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Morphometric identification of *Varroa destructor* and its plasticity by the exposure to thymol

Identificación morfométrica de *Varroa destructor* y su plasticidad por la exposición a timol

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ABSTRACT

Currently, there are four *Varroa* species identified worldwide, which present a high interspecific and intraspecific variability. The objective of this investigation was to identify the predominant species of *Varroa* and the effect of thymol on the plasticity of the mite. To determine the species and effect of thymol on the plasticity of the mite, 150 specimens from 65 hives and 54 from 17 beehives exposed to thymol for 28 days were morphometrically tested. According to the morphometric measurements, the mites were identified as *Varroa destructor*, there being no morphometric evidence of the infestation by other Varroa species. Mite populations differed among apiaries (P≤ 0.05), so that variables genital shield width (P=0.013), genital shield length (P=0.002) and genital shield width (P=0.026) were plus variants. We found 8 morphotypes, observing differences between the means of the genital shield length for thymol effect (P≤ 0.05). It is concluded that *Varroa destructor* exposed to thymol presents intraspecific morphometric variability for adaptation to the selection pressure imposed by the acaricide.

Keywords: Morphometry, pathogen, acaricide.

RESUMEN

Actualmente existen cuatro especies de *Varroa* identificadas en todo el mundo, las cuales presentan una alta variabilidad interespecífica e intraespecifica. El objetivo de esta investigación fue identificar la especie predominante de Varroa y el efecto del timol sobre la plasticidad del ácaro. Para determinar la especie y el efecto del timol sobre la plasticidad del ácaro se analizaron morfométricamente 150 especímenes de 65 colmenas y 54 de 17 colmenas expuestas a timol por 28 días. De acuerdo a las medidas morfométricas, los ácaros fueron identificados como *Varroa destructor,* no habiendo evidencia morfométrica de la infestación por otras especies del genero *Varroa.* Las poblaciones de ácaros difieren entre apiarios (P≤ 0.05), por lo que las variables ancho del escudo genital (P=0.013), largo del escudo genital (P=0.002) y ancho del escudo anal (P=0.026) fueron más variantes. Se encontraron 8 morfotipos, observándose diferencias entre las medias de largo del escudo genital por efecto del timol (P≤ 0.05). Se concluye que *Varroa destructor* expuesta al timol presenta una variabilidad morfométrica intraespecífica por la adaptación a la presión de selección impuesta por el acaricida.

Palabras claves: Morfometría, patógeno, acaricida.

INTRODUCTION

Varroasis is the main parasitic disease affecting the bee (*Apis mellifera*) worldwide; this disease is caused by at least four identified species: *Varroa jacobsoni Oudemans*, *Varroa underwoodi*, *Varroa rindereri* and *Varroa destructor* (Oudemans, 1904; Dauphiné *et al*., 1987; De Guzman *et al*., 1996; Anderson *et al*., 2000). These four *Varroa* species directly affect *A. mellifera* pups at the most sensitive stages of their ontogenetic development, feeding mainly on the haemolymph of their host, causing: weight loss, decreased flight performance, increased premature search of food, diminution of learning capacity, diminution of the return rate and reduction of the average life of the bees; also that they present a pathogenic function of many viral diseases associated with disorders of colony collapse; These negative causes are considered the most destructive disease of honey bees (De Jong *et al*., 1982, Duay *et al*., 2003, Kralj *et al*., 2006, Cox *et al*., 2007, de Miranda *et al*. , 2010).

Elucidating the potential of genetic and phenotypic variability related to the global distribution process, gave rise to a series of studies on the intraspecific morphological differentiation of the parasite (Delfinado *et al*., 1989, De Guzman *et al*., 1996). The plasticity of the *Varroa* mite is influenced by the geographical variability, the natural climatic conditions and also with the different host species (Boudagga *et al*., 2003, Maggi *et al*., 2009, Akinwande *et al*., 2012, Badejo *et al*., 2013, Aude *et al*., 2016; Dadgostar *et al*., 2018). In this sense it has been shown that there is a great phenotypic plasticity within the same population of mites in different species of bees worldwide (Akimov *et al*., 2004); this plasticity is classically defined as the phenotypic adjustment of an organism to the environment, that is to say adaptation through morphological, physiological and behavioral changes of individuals; allowing the maintenance of the individual aptitude, and therefore leads to the persistence of the population and the species (Pigliucci, 2005; Nussey *et al*., 2007).

