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Presence of deformed wing virus and Kashmir bee virus in Africanized honey bee colonies in Costa Rica infested with *Varroa destructor*

RAFAEL A CALDERÓN, JOHAN VAN VEEN, HENRY GARCE AND MARIO E ESQUIVEL

Newly emerged bees with damaged wