et al.*, 2000).

Recently, three different mutations have been associated with resistance to pyrethroids in *V. destructor*. They all occur in the same residue of the voltage-gated sodium channel, a large transmembrane protein that is essential for the generation and propagation of nerve impulse transmission. This is residue 925, located in the transmembrane segment 5 of domain II (IIS5), which is predicted to be a key point for the binding of pyrethoids within the channel protein (O'Reilly *et al.*, 2014). Until now, in resistant mites collected in Europe only the substitution of a leucine to a valine (L925V) caused by a C/G substitution (ctg→gtg) has been reported (Gonzalez-Cabrera *et al.*, 2013; Hubert *et al.*, 2014), whilst in Southeastern USA two alternative mutations (L925M and L925I) have been documented, caused by different polymorphisms (ctg→atg and ctg→ata) (González-Cabrera *et al.*, 2016).

A recent survey managed by Gonzales-Cabrera *et al.* (2018) provided evidence of the wide distribution of mutation L925V across Europe, incorporating mites collected in several countries, including Italy. In that work, Italian samples were derived from one single region (Sicily), and a consistent fraction carried the resistant allele, specifically 39% homozygous and 6.8% heterozygous genotypes for the L925V mutation. No further data on the spread of resistant mites in other parts of the country are available, despite the first notification of the ineffective treatment with tau-fluvalinate being derived from this country. Previous studies highlighted the importance of regular surveys to monitor the susceptibility

to the acaricide used for *Varroa* mite control (Spreafico *et al.*, 2001). The main aim of this study was to obtain further data regarding the extent of *V. destructor* populations in Italy, focusing on the Lombardy region, the first area where pyrethroid control of this pest failed.

It is known from the literature that pyrethroid resistance in arthropods could also be influenced by enhanced esterase activity (Panini *et al.*, 2016). Specifically, higher levels of specific esterases have been reported to be involved in *Varroa* mites resistant to tau-fluvalinate in Europe and Israel (Sammataro *et al.*, 2005). This was derived indirectly from laboratory bioassays, set up to assess different levels of susceptibility among mite populations collected in different field treatment conditions (Milani, 1995). To determine the involvement of detoxification systems, bioassays with specific enzymatic inhibitors, such as piperonyl butoxide (PBO), were performed by other authors (Hillesheim *et al.*, 1996).

Initial attempts to detect directly esterase activity were performed (Gerson *et al.*, 1991). More recently, Mozes-Koch *et al.* (2000) found a positive correlation between resistant mites and their enzymatic detoxification systems: mites that had survived exposure to different concentrations of tau-fluvalinate showed increased monooxygenase and esterase activities. Esterase activity was explored further by determining total esterase activity in relation to different laboratory substrates and zymographic studies to assess inhibitor effects (Dmitryjuk *et al.*, 2014). Direct biochemical assays have been widely used to detect esterase-based resistance mechanisms (Panini *et al.*, 2017a; 2017b). So the second part of this work investigated the potential of a rapid biochemical assay in detecting esterase activity in individual *Varroa* mites to highlight any significant differences among populations.

Materials and methods

Mite samples

Varroa mites were collected in 2017 and 2018, from June to August, in 43 different localities of the Lombardy region. Table 1 summarizes the number of samples analysed for each province.

Samples were mainly provided directly by beekeepers, through sticky boards positioned at the bottom of the colonies or pieces of honeycomb. In the latter form, mites were collected following the procedure described by Dietemann *et al.* (2013). Adult mites were placed in tubes and stored at −20 °C until DNA extraction.

Subsequently, live *V. destructor* specimens were collected in 7 additional localities: 5 from Milano province (MI 11E - MI 15E), 1 from Bergamo (BG 02E) and 1 from Pavia (PV 02E), and stored at −80 °C for enzymatic and molecular analysis.

gDNA extraction and TaqMan assay

Genomic DNA was extracted from single mites as already described (Panini *et al.*, 2014). From each apiary n = 12 specimens were considered (if available), with more than 600 *Varroa* mites analyzed in total (table 1).