Although the different genotypes of *Varroa* are known, little is known about the epigenetic characteristics and phenotypic differences of *Varroa* populations that affect honey bees in this region; in this sense, it is necessary to use morphometric discrimination techniques (Delfinado *et al*., 1989), which are based on the analysis of measurements of some segment of the body and that mainly uses the concepts of size and shape (López *et al*., 2002). In this sense, by means of morphometric analysis, the *Varroa* species was identified and its plasticity varies after treatment with thymol.

MATERIAL AND METHODS

Location of the experimental area

The investigation was carried out in the Tepic municipality, Nayarit, Mexico; located at 21° 51'and 21° 24' north latitude, 104° 34'and 105° 05' west longitude at 915 m a.s.l. In the zone the subhumid warm climate predominates with rains in summer, and the semi-warm subhumid with rains in winter; the average annual rainfall is 1,121 mm and the average temperature is 21.1° C (Fernández *et al*., 2010).

Obtaining samples

Approximately 300 bees were collected that were located between the third and fourth chambers of the breeding chamber, said bees were placed in containers with 70%

alcohol. Samples were collected on day zero (prior to the first application of thymol) and at the end of treatment on day 28. *Varroa* specimens were obtained using the methodology of De Jong *et al*. (1982), in the Functional Biology Laboratory of the Academic Unit of Veterinary Medicine and Zootechnics of the Autonomous University of Nayarit.

Experimental units

To determine *Varroa* species and morphometric variability, 150 *Varroa* specimens from 65 hives were analyzed, and to determine the effect of thymol on *Varroa* plasticity, 54 specimens were taken from 17 hives with the presence of the mite before and after the thymol application of the beehives presented the following characteristics: seven spaces between racks full of adult bees, queen with posture and a honey cast with pollen; these beehives were from 5 apiaries destined for the formation of queen bees fertilization nuclei.

Processing of mites

Varroa mites were processed for observation following the techniques described by Krantz (1978) and Maggi *et al*. (2009). Each mite was placed in 50 % lactic acid for 2 hours at 100 °C; subsequently the mites were stored in alcohol at 50% v / v until observed. The morphometric characters were measured using a stereoscopic microscope with an eye micrometer at 20X.

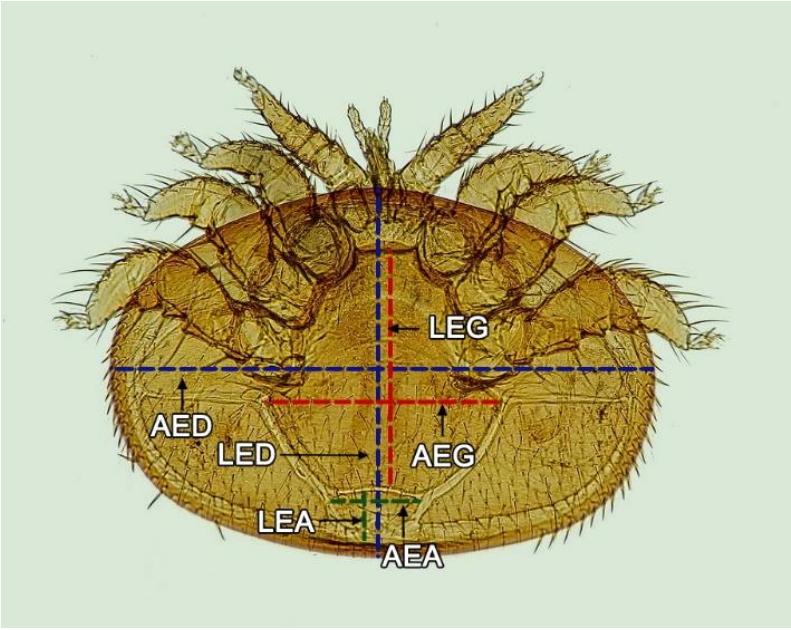

Figure 1. Variables measured of *Varroa*: (AED) dorsal shield width, (LED dorsal shield length, (AEG) genital shield width, (LEG)), genital shield length, (AEA) shield width anal, (LEA) length of the anal shield

Morphometry

Six variables were measured (Figure 1) in each of the specimens: dorsal shield width (AED), dorsal shield length (LED), genital shield width (AEG), genital shield length (LEG), shield width anal (AEA) and length of the anal shield (LEA).

