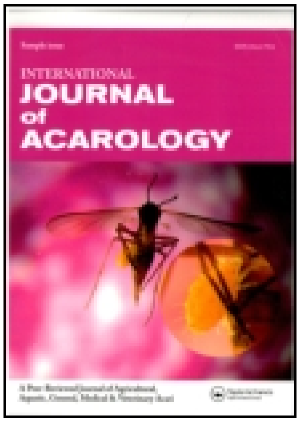

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Susceptibility of *Varroa destructor* (Gamasida: Varroidae) to four pesticides used in three Mexican apicultural regions under two different management systems

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SUSCEPTIBILITY OF *VARROA DESTRUCTOR* (GAMASIDA: VARROIDAE) TO FOUR PESTICIDES USED IN THREE MEXICAN APICULTURAL REGIONS UNDER TWO DIFFERENT MANAGEMENT SYSTEMS

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ABSTRACT – Mexico has five apicultural regions wich are defined according to their blooming period and geography. *Varroa destructor* Anderson and Trueman is controlled with pesticides and alternative treatments in all these regions. To determine the concentration-mortality response lines of *V. destructor,* bioassays with four pesticides that are used for its control in the Gulf of Mexico, Yucatan Peninsula and Central-Highland regions were conducted. The Burgerjon spraying tower was used to apply known concentrations of flumethrin, fluvalinate, amitraz and coumaphos. Lethal concentrations 50 (LC_{50}) and resistance indexes were calculated with Probit analyses. In the Gulf region, the resistance indexes were: flumethrin, 659.43; fluvalinate, 21.83; amitraz, 12.77; coumaphos, 1.49×. In the Central-Highland region: flumethrin, 243.43; fluvalinate, 19.04; amitraz, 8.56; coumaphos, 1.22 x. In the Yucatan Peninsula region: flumethrin, 4057.32; fluvalinate, 199.57; amitraz, 26.55; coumaphos, 3.93×. These results suggest a resistance to flumethrin, fluvalinate and amitraz, with similar values in migratory beekeeping regions, and higher values in the non-migratory region. Coumaphos remained effective in *V. destructor* populations of the Gulf and Central-Highland regions.
Key words – Resistance, Mexico, flumethrin, fluvalinate, amitraz, coumaphos, Burgerjon, migratory, transhumance, *Apis mellifera*.

INTRODUCTION

Varroosis is a parasitic disease caused by the mite *Varroa destructor* Anderson and Trueman (hereafter Varroa). It is the most damaging disease affecting honey bees (*Apis melifera* L.) throughout the world (Dietz and Hermann, 1988; Guzmán-Novoa *et al.*, 1999). Since its detection in Mexico in 1992, several chemical treatments have been used to control it, including fluvalinate, flumethrin and amitraz in specific presentation for bees. Home-made treatments with flumethrin-, coumaphos- and amitraz-based pesticides used for livestock protection, and alternative treatments containing formic acid, oxalic acid or thymol, among others, are also used by beekeepers (Otero-Colina, 1991, 1993; Rodríguez-Dehaibes *et al.*, 1992; Colin *et al.*, 1994; SAGARPA, 2002).

Inappropriate use of pesticides has resulted in resistant Varroa populations in several countries including Italy, France, USA and Argentina (Colin *et al.*, 1994; Milani, 1995; Elzen *et al.*, 1999a, b; Thompson *et al.*, 2002; Pettis, 2004; Rodríguez-Dehaibes *et al.*,

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2005; Maggi *et al.*, 2009). In Mexico, resistance to flumethrin and amitraz was documented in 2005 (Rodríguez-Dehaibes *et al.*, 2005).

Mexico has five apicultural regions: North, Central-Highland, Pacific, Gulf, and Yucatan Peninsula. Each of these is characterized by different types of bloom, climate and two management systems: fixed or sedentary beekeeping**,** practiced all year round in the same area, and migratory or transhumant beekeeping, the most profitable and intensive one, in which hives are moved depending on the flowering seasons. There are two important transhumant routes in Mexico. The first one covers the states of Tamaulipas, San Luis Potosí, Jalisco and Zacatecas in the North and Pacific apicultural regions, while the second and most important one – in the Central-Highland and the Gulf regions – covers the states of Veracruz, Tlaxcala, Puebla, Mexico, Oaxaca and Morelos, all of them renowned for their honey production. Fixed beekeeping takes place mainly in the states of Campeche, Yucatan and Quintana Roo in the Yucatan Peninsula. Almost 50% of Mexican beekeepers belong to this region, and contribute more than 35% of the national honey production (SAGAR, 2000).