TaqMan assay was performed to detect L925V mutation, using primers (forward: Vd_L925V_F; reverse: Vd_L925V_R) and probes (wild-type: Vd_L925_V; mutant: Vd_L925_M) (Gonzalez-Cabrera *et al.*, 2013). ZNA-probes (Metabion International AG) were synthesized with a 3' non-fluorescent quencher and labelled at the 5' end with HEX (535 nm excitation and 556 nm emission) or 6-FAM (495 nm excitation and 520 nm emission), respectively for the wild-type and the mutant probe. The PCR mixture contained 0.9 µM of each primer, 0.2 µM of each probe, 5 µL 2X iTaq Universal Probes SuperMix (Biorad) and 1.5 µL genomic DNA, in a final volume of 10 µL. PCRs were run on a BioRad CFX96^{TM} Real-Time System with the following cycling conditions: denaturation at 95 °C for 5 min, followed by 40 cycles of denaturation 5 sec at 95 °C and annealing/extension 30 sec at 60 °C.

RFLP assay

Samples with the susceptible genotype were randomly screened with a PCR-RFLP assay to verify the absence of the other two possible mutations described on the same locus (L925I and L925M), following published protocol (Millán-Leiva *et al.*, 2018). The PCR reaction contained 0.4 µM of each primer (forward: 1,273iF; reverse: 1,973iR), 12.5 µL DreamTaq Green PCR MasterMix 2X (Thermo Scientific) and 1 µL genomic DNA, in a final volume of 25 µL. PCRs were run on a BioRad CFX96^{TM} with the following cycling conditions: denaturation at 95 °C for 2 min, followed by 35 cycles of denaturation 30 sec at 95 °C, annealing 20 sec at 60 °C and extension 1 min at 72 °C, with a final elongation for 5 min at 72 °C. The digestion mixtures were then assembled with 2 µL of the PCRs, 2.5 U of Psp124BI (Sib Enzyme) and 2 µL of 10X SE buffer Psp124BI, with a final volume of 10 µL. The reaction was incubated at 37 °C for 1h and 30 min, then loaded onto a 2% TBE agarose gel.

4-nitrophenyl acetate (pNA) assay

Ten specimens/locality were screened for total esterase activity using a colorimetric assay with the model substrate 4-nitrophenyl acetate (pNA) (Mozes-Koch *et al.*, 2000), according to the previously published method (Pocker and Stone, 1967). A 100 mM pNA stock was prepared in acetone and diluted in 20 mM phosphate buffer pH 7.0 (final concentration 2 mM). Individual *Varroa* mites were homogenized in 100 µL of 20 mM phosphate buffer pH 7.0 and centrifuged at 4 °C at 10000 × g for 1 min. The supernatant was used as the enzyme source and 10 µL were transferred to single wells of a 96-well microplate, containing 140 µL of 20 mM phosphate buffer pH 7.0. The reaction was started with the addition of 100 µL/well of 2 mM pNA previously prepared (final concentration 0.8 mM; total volume 250 µL/well).

Esterase activity was measured as the rate of hydrolysis of pNA to 4-nitrophenol and acetate; the assay was performed in a microplate reader (MultiScanGo, Thermo Scientific) taking readings every 5 seconds at 405 nm for 15 min. Slopes were recorded as the rate of optical density change per minute at 405 nm (OD_{405}/min).

Table 1. Total number of *V. destructor* specimens analysed and percentages of genotypes split into province and apiary. SS, homozygous wild-type; SR, heterozygous; RR, mutant homozygous.