Preparation of thymol

Thymol crystal previously pulverized with a purity of 98.0% was used. The treatment consisted of mixing 8 g of thymol and 32 g of icing sugar to obtain a concentration of 20%.

Application of the treatment

For the application, packages with 40 g of the mixture were prepared, which were scattered on 20 x 20 cm paper boxes on the breeding chamber. The treatment was applied 3 times with intervals of 7 days.

Statistical analysis

To determine the morphometric differences of *Varroa* among the apiaries, a comparison of means with a one-way ANOVA test was performed, variables that had significant differences were subjected to a second post hoc multiple comparison analysis by means of a comparison of Tukey means. A correlation analysis was performed among the variables under study. To relate the plasticity with the thymol-based treatment, a Pearson correlation was performed. To determine the morphotypes, an analysis of average K-clusters was made; for this, the Software Statistical Package for the Social Sciences (SPSS) version 20 was used (IBM, 2011).

RESULTS

According to (Anderson *et al*., 2000) it was determined that the predominant species in this region is *Varroa destructor* with averages of AED (1688.40 ± 33.46 µm) and LED (1128.10 ± 25.76 µm). The populations of mites differed (P≤ 0.05) among apiaries (Table 1), so it was found that the variables AEG (P = 0.013), LEG (P = 0.002) and AEG (P = 0.026) were more discriminant in comparison AED (P = 0.086), LED (P = 0.16) and the LEA (P = 0.34). The LEG variable was the one that presented a correlation (P≤ 0.05) with all the variables studied; however, its correlation was greater in relation to the AED and AEG variables (Table 2).

Table 1. Mean of the variables studied (µm) belonging to the populations of *Varroa destructor* of 5 apiaries evaluated.

Apiary	AED	LED	AEG	LEG	AEA	LEA
1	1696 a	1140 a	721 a	593 a	276 ab	129 a
2	1684 a	1118 a	718 ab	575 ab	286 a	132 a
3	1666 a	1119 a	679 b	557 b	271 b	125 a
4	1691 a	1138 a	715 ab	594 ac	283 ab	134 a
5	1705 a	1125 a	725 ac	595 ac	286 ac	131 a

(AED) width of the dorsal shield, (LED) length of the dorsal shield, (AEG) width of the genital shield, (LEG) length of the genital shield, (AEA) width of the anal shield and (LEA) length of the anal shield.

Table 2. Identification of discriminant variables of *Varroa destructor* by Tukey analysis

Variable	F	Sig.
AED	2.181	.086
LED	1.706	.165
AEG	3.569	**.013**
LEG	4.946	**.002**
AEA	3.039	**.026**
LEA	1.143	.348

(AED) width of the dorsal shield, (LED) length of the dorsal shield, (AEG) width of the genital shield, (LEG) length of the genital shield, (AEA) width of the anal shield and (LEA) length of the anal shield.

Eight morphotypes were identified; morphotype A was established in mites collected in apiary 1; the morphotype B was found in mites belonging to the apiaries 1, 2; 4 and 5; the morphotypes C, F and H were found in mites of the 5 apiaries; morphotype D was established in mites of apiary 3; the morphotype E was found in mites of apiaries 1, 2, 4 and 5; the morphotype G was found in mites of apiaries 2 and 3. The mean of each variable studied belonging to the eight morphotypes of *V. destructor* and the results of ANOVA is shown in (Table 3 and 4). By means of an equality analysis (Table 5) a correlation (P≤ 0.05) was found between the treatment and the variables AED (P = 0.065) and LEG (P = 0.002).