All this leads to our first hypothesis: Varroa should have similar susceptibility levels to those pesticides used for its control in the apicultural regions located on the same migratory route due to the intense exchange of bees and, therefore, of mites. We also stated as hypothesis that there must be differences in the susceptibility levels from the Yucatan Peninsula region where beekeeping is sedentary, and has no population exchange, compared to those of the transhumant routes mentioned above.

When estimating levels of resistance, it is important to use the same method to obtain an adequate comparison over time. Pérez Santiago *et al.* (2000) and Rodríguez-Dehaibes *et al.* (2005) used aspersion with the Burgerjon tower to determine lethal concentrations 50 (LC_{50}) for Varroa. The aim of this research was to determine the LC_{50} for Varroa to flumethrin, fluvalinate, amitraz and coumaphos in three apicultural regions in order to estimate the resistance in time with respect to geographical variation and management systems.

MATERIALS AND METHODS

Adult females of Varroa were used in all bioassays. Mites were obtained from bee apiaries in Tejería, Veracruz (Gulf region), Texcoco, Mexico (Central-Highland region) and Sabancuy, Campeche (Yucatan Peninsula region) (Fig. 1); one apiary per region,

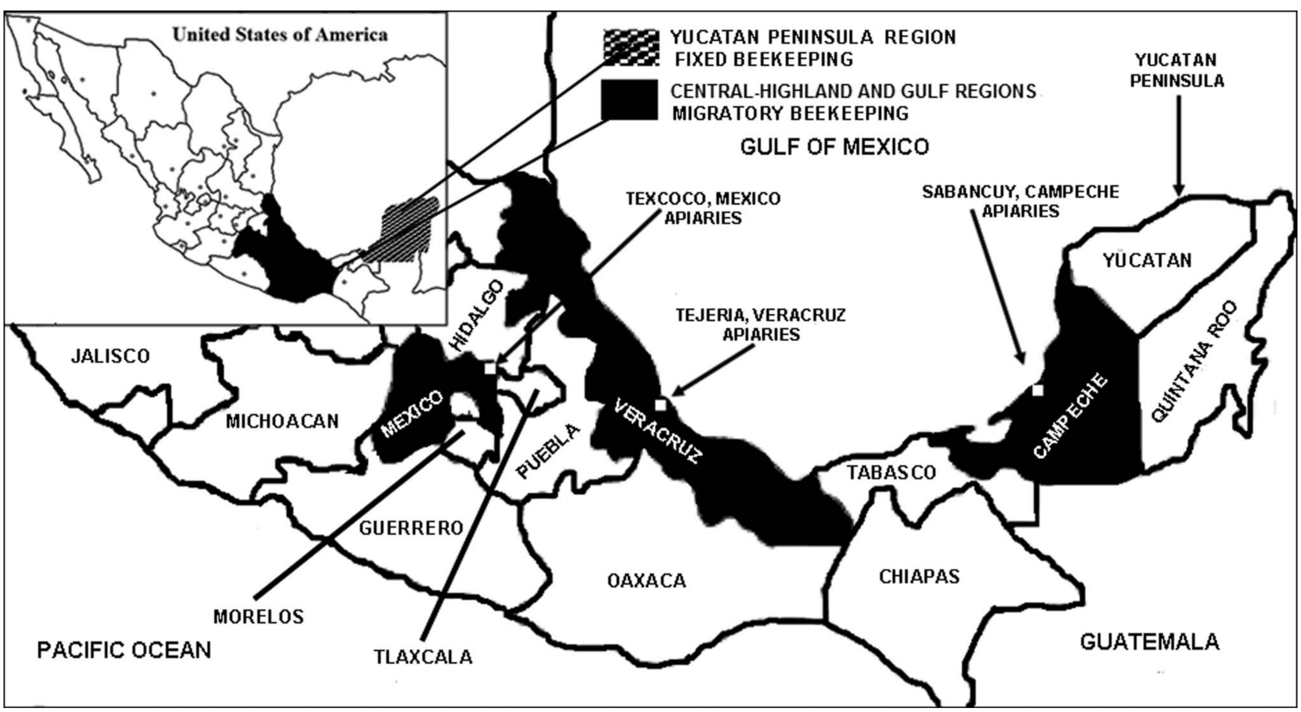

Fig. 1. Location of apiaries, source of Varroa females used in the bioassays, from two of the most important apicultural regions in Southeastern Mexico.

30 hives each one. There is an intense migration or transhumance in the first two regions; beehives are moved between the states of Veracruz, Puebla, Mexico, Tlaxcala, Oaxaca and Morelos, but not in the Yucatan Peninsula region. Mites were collected with a fine brush from capped cells occupied by drone pupae. They were kept in modified disposable Petri dishes (with a ventilation hole) in an incubator chamber at 33°C and 60% R.H, supplied with drone pupae as food for a maximum of 24 h before the bioassays.

The Burgerjon tower (Burgerjon, 1956) was used for bioassays with amitraz (Taktic ®, liquid, 12.5%, Hoechst, Frankfurt, Germany), flumethrin (Bayticol®, concentrated emulsion 3%, Bayer, Leverkusen, Germany), fluvalinate (Mavrik ® Perimeter 22.3%, flowable liquid, Wellmark, Schaumburg, IL, USA) and coumaphos (Asuntol ® liquid 20%, Bayer, Leverkusen, Germany). Pesticide dilutions were prepared with double distilled water as solvent immediately before each bioassay. Pesticides were administered according to the method described by Pérez Santiago *et al.* (2000) and Rodríguez-Deahibes *et al.* (2005). The study was conducted at the Laboratorio de Apicultura de la Posta Zootécnica Torreón del Molino, Facultad de Medicina Veterinaria y Zootecnia, Universidad Veracruzana, in Veracruz, Mexico.

The interval between the highest concentration that does not kill any Varroa and the lowest concentration that kills an entire population was calculated. Subsequently, five logarithmically separated concentrations within this response range were prepared. Fourteen mites were used per replicate, with four replicates for each dilution. A control, which was sprayed with double distilled water, was also included.

Once treated, mites were placed in modified disposable Petri dishes with three drone pupae as food and a small cotton ball moistened with double distilled water. Specimens were kept in the incubator for 24 h. Mortality was subsequently determined with a stereoscopic microscope. A Varroa was considered dead when it did not move in response to the contact with a fine brush.

Mortality obtained from each dilution of each pesticide per replicate was corrected against the control mortality with Abbott's formula (Abbott, 1925). The logarithmic concentration-mortality lines and the LC_{50} were calculated with Probit analysis. Resistance indexes were obtained by dividing the current LC_{50} by the lowest LC_{50} obtained by Pérez Santiago (1995), Pérez Santiago *et al*. (2000) or Rodríguez-Dehaibes *et al.* (2005). The LC_{50} 95% confidence limits overlap was used to determine if a population had a similar response to the toxic (Robertson and Preisler, 1992).

RESULTS

In the case of the LC_{50} of flumethrin from Veracruz and Texcoco obtained in this assay, as well as those from Veracruz collected by Rodríguez-Dehaibes *et al.* (2005), response lines were displaced almost three logarithmic cycles compared to those of Pérez Santiago *et al.* (2000), which are considered the baseline for this study. This finding indicates the development of resistance of Varroa to flumethrin, as shown by high resistance indexes (Table 1). The 95% confidence intervals for Veracruz and Texcoco did not overlap, indicating a significantly higher level of resistance in the first place. The LC_{50} found in the Yucatan Peninsula was displaced four logarithmic cycles in relation to the baseline, placing the Yucatan Peninsula as the region with the highest development of resistance to flumethrin.

Populations from Veracruz and Texcoco showed similar LC_{50} for fluvalinate, although the slope was steeper in the Varroa population from Veracruz, which means that the population could be more homogeneous in its response to the pesticide. Nevertheless, the 95% confidence limits of these two LC_{50} overlapped, suggesting that there is no significant difference between the two Varroa populations. The three slopes were similar when compared to the baseline, although the LC_{50}'s in this study were displaced more than one logarithmic cycle which, in the case of fluvalinate, could mean that those Varroa populations are beginning the process of susceptibility loss (Table 1). The slope in the Yucatan Peninsula was less steep, indicating a more heterogeneous response to the toxic. Furthermore, the confidence limits 95% of LC_{50} did not overlap, suggesting that resistance level in the Varroa population from Yucatan Peninsula is significantly higher from the baseline and also from the two other populations.