Province	Apiary (Locality)	Specimens	SS(%)	SR(%)	RR(%)
Bergamo		*42*	*83.3*	*2.4*	*14.3*
	BG 01 (Caprino Bergamasco)	42	83.3	2.4	14.3
Brescia		*175*	*90.3*	*2.9*	*6.9*
	BS 01 (Milzano)	17	100.0	0.0	0.0
	BS 02 (Sabbio Chiese)	32	93.8	0.0	6.3
	BS 03 (Brescia)	4	75.0	25.0	0.0
	BS 06 (Brescia)	2	100.0	0.0	0.0
	BS 07 (S. Polo Borgosatollo)	12	100.0	0.0	0.0
	BS 08 (Manerbio)	12	100.0	0.0	0.0
	BS 09 (Bedizzole)	12	100.0	0.0	0.0
	BS 10 (Desenzano)	12	100.0	0.0	0.0
	BS 11 (Cortine di Nave)	12	33.3	8.3	58.3
	BS 12 (Calvisano)	12	91.7	0.0	8.3
	BS 13 (Alfianello)	12	100.0	0.0	0.0
	BS 14 (Sale Marasino)	12	100.0	0.0	0.0
	BS 15 (Monticelli Brusati)	12	66.7	25.0	8.3
	BS 16 (Brescia)	12	91.7	0.0	8.3
Como		*86*	*82.6*	*1.2*	*16.3*
	CO 02 (Laglio)	53	92.5	0.0	7.5
	CO 03 (Como)	12	50.0	0.0	50.0
	CO 04 (Como)	12	83.3	0.0	16.7
	CO 05 (Como)	4	100.0	0.0	0.0
	CO 06 (Como)	5	40.0	20.0	40.0
Cremona		*98*	*93.9*	*1.0*	*5.1*
	CR 01 (Cremona)	20	100.0	0.0	0.0
	CR 02 (Cremona)	18	94.4	0.0	5.6
	CR 03 (Pescarolo)	12	100.0	0.0	0.0
	CR 04 (Olmeneta)	12	91.7	0.0	8.3
	CR 05 (Gadesco)	12	83.3	8.3	8.3
	CR 06 (Monasterolo)	12	100.0	0.0	0.0
	CR 07 (Picenardi)	12	83.3	0.0	16.7
Lodi		*7*	*85.7*	*0.0*	*14.3*
	LO 01 (Zelo Buon Persico)	7	85.7	0.0	14.3
Mantova		*3*	*100.0*	*0.0*	*0.0*
	MN 01 (Castiglione delle Stiviere)	3	100.0	0.0	0.0
Milano		*175*	*80.0*	*2.9*	*17.1*
	MI 01 (Cassano d'Adda)	6	83.3	0.0	16.7
	MI 02 (Solaro)	31	80.6	0.0	19.4
	MI 03 (Corneliano Bertario)	24	87.5	0.0	12.5
	MI 04 (Montanaso Lombardo)	42	97.6	0.0	2.4
	MI 05 (Paderno Dugnano)	12	66.7	16.7	16.7
	MI 06 (Buscate)	12	8.3	0.0	91.7
	MI 07 (Cernusco sul Naviglio)	12	83.3	16.7	0.0
	MI 08 (San Donato Milanese)	12	41.7	8.3	50.0
	MI 09 (Cambiago)	12	100.0	0.0	0.0
	MI 10 (Segrate)	12	100.0	0.0	0.0
Monza Brianza		*12*	*100.0*	*0.0*	*0.0*
	MB 01 (Cornate d'Adda)	12	100.0	0.0	0.0
Pavia		*6*	*100.0*	*0.0*	*0.0*
	PV 01 (Zavattarello)	6	100.0	0.0	0.0
Sondrio		*6*	*100.0*	*0.0*	*0.0*
	SO 01 (Albosaggia)	6	100.0	0.0	0.0
Varese		*2*	*100.0*	*0.0*	*0.0*
	VA 02 (Samarate)	2	100.0	0.0	0.0
	Total	*612*	*86.8*	*2.1*	*11.1*

Total protein content was measured using the Bradford assay (Biorad Protein Assay Kit) according to the manufacturer's instructions, to convert activity values to OD_{405}/min/mg_{prot}.