Table 3. Mean of the variables studied (µm) belonging to the 8 morphotypes of *V. destructor*

MORPHOTYPES	AED	LED	AEG	LES	AEA	LEA
A	1583	1042	533	483	267	108
B	1707	1147	738	589	290	139
C	1699	1146	683	569	281	137
D	1692	1092	642	583	258	125
E	1708	1134	718	613	276	131
F	1653	1115	707	589	281	124
G	1629	1142	717	533	263	117
H	1700	1109	725	565	282	127

(AED) width of the dorsal shield, (LED) length of the dorsal shield, (AEG) width of the genital shield, (LEG) length of the genital shield, (AEA) width of the anal shield and (LEA) length of the anal shield.

Table 4. ANOVA hierarchical cluster k averages of 8 *V. destructor* morphotypes

Variable	Mean	F	Sig.
AED	5673.763	15.000	**.000**
LED	2958.600	10.197	**.000**
AEG	7459.992	32.075	**.000**
LEG	4172.858	19.927	**.000**
AEA	372.733	2.415	**.035**
LEA	369.098	4.720	**.001**

(AED) width of the dorsal shield, (LED) length of the dorsal shield, (AEG) width of the genital shield, (LEG) length of the genital shield, (AEA) width of the anal shield and (LEA) length of the anal shield

Table 5 Equality tests of the means before and after the treatment of the variables of *V. destructor*

Variables	Lambda of Wilks	F	Sig.
AED	.904	3.631	.065
LED	.988	.412	.525
AEG	.944	2.010	.165
LEG	.745	11.615	**.002**
AEA	.941	2.115	.155
LEA	1.000	.000	1.000

(AED) width of the dorsal shield, (LED) length of the dorsal shield, (AEG) width of the genital shield, (LEG) length of the genital shield, (AEA) width of the anal shield and (LEA) length of the anal shield.

DISCUSSION

The results found in this investigation correspond to the species of *Varroa* destructor; since the variables AED and LED are similar, but lower than those found in different parts of the world (Anderson *et al*., 2000; Zhang, 2000 ;, Boudagga *et al*., 2003; Maggi *et al*., 2009; Kelomey *et al*. ., 2016); however, our results coincide with the general average described for this mite (Anderson *et al*., 2000); these morphometric differences are due to the interaction between the parasite and its host. It has been observed that when the host has body variation, the parasite also changes this condition (Giménez *et al*., 2017); that is, the morphometric variations of the mite depend on the *Apis mellifera* lineage that *Varroa destructor* parasitizes; in this sense George *et al*., (2004) have shown that the parasite biomass is controlled by the host metabolic rate, in the same way the cell size of the combs affects the body size of the host and consequently mite size (Borsuk *et al*., 2012).

Varroa's plasticity studies have found morphometric variations in large regions and countries such as Iran, Argentina, and Ukraine; finding 17 morphotypes per study (Akimov *et al*., 2004; Maggi *et al*., 2009; Dadgostar *et al*., 2018); however, in a group of beehives of local producers, 8 morphotypes were found, which means a great morphometric variability in a population of closely related bees, which is why we consider that this variation depends on the selection regimes within the habitats, the migration, the different epochs of bee reproduction and the possible mutations that the mite could present in time (Van Tienderen, 1991, Akimov *et al*., 2004, Carroll et al., 2007).

The phenotypic plasticity was clearly observed after 28 days of having applied a natural acaricide, reason why we could observe a significant reduction in the AED and LEG; so we agree with Maggi *et al*. (2009) who has reported the morphometric plasticity of *Varroa destructor* in different regions of South America. This morphometric plasticity has been documented in other species where they have found an association between body size and susceptibility to drugs (Bridges *et al*., 2001, Oliveira *et al*., 2007, Yarahmadi *et al*., 2009). In this sense, the ability of parasites to adjust their phenotype to a pesticide is considered as a strategy of adaptation to the intense selection pressure imposed by the miticide, causing plastic responses in body or ontogenetic allometry (Wu *et al*., 2003).

CONCLUSION

100% of the mites evaluated belong to the *V. destructor* species. 8 clearly differentiated morphotypes were found, which allowed us to understand the intraspecific morphometric variability of *V. destructor* in populations of *A. mellifera* geographically related. A correlated positive plasticity was observed between the acaricide and the LEG decrease; then the post-treatment plasticity is the result of adaptation, due to the selection pressure imposed by the acaricide; having indications that the mite adapts by means of its morphological variability to the adverse conditions for its survival and to the colonies of bees that parasitize.

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