

# *Acarapis woodi* (Rennie), honey bee endoparasite: from the pest of the century to a forgotten case in Mexico

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## ABSTRACT

**Objective:** To determine the incidence of *Acarapis woodi* (Rennie) infestation in the tracheae of honey bees (*Apis mellifera* L.) from colonies not previously treated against the *Varroa destructor* Anderson and Trueman mite and to discuss the current status of *A. woodi* in Mexico, based on our observations and historical information.

**Design/Methodology/Approach:** Samples of the thoracic tracheae of honey bees from colonies never treated against the *V. destructor* mite were taken in search of *A. woodi*. A 20-worker sample was collected from each colony, preserved in 70% ethanol, and examined no later than two days after the collection. Foraging workers were selected from the lateral combs, the entrance of the hive, and the inner lid, the places where the oldest bees can be found and which have a higher infestation probability trend. The specimens were diagnosed using the dissection technique and observed under a microscope.

**Results:** None of the examined honey bees' tracheae —and therefore none of the colonies— were infested.

**Study limitations/Implications:** Although only one apiary was studied, a general trend can be observed in Mexico and many other countries in the Americas, especially in tropical and subtropical sites: after a shock wave of *A. woodi* infestations, they were less frequent, until their detection became very difficult.

**Findings/Conclusions:** The decreasing prevalence of *A. woodi* has been attributed to climatic preferences and treatments against *V. destructor*. However, this reduction could be the result of resistance among honey bees.

**Keywords:** *Apis mellifera*, tracheal mite infestation, population, diagnosis.

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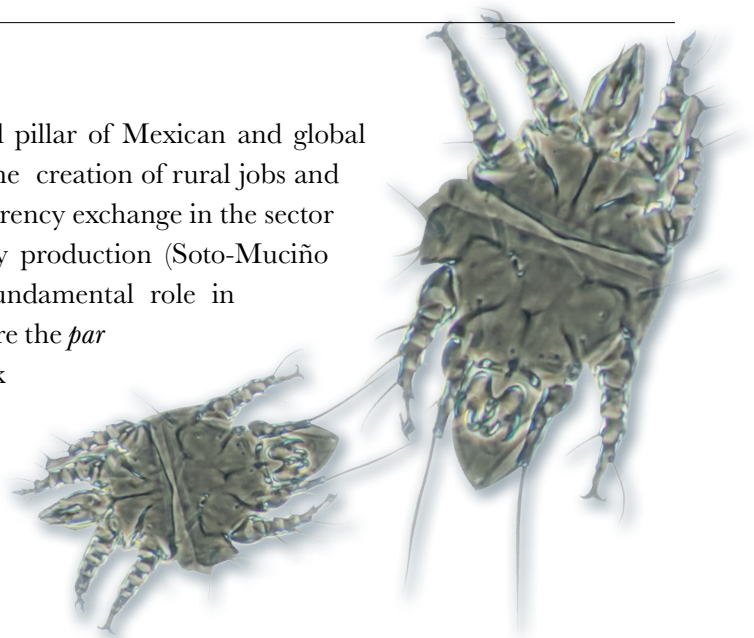
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## INTRODUCTION

Beekeeping is a fundamental pillar of Mexican and global agriculture, both as a result of the creation of rural jobs and as the third source of foreign currency exchange in the sector due to the high levels of honey production (Soto-Muciño *et al.*, 2017). Insects play a fundamental role in ecosystems as pollinators. Bees are the *par excellence* participants in this task and consequently they have enormous economic and ecological importance. Most of the food that