The remaining homogenate volume of such specimens was used for DNA extraction and genotyping according to "gDNA extraction and TaqMan assay" paragraph.

Results

Target-site resistance

TaqMan PCR enabled the genotyping of single *V. destructor* specimen collected over the entire Lombardy region, to determine the presence of the wild-type or resistant allele related to mutation L925V. Frequencies (%) of the 3 possible genotypes (SS, homozygous wild-type; SR, heterozygous; RR, mutant homozygous) are reported in table 1. In general, the amount of the resistant allele was low in the tested populations, with small percentages of homozygous resistant specimens (11.1%) and rare individuals showing the heterozygous genotype (2.1%). This trend is not homogeneous among the provinces: some of them showed higher % of the resistant allele (Milano 17% and Como 16%, followed by Bergamo and Lodi 14% each), whilst others were fully susceptible (Mantova, Pavia, Sondrio and Varese). Data for each province are shown in figure 1.

PCR-RFLP analysis confirmed the susceptible genotype of the analysed samples, excluding the presence of mutations L925I and L925M.

Esterase activity

The pNA esterase assay showed it is possible to measure directly the esterase activity of single *Varroa* mite. The analyses were performed on 6 populations collected from Bergamo and Milano (BG 02E and MI 11E-MI 15E), two of the provinces which had shown high frequencies of the mutated allele. One additional population from Pavia, where only susceptible mites were detected from the molecular screening, was also investigated (PV 02E).

Ten specimens/localities were tested. Although esterase activities showed very low levels of variability, one way ANOVA revealed statistically significant differences ($F_{6,40}$ = 9.681; $p < 0.0001$) among populations (figure 2). Student-Newman-Keuls *post hoc test* separates esterase activity means in two groups, with populations BG 02E, MI 13E and MI 14E lower than the others. None of the specimens assayed for esterase activity carried the L925V mutation (data not included in table 1).