The Veracruz and Texcoco Varroa populations LC_{50} for amitraz were very similar (Table 1), and their 95% confidence limits overlapped, indicating that they are not significantly different. On the other hand, LC_{50} for the Yucatan Peninsula had a steeper slope compared to that of Veracruz, with no overlap in their confidence limits, indicating a significant difference. The baseline showed a similar slope to that of Veracruz, but was separated from both populations by one logarithmic cycle on its LC_{50}, suggesting the development of moderate resistance at Veracruz and Texcoco, and even more at the Yucatan Peninsula.

For coumaphos, Varroa from Veracruz and Texcoco showed similar LC_{50}'s and slopes to the baseline (Table 1) and their confidence limits overlap, suggesting similar susceptibility. These results suggest

Table 1. *Varroa destructor* mean lethal concentrations (LC_{50}) and resistance indexes to flumethrin, fluvalinate, amitraz and coumaphos, obtained from the Central-Highland, Gulf and Yucatan Peninsula apicultural regions, Mexico. Compared to the baselines and shown by the resistance indexes, significantly higher LC_{50}'s of all pesticides were found, except coumaphos in the Gulf and Central-Highland regions. The highest resistance indexes were consistently found in the Yucatan Peninsula.

Pesticides/apicultural regions	LC_{50}	Confidence limits 95% (LC_{50})	Slope	Resistance index
Flumethrin				
Gulf (Tejería, Veracruz, 2006)	0.577	0.4439–0.7648	1.0659	659.43 c
Central-Highland (Texcoco, Mexico, 2006)	0.213	0.1689–0.2675	1.3153	243.43 b
Yucatan Peninsula (Sabancuy, Campeche, 2007)	3.550	3.0124–4.2098	1.8051	4, 057.14 d
Baselines: Pérez Santiago *et al.,* 2000	0.000875	0.0002–0.0065	0.4401	−a
Fluvalinate				
Gulf (Tejería, Veracruz, 2006)	4.083	3.3593–5.0336	1.5788	21.83 b
Central-Highland (Texcoco, Mexico, 2006)	3.560	2.6119–4.7461	1.0857	19.04 b
Yucatan Peninsula (Sabancuy, Campeche, 2007)	37.320	24.9479–55.2944	0.7131	199.57 c
Baselines: Pérez Santiago *et al.* 2000	0.187	0.1253–0.2893	0.8103	−a
Amitraz				
Gulf (Tejería, Veracruz, 2006)	2.937	2.1527–4.4246	1.0276	12.77 b
Central-Highland (Texcoco, Mexico, 2006)	1.969	1.6111–2.4331	1.5610	8.56 b
Yucatan Peninsula (Sabancuy, Campeche, 2007)	6.107	5.0600–7.5176	1.7313	26.55 c
Baselines: Pérez Santiago *et al.* 2000	0.23	0.1365–0.3668	0.8813	−a
Coumaphos				
Gulf (Tejería, Veracruz, 2006)	6.244	5.2373–7.4822	1.9144	1.49 a
Central-Highland (Texcoco, Mexico, 2006)	5.115	4.0024–6.6175	1.1627	1.22 a
Yucatan Peninsula (Sabancuy, Campeche, 2007)	16.486	13.6341–19.8938	1.7078	3.93 b
Baselines: Pérez Santiago *et al.,* 2000	4.190	2.6305–6.6368	1.1621	−a

Note: Resistance indexes are significantly different when baselines and compared pesticide LC_{50} confidence limits do not overlap (Robertson and Preisler, 1992).

that susceptibility levels to the pesticide are either maintained or are very similar to the baselines. The LC_{50} seen for the Yucatan Peninsula was displaced less than 1 logarithmic cycle with respect to the baseline, with a resistance index of 3.96.

DISCUSSION

Right after Varroa was discovered, two pesticides were authorized in Mexico for their use to protect honey bees: fluvalinate and flumethrin (both from the toxicological group of pyrethroids). Five years later, the formamidine pesticide amitraz was authorized (SAGARPA, 2002). In addition, beekeepers from several regions have prepared homemade products using the above mentioned pesticides as well as coumaphos, an organophosphate, which is used in agriculture and livestock protection. The use of these pesticides has caused selection pressure and differed from place to place (Rodríguez-Dehaibes *et al*., 2005).