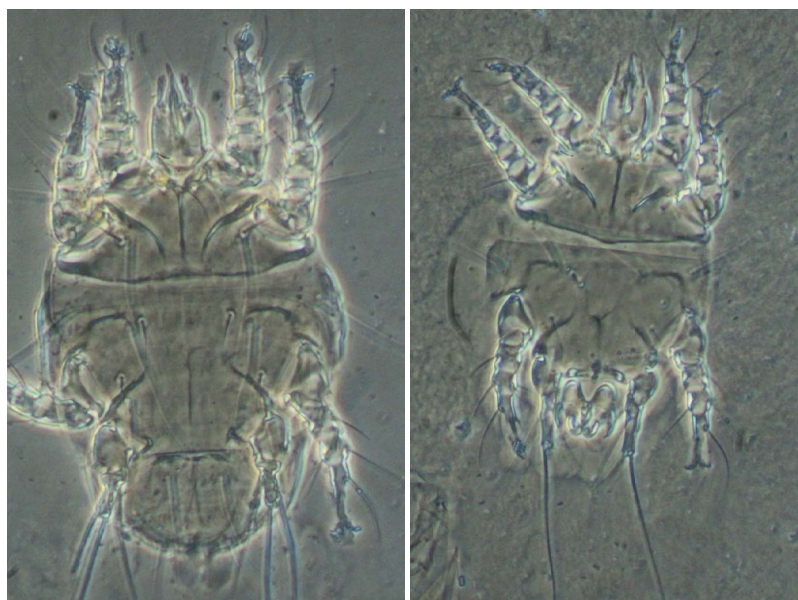


humans consume and trade directly or indirectly depends on pollination by bees (Pantoja *et al.*, 2014).

Despite their benefits, a significant reduction in the abundance of pollinators has been reported worldwide, as a result of environmental deterioration, intensive agriculture, the use of pesticides, the introduction of non-native species, diseases, and climate change (González-Varo *et al.*, 2013). Cereals that depend on anemophily and are part of the human diet (such as corn and wheat) may not be affected to such a degree by the decrease in bees. Nevertheless, forage crops used for meat and dairy production will undoubtedly be severely affected by pollinator decline (Spivak *et al.*, 2010).

The *Acarapis woodi* (Rennie) mite (Figure 1) is an obligate parasite of *Apis mellifera* L. (Pettis and Wilson, 1996) and *A. cerana* Fabricius (Maeda and Sakamoto, 2016) bees. It lives inside the tracheae of bees, especially the tracheal trunks (Figure 2), but can also be found in other parts of the respiratory system, including the air sacs (Wilson *et al.*, 1997). Its entire life cycle takes place within this microhabitat, but adult females can leave the bees in search of another specimen to parasitize, usually young adult bees less than four days old (Hoy, 2011). These mites feed by perforating the tracheae of bees and sucking the hemolymph that bathes them; as a result of their feeding, the tracheae turn dark and may even collapse (Figure 3) (Scott-Dupree and Otis, 1992).

In 1916 *A. woodi* was discovered in Scotland, UK (Bailey, 1999). A few years earlier, a pronounced mortality of bee colonies was recorded in the Isle of Wight, southern England, an event known as “the Isle of Wight disease” (Bailey, 1964). When *A. woodi* was found to be an endoparasite of bees, it was associated with and blamed for the disease. The mite was even said to have been discovered on the Isle of Wight, not in Scotland. The nature and intensity of the damage caused by *A. woodi* in bees have been the subject of much debate: some maintain that bee mortality in the Isle of Wight was caused by this mite (Adam,



**Figure 1.** Photomicrographs of *Acarapis woodi*. Adult female, left. Adult male, right.



**Figure 2.** Tracheal trunk of a bee (*Apis mellifera*) filled with numerous specimens of *Acarapis woodi*.



**Figure 3.** Melanization of the trachea of bees (*Apis mellifera*) caused by the infestation by *Acarapis woodi*.

1968), while others (Bailey, 1958, 1961, 1964, 1999) argue that the most evident damage is the reduction in the longevity of adult bees and a greater mortality of colonies at the end of winter, when a high percentage of workers have been parasitized.

Despite Bailey's insistent arguments (1958, 1961, 1964, 1999), *A. woodi* was considered a major threat to beekeeping throughout the Americas. Consequently, in 1922 the United States of America restricted the import of bees from anywhere in Europe (Menapace and Wilson, 1980). In the years following its discovery, *A. woodi* spread to several countries in Europe, Africa, and South Asia (Morse, 1978), undoubtedly as a result of the transport of parasitized bees. However, the entire American continent was assumed to be free of its presence. Nevertheless, *A. woodi* was detected for the first time in the American continent in 1971, specifically in Brazil (Nascimento *et al.*, 1971). It was just a matter of time before it spread to neighboring countries.