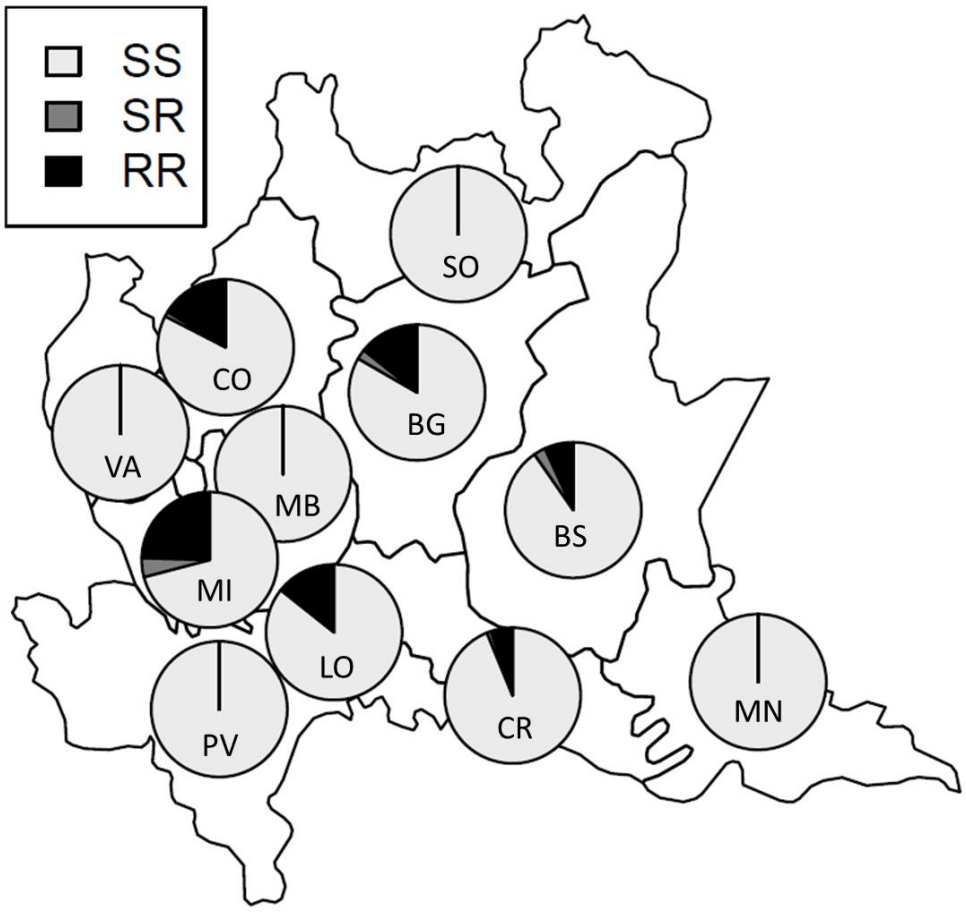

Figure 1. Lombardy distribution map (%) of the resistant mites collected among different localities in 2017 and 2018. Possible genotypes: SS, homozygous wild-type (light grey); SR, heterozygous (dark grey); RR, mutant homozygous (black).

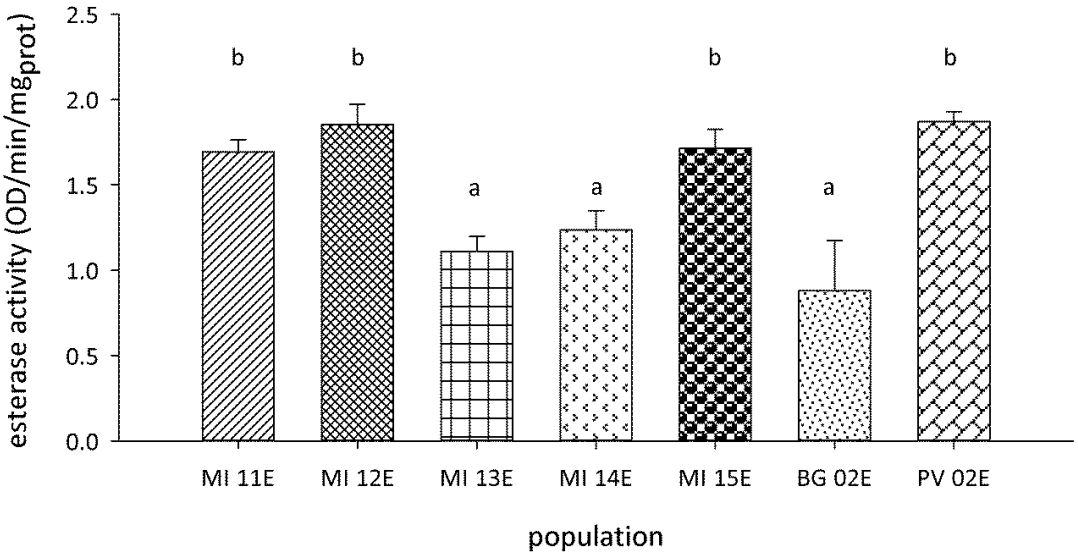

Figure 2. Esterase activities (OD/min/mg_{prot}) of 7 different populations of *V. destructor*. Bars with the same letter do not differ at $p = 0.05$ level (Student-Newman-Keuls post hoc test).

Discussion and conclusions

The emergence of resistance to the pyrethroid tau-fluvalinate in *V. destructor* has become a vital concern for beekeepers worldwide. After the first detection of resistance in the Lombardy region in 1995 (Milani, 1995; Martin, 2004), no further data were forthcoming regarding the spread of resistant mites in this territory, despite the increasing number of reports from across and outside Europe. The importance of the phenomenon is further enhanced by the subsequent correlation of resistance with a target-site mutation located in the gene coding for the sodium channel protein (Gonzalez-Cabrera *et al.*, 2013).