Varroa's response to the studied pesticides has been consistently similar in the Gulf and Central-Highland beekeeping regions, represented by mites collected from Tejería, Veracruz, and Texcoco, Mexico, respectively. The reason might be that both populations are found within the same migratory route, where bee populations and their parasites are constantly interchanging. The baselines were obtained in 1995 in Córdoba, Veracruz, in the Gulf region, and enabled us to compare the evolution of resistance in time and in the same migratory route.

Varroa's high rate of resistance to flumethrin could be due to the beekeepers continuous use of homemade preparations with this pesticide (acquired from commercial pesticides sold for use in cattle regions, as personally seen by the authors). The resistance index of fluvalinate is relatively lower compared to that found for flumethrin. Fluvalinate was the first pesticide used in 1992 in commercial formulation for bees, and beekeepers also used it in homemade treatments (Rodríguez-Dehaibes *et al*., 2005). Since 1998, however, there are no agricultural pesticides in Mexico with fluvalinate as active ingredient, thus delaying the development of resistance. Thompson *et al*. (2002) found decreased efficacy of flumethrin and fluvalinate (2–5%) on Varroa strains presenting LC_{50} values 13 and 11 times higher than that of a susceptible stain. Populations in this study subjected to flumethrin and fluvalinate had resistance indexes from 19 to 4057×, thus are considered resistant to these pyrethroids. Extremely high resistance indexes have been associated to target site insensitivity, often associated with other resistance mechanisms. In this case, up to four amino acid mutations in the sodium channel (Wang *et al*., 2002) alone, or associated with increased monooxygenases (Hillesheime *et al*., 1996; Mozes-Koch *et al*., 2000), might be responsible for this exacerbated case of pyrethroid resistance. In the case of formamidines (amitraz), resistance indexes above five clearly show the development of resistance (Li *et al.*, 2004), although compared to the pyrethroids, this pesticide has been less used. Resistance indexes above five in the case of formamidines (amitraz), clearly show the development of resistance (Li *et al.*, 2004), although compared to the pyrethroids this pesticide has been less used. It is worth notice that the LC_{50} found by Rodríguez-Dehaibes *et al.* (2005) represents an intermediate link in the development of resistance to amitraz in time, although probably with a more homogeneous response to the pesticide.

Compared with all pesticides studied, our results regarding coumaphos do not differ from those found by Pérez in 1995, with a slight increment in the Yucatan Peninsula. The low resistance levels in the beekeeping regions in our study match the scarce use of this pesticide. Mexico has not approved the registration of coumaphos for bees, and its use in homemade preparations in our studied regions has been uncommon.

When the resistance indexes of mites collected in the Yucatan Peninsula are compared against those from the Gulf region and Central-Highland, we obtained very high values for flumethrin and fluvalinate, possibly due to a couple of factors: the continuous use of these pesticides, as well as the fact that there is no bee hive mobilization in this region, so bee parasites are easily selected and have a high degree of genes resistant to the used pesticides. Furthermore, both products are pyrethroids, being more likely to share resistance mechanisms (enzymatic or point mutations). Likewise, resistance to amitraz and coumaphos is less pronounced, although seemingly, there is a resistance development for amitraz and incipient in coumaphos.

The continuous presence of various pesticides accumulated in the wax and pollen in the hive also could be responsible of future losses in susceptibility of Varroa to this and other pesticides (Mullin *et al*., 2010), and must be taken into consideration.

The lack of a comprehensive management program for Varroa is evident, and it is supported by the resistance indexes found, in a higher degree for flumethrin and fluvalinate, and to a lesser extent for amitraz. Most ideas to decrease the development of resistance include the rotation of acaricides. Milani (1999) suggested that rotating chemical and non-chemical products, combined with bees resistant to Varroa, should delay the onset of resistance. In Minnesota, USA, fluvalinate-resistant mites were also resistant to amitraz, but not to coumaphos (Elzen *et al.*, 2000). Thompson *et al.* (2002) did not show cross resistance between any of these two pyrethroids and amitraz or coumaphos; however they suggested cross resistance between flumethrin and fluvalinate, as well as did Rodríguez-Dehaibes *et al.* (2005) in Veracruz, Mexico. Coumaphos as well as alternative treatments with different modes of action must be an option in a resistance management program for Varroa in Mexico.

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