The finding of *A. woodi* in the Americas caused alarm, given the obvious risk that it could spread both to the north and the south, and the consequent and significant damage that it would cause to bees in various countries. Researches were carried out to determine its northwards advance. As a result, it was discovered in 1980 in Colombia (Menapace and Wilson, 1980), in 1982 in Mexico (Wilson and Nunamaker, 1982), and in 1984 in the United States (Delfinado-Baker, 1984).

Based on the somewhat alarmist data of Adam (1968), Menapace and Wilson (1980), and others, the idea was posited that *A. woodi* would cause severe damage to bees in the American continent. Consequently, activities were conducted to assess its levels of infestation and damage, to determine if Africanized bees—which had recently reached Mexico—were carriers of the said mite, and to contain its spread.

Products were developed to control it (García Figueroa and Arechavaleta-Velasco, 2018). Among other relevant results, Romero-Vera and Otero-Colina (1992) determined that, although Africanized bees can indeed be parasitized by *A. woodi* (with infestation levels similar to those of European bees), the first migratory Africanized bees that reached North America were not carriers of said mite. Instead, they acquired it from previously parasitized European bees in Mexico.

After initially fearing the presence of *A. woodi* in Mexico, beekeepers started to notice that the problem was not as serious as they had been led to believe (testimonial information collected by the authors). Since the diagnosis of the presence of *A. woodi* involved a very laborious method, it was rarely carried out by beekeepers; nevertheless, bee mortality comparable to that of the Isle of Wight disease and potentially associated with *A. woodi* parasitism was never observed. Those who did conduct a diagnosis noticed that, as the years went by, the infestation levels decreased and parasitized bees became rare (García Figueroa and Arechavaleta-Velasco, 2018).

Several hypotheses were put forward to explain the reduction in the intensity of bee parasitism by *A. woodi*: that the climate in tropical and subtropical areas, such as Mexico, is not favorable for the development and increase of their populations (Bailey, 1958); that applying acaricides to control the *V. destructor* mite, detected in Mexico in 1992 (Rodríguez-Dehaibes *et al.*, 1992), had a collateral effect against *A. woodi* (Martínez-Puc *et al.*, 2011); and that bees developed resistance to infestation by *A. woodi* (Pettis and Pankiw, 1998).

The aim of this work is to describe the current state of bee parasitism by *A. woodi* in Mexico and propose a hypothesis to explain the reduction in infestation levels. Information has been taken from numerous studies conducted throughout the years in which this mite has been present in the country and it was complemented with observations made in an apiary of Euro-African bee colonies in which no treatments against *V. destructor* have ever been applied.

## MATERIALS AND METHODS

### Study group

The research was carried out in February 2019 in the experimental apiary of the Posgrado en Recursos Genéticos y Productividad Genética, Colegio de Postgraduados - Campus Montecillo, Texcoco, Estado de México (19° 17' N and 98° 54' W; 2,240 m.a.s.l.),



where  $n=20$  bees were selected from each of 54 previously selected hives. The samples were dissected in the laboratory within two days after the collection.

### Sample collection

The samples were collected in the morning (9:00 a.m.), when the foraging activity begins, to avoid stressing the bees. Adult bees were selected from the inside lid of the hive, the edge combs, and the entryway. When hives had stacked supers, bees were collected from the honey combs, the place where the older foraging workers can be found. The worker bees were put in wide-mouthed plastic jars with 30 mL of 70% ethyl alcohol, which caused their immediate death and enabled their short-term conservation. The samples were then taken to the Entomology and Acarology laboratory for diagnosis.

### Dissection technique

The 20-bee sample was placed on a paper towel to remove the excess alcohol, whose aqueous consistency could hinder cutting during dissection. Another towel was placed on top of the sample for better absorption, applying light pressure by hand. The sample was left to rest for about 2 min. Meanwhile, five separate drops of lactic acid were put on a slide. Each bee was placed between the thumb and forefinger of the researchers, with its ventral area facing up and the head was removed with entomological tweezers (the first pair of legs usually come out with the head). Fine-tipped scissors were used to make a 2 to 3-mm thick cut parallel to the one that resulted from the removal of the head. The tracheae were left exposed along with the spiracles—which are the entry route for mites. Finally, a thorax slice was taken with the same entomological tweezers and placed on one of the five drops of lactic acid on the slide. This technique was repeated four times, with five bees per slide.