Today in Italy there is a restricted number of active ingredients authorized for the control of *V. destructor* infestations. One of the most used acaricides was tau-fluvalinate but, in recent years, its use has been reduced after consistent failures of the product. Historical documents were not readily available regarding treatments used against *V. destructor* from the beekeepers during this survey. However, the majority indicated they had replaced tau-fluvalinate with other treatments (mainly oxalic acid) and currently pyrethroids have a very limited application. Since it has been demonstrated that high resistance levels to tau-fluvalinate quickly revert to susceptibility in the absence of treatment (Milani and Della Vedova, 2002; González-Cabrera *et al.*, 2018), the importance of investigating the resistance status of populations in the territory is of primary importance to maintain efficacy of this compound. We have no data about the presence of target-site mutations to explain treatment failures experienced in the past but TaqMan assays performed on mites collected around several localities in Lombardy in 2017-2018 showed that the mutation is present in this area; however, low frequencies of the resistant allele were detected. The majority of the resistant mites found were in the homozygous form (11%), and only a small proportion possessed the heterozygous genotype (2%). This is in line with published data which discussed the differences between SR and RR genotype frequencies (Solignac *et al.*, 2005; González-Cabrera *et al.*, 2018). Furthermore, frequencies of resistant mites are not homogeneous among the provinces, with peaks of the homozygous genotype in some localities and fully susceptible populations in others. Considering this, our data suggest a reduction of the frequency of target-site resistance to tau-fluvalinate, suggesting it could still be used, in rotation with other products, against the parasite.

Biochemical analyses of esterase activities in this study represent a preliminary investigation on the topic, which suggests some considerations for future work. Here we developed an assay which enabled measurements of esterase activity on individual mites, essential for the accurate evaluation of the possible involvement of enhanced levels of esterases in acaricide detoxification. Our results revealed significant differences in esterase activity between populations. Nevertheless, it is not possible to correlate such data to metabolic resistance as the limited number of specimens precluded bioassays. The protocol allowed parallel gDNA extraction and evaluation for the presence of target-site mutations. This could be useful in the future to assess the presence of multiple resistance mechanisms.

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References

BAXTER J., EISCHEN F., PETTIS J., WILSON W., SHIMANUKI H., 1998.- Detection of fluvalinate resistance by *Varroa mites* in United States.- *American Bee Journal*, 138: 291-295.

DIETEMANN V., NAZZI F., MARTIN S. J., ANDERSON D. L., LOCKE B., DELAPLANE K. S., WAUQUIEZ Q., TANNAHILL C., FREY E., ZIEGELMANN B., 2013.- Standard methods for varroa research.- *Journal of Apicultural Research*, 52: 1-54.

DMITRYJUK M., ŻÓŁTOWSKA K., FRĄCZEK R., LIPIŃSKI Z., 2014.- Esterases of *Varroa destructor* (Acari: Varroidae), parasitic mite of the honeybee.- *Experimental and Applied Acarology* 62: 499-510.

FERNANDEZ N., OMAR G., 1997.- New indications of decrease in the efficiency of the active ingredient fluvalinate.- *Boletín del Colmenar*, 4: 10-11.

GERSON U., MOZES-KOCH R., COHEN E., 1991.- Enzyme levels used to monitor pesticide resistance in *Varroa jacobsoni*.- *Journal of Apicultural Research*, 30: 17-20.

GONZALEZ-CABRERA J., DAVIES T. E., FIELD L. M., KENNEDY P. J., WILLIAMSON M. S., 2013.- An amino acid substitution (L925V) associated with resistance to pyrethroids in *Varroa destructor*.- *PLoS ONE*, 8: e82941.

GONZÁLEZ-CABRERA J., RODRÍGUEZ-VARGAS S., DAVIES T. E., FIELD L. M., SCHMEHL D., ELLIS J. D., KRIEGER K., WILLIAMSON M. S., 2016.- Novel mutations in the voltage-gated sodium channel of pyrethroid-resistant *Varroa destructor* populations from the southeastern USA.- *PloS ONE*, 11: e0155332.

GONZÁLEZ-CABRERA J., BUMANN H., RODRÍGUEZ-VARGAS S., KENNEDY P. J., KRIEGER K., ALTREUTHER G., HERTEL A., HERTLEIN G., NAUEN R., WILLIAMSON M. S., 2018.- A single mutation is driving resistance to pyrethroids in European populations of the parasitic mite, *Varroa destructor*.- *Journal of Pest Science*, 91: 1137-1144.

GRACIA-SALINAS M., FERRER-DUFOL M., LATORRE-CASTRO E., MONERO-MANERA C., CASTILLO-HERNÁNDEZ J., LUCIENTES-CURD J., PERIBANEZ-LOPEZ M., 2006.- Detection of fluvalinate resistance in *Varroa destructor* in Spanish apiaries.- *Journal of Apicultural Research*, 45: 101-105.

HILLESHEIM E., RITTER W., BASSAND D., 1996.- First data on resistance mechanisms of *Varroa jacobsoni* (Oud.) against tau-fluvalinate.- *Experimental & Applied Acarology*, 20: 283-296.

HUBERT J., NESVORNA M., KAMLER M., KOPECKY J., TYL J., TITERA D., STARA J., 2014.- Point mutations in the sodium channel gene conferring tau-fluvalinate resistance in *Varroa destructor*.- *Pest Management Science*, 70: 889-894.

LODESANI M., COLOMBO M., SPREAFICO M., 1995.- Ineffectiveness of Apistan® treatment against the mite *Varroa jacobsoni* Oud in several districts of Lombardy (Italy).- *Apidologie*, 26: 67-72.

MAGGI M., TOURN E., NEGRI P., SZAWARSKI, N., MARCONI A., GALLEZ L., MEDICI S., RUFFINENGO S., BRASESCO C., DE FEUDIS L., QUINTANA S., SAMMATARO D., EGUARAS M., 2016.- A new formulation of oxalic acid for *Varroa destructor* control applied in *Apis mellifera* colonies in the presence of brood.- *Apidologie*, 47: 596-605.

MARTIN S. J., 2004.- Acaricide (pyrethroid) resistance in *Varroa destructor*.- *Bee World*, 85: 67-69.

MILANI N., 1995.- The resistance of *Varroa jacobsoni* Oud to pyrethroids: a laboratory assay.- *Apidologie*, 26: 415-429.

MILANI N., DELLA VEDOVA G., 2002.- Decline in the proportion of mites resistant to fluvalinate in a population of *Varroa destructor* not treated with pyrethroids.- *Apidologie*, 33: 417-422.

MILLÁN-LEIVA A., HERNÁNDEZ-RODRÍGUEZ C. S., GONZÁLEZ-CABRERA J., 2018.- New PCR–RFLP diagnostics methodology for detecting *Varroa destructor* resistant to synthetic pyrethroids.- *Journal of Pest Science*, 91: 937-941.

MOZES-KOCH R., SLABEZKI Y., EFRAT H., KALEV H., KAMER Y., YAKOBSON B., DAG A., 2000.- First detection in Israel of fluvalinate resistance in the varroa mite using bioassay and biochemical methods.- *Experimental & Applied Acarology*, 24: 35-43.

NAZZI F., BROWN S. P., ANNOSCIA D., DEL PICCOLO F., DI PRISCO G., VARRICCHIO P., DELLA VEDOVA G., CATTONARO F., CAPRIO E., PENNACCHIO F., 2012.- Synergistic parasite-pathogen interactions mediated by host immunity can drive the collapse of honeybee colonies.- *PLoS Pathogens*, 8: e1002735.