### Microscopic observation

Once the thoracic muscles of the bees softened as an effect of lactic acid, a stereoscopic microscope was used to separate the thoracic tracheae at the closest point to the spiracle and two dissecting forceps were used to expose any mites that might be inside. Subsequently, the tracheae were mounted between slides with lactic acid as a preparation medium. The samples were observed using a phase contrast microscope with 100x and 400x magnifications. Previous preparations from the mite collection of the Laboratorio de Acarología of the Colegio de Postgraduados show that, even without stained material, this microscope provides a clear view of the mites inside the tracheae, as well as of the dark spots that result from the infestation by *A. woodi*.

## RESULTS AND DISCUSSION

No bees from the examined colonies were infested by *A. woodi* (Table 1). This result agrees with the findings of Martínez-Puc *et al.* (2011) and Martínez-Cesáreo *et al.* (2016), who did not detect infestation by *A. woodi* mite in bee colonies from the states of Yucatán and Estado de México, respectively. For their part, García-Figueroa and Arechavaleta-Velasco (2018) estimated a 0.02% prevalence of infested bees in the state of Morelos. The

**Table 1.** Results of the dissection test carried out to observe mites in the tracheae of sampled bees.

Number	ID-Hive	Result	Number	ID-hive	Result
1	075	(-)	28	207	(-)
2	074	(-)	29	034	(-)
3	053	(-)	30	083	(-)
4	021	(-)	31	049	(-)
5	064	(-)	32	07	(-)
6	001	(-)	33	087	(-)
7	003	(-)	34	073	(-)
8	038	(-)	35	079	(-)
9	095	(-)	36	080	(-)
10	026	(-)	37	023	(-)
11	086	(-)	38	104	(-)
12	066	(-)	39	122	(-)
13	030	(-)	40	062	(-)
14	184B	(-)	41	155	(-)
15	084	(-)	42	069	(-)
16	04	(-)	43	209	(-)
17	018	(-)	44	052	(-)
18	150	(-)	45	024	(-)
19	070	(-)	46	092	(-)
20	013	(-)	47	119	(-)
21	060	(-)	48	048	(-)
22	09	(-)	49	08	(-)
23	06	(-)	50	036	(-)
24	081	(-)	51	187	(-)
25	056	(-)	52	122	(-)
26	112	(-)	53	020	(-)
27	015	(-)	54	019	(-)

ID-hive=hive identification; Number=sequence number; (-)=negative for mites.

absence of infestation by *A. woodi* or infestation levels so low that they are undetectable with the methods used seem to be a widespread phenomenon in sites with a tropical or subtropical climate in the Americas. For example, Calderón *et al.* (2007) detected this mite in 1.1% of the bees they examined in Costa Rica, while Peixoto *et al.* (2019) and Szawarski *et al.* (2017) did not detect it in Brazil and Argentina, respectively.

Numerical data for the infestation of bees by *A. woodi* reported by various authors almost one century after its discovery (Bailey, 1999) are difficult to compare, as a result of the wide differences in the techniques used to quantify parasitism. The method usually

used to observe *A. woodi* has been to dissect the thorax of adult worker bees and view them under stereoscopic or biological microscopes. However, even that method could have variations. For example, Martínez Puc *et al.* (2011) only detached the head of the bees along with the first pair of legs, exposing the thoracic tracheae and, without further cuts, observed them frontwise with a stereoscopic microscope. Using a more refined method, Calderón *et al.* (2007) made a second cut after they detached the head and the first pair of legs to obtain an approximately 2-mm thick thorax segment. They placed that segment in 10% potassium hydroxide to macerate the muscles, separated the thoracic tracheae to make a more detailed observation, and even counted the *A. woodi* specimens.

Since dissecting the thoracic discs for observation involves a very laborious method, the number of bees examined per hive is usually low. It is hard to detect mild infestations with samples of 10 (Wilson and Nunamaker, 1982) or 30 specimens (Calderón *et al.*, 2007).

Other methods based on DNA analysis have been recently developed to detect *A. woodi* in bees (Garrido-Bailón *et al.*, 2012; Pietropaoli *et al.*, 2022). These methods for the observation of the interior of the tracheae of the bees are more sensitive and less laborious than those involving the dissection of the specimens. However, they can only attest to the presence or absence of *A. woodi* in the samples.