O'REILLY A. O., WILLIAMSON M. S., GONZÁLEZ-CABRERA J., TURBERG A., FIELD L. M., WALLACE B. A., DAVIES T. E., 2014.- Predictive 3D modelling of the interactions of pyrethroids with the voltage-gated sodium channels of ticks and mites.- *Pest Management Science*, 70: 369-377.

PANINI M., DRADI D., MARANI G., BUTTURINI A., MAZZONI E., 2014.- Detecting the presence of target-site resistance to neonicotinoids and pyrethroids in Italian populations of *Myzus persicae*.- *Pest Management Science*, 70: 931-938.

PANINI M., MANICARDI G. C., MOORES G., MAZZONI E., 2016.- An overview of the main pathways of metabolic resistance in insects.- *Invertebrate Survival Journal*, 13: 326-335.

PANINI M., TOZZI F., BASS C., ZIMMER C. T., FIELD L., BORZATTA V., MAZZONI E., MOORES G., 2017a.- The interactions between piperonyl butoxide and analogues with the metabolic enzymes FE4 and CYP6CY3 of the green peach aphid *Myzus persicae* (Hemiptera: Aphididae).- *Pest Management Science*, 73: 371-379.

PANINI M., TOZZI F., BASS C., ZIMMER C. T., FIELD L., BORZATTA V., MAZZONI E., MOORES G., 2017b.- Biochemical evaluation of interactions between synergistic molecules and phase I enzymes involved in insecticide resistance in B- and Q-type *Bemisia tabaci* (Hemiptera: Aleyrodidae).- *Pest Management Science*, 73: 1873-1882.

POCKER Y., STONE J. T., 1967.- The catalytic versatility of erythrocyte carbonic anhydrase. III. Kinetic studies of the enzyme-catalyzed hydrolysis of *p*-nitrophenyl acetate.- *Biochemistry*, 6: 668-678.

ROSENKRANZ P., AUMEIER P., ZIEGELMANN B., 2010.- Biology and control of *Varroa destructor*.- *Journal of Invertebrate Pathology*, 103: S96-S119.

SAMMATARO D., UNTALAN P., GUERRERO F., FINLEY J., 2005.- The resistance of varroa mites (Acari: Varroidae) to acaricides and the presence of esterase.- *International Journal of Acarology*, 31: 67-74.

SOLIGNAC M., CORNUET J., VAUTRIN D., LE CONTE Y., ANDERSON D., EVANS J., CROS-ARTEIL S., NAVAJAS M., 2005.- The invasive Korea and Japan types of *Varroa destructor*, ectoparasitic mites of the Western honeybee (*Apis mellifera*), are two partly isolated clones.- *Proceedings of the Royal Society B: Biological Sciences*, 272: 411-419.

SPREAFICO M., EÖRDEGH F. R., BERNARDINELLI I., COLOMBO M., 2001.- First detection of strains of *Varroa destructor* resistant to coumaphos. Results of laboratory tests and field trials.- *Apidologie*, 32: 49-55.

STARA J., PEKAR S., NESVORNA M., ERBAN T., VINSOVA H., KOPECKY J., DOSKOCIL I., KAMLER M., HUBERT J., 2019.- Detection of tau-fluvalinate resistance in the mite *Varroa destructor* based on the comparison of vial test and PCR-RFLP of kdr mutation in sodium channel gene.- *Experimental & Applied Acarology*, 77: 161-171.

THOMPSON H. M., BROWN M. A., BALL R. F., BEW M. H., 2002.- First report of *Varroa destructor* resistance to pyrethroids in the UK.- *Apidologie*, 33: 357-366.

WANG R., LIU Z., DONG K., ELZEN P. J., PETTIS J., HUANG Z., 2002.- Association of novel mutations in a sodium channel gene with fluvalinate resistance in the mite, *Varroa destructor*.- *Journal of Apicultural Research*, 41: 17-25.

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