The values obtained from the above-mentioned methods may also vary. Scientists usually estimate only the percentage of infested bees (Calderón *et al.*, 2007). Others estimate infestation levels according to the number of specimens found in the tracheae (Otis, 1986; Romero-Vera and Otero-Colina, 1992). Still others look for mites in one or both tracheae (Bailey, 1958). However, specifically in the American continent (and more precisely in Mexico), all values obtained from the diagnostic methods indicate a net reduction in *A. woodi* infestation compared to the first years after the detection of this mite in the country. Back then, Eischen *et al.* (1990) estimated that 60% of the colonies were infested, while Romero-Vera and Otero-Colina estimated an almost 100% infestation and comparable levels of severity (number of *A. woodi* specimens in individual bees) among Africanized and European bees. These results contrast both with the most recent data (Martínez Puc *et al.*, 2011; Martínez-Cesáreo *et al.*, 2016) and with this research.

Although *A. woodi* infestation has ostensibly decreased up to the point where it has almost disappeared, several hypotheses haven't been posited to explain this phenomenon. One of them is that the climate in Mexico is not favorable for the development of high mite populations (Bailey, 1958). However, this is a questionable proposal, since Mexico has a wide variety of climates and more than one scenario must be taken into consideration. The works of Eischen *et al.* (1990) and Romero-Vera and Otero-Colina (1992) stand out among the first instances in which *A. woodi* was detected in Mexico. The former observed 60% of infested colonies (40% of them with  $\geq 30\%$  infested workers) in the states of Nuevo León and Tamaulipas, while the latter detected approximately 10% of bees severely infested with *A. woodi* (more than 15 mites in their tracheae) in 515 of 516 hives in the states of Chiapas, Quintana Roo, and Tabasco.

The second hypothesis postulates that the pesticides used to control *V. destructor*—which was detected in Mexico in 1992 (Rodríguez-Dehaibes *et al.*, 1992)—can also control *A. woodi* (Martínez Puc *et al.*, 2011; García-Figueroa and Arechavaleta-Velasco, 2018). No

tests have been conducted to determine the effect of these pesticides on *A. woodi*, most likely because this mite became increasingly difficult to detect and no obvious damage was observed that could be associated with its parasitism. However, this hypothesis ignores that the control of *V. destructor* was never widespread. Many beekeepers never treated their hives against this mite and wild colonies—whose numbers are unknown, but which undoubtedly exist—were not treated against *V. destructor* and therefore would have served as *A. woodi* reservoirs and sources of contagion. The results of this work confirm past reports: the sampled colonies have never been treated against *V. destructor*.

Therefore, the most feasible hypothesis is that bees developed resistance to *A. woodi* over the years. This hypothesis is particularly suitable in the case of Mexico, but does not rule out that other hypotheses can be valid to a lesser extent, since they are not mutually exclusive. During the initial stages of the establishment of *A. woodi* in Mexico, the percentage of infested bees and infestation levels were high (Eischen *et al.*, 1990; Romero-Vera and Otero-Colina, 1992), but these figures have decreased over the years. These results would confirm that bees with some degree of resistance gradually replaced vulnerable bees.

Determining the resistance mechanism against the potential presence of *A. woodi* in Mexico would be difficult, mainly given the almost complete lack of biological material infested by *A. woodi* that could be compared with healthy samples. Bees groom each other (Pettis and Pankiw, 1998) and perhaps this practice could be a valid defense mechanism in the case of Mexico. A physical barrier—such as setae or the size of the spiracles—might prevent mites from entering the tracheae of bees. As stated in the title of this paper, the much-feared entry of *A. woodi* into Mexico became a practically forgotten topic. However, this threat should not be overlooked, because once an organism develops resistance to a parasite, the parasite also evolves to evade such resistance—a well-known fact in the field of parasitology, particularly in the study of bee parasites (Cobey, 1997).

## CONCLUSIONS

The infestation of bees by the *A. woodi* tracheal mite shows a decreasing trend, both in the material studied and in Mexico and other American countries. The cause of this phenomenon seems to be the resistance developed by bees.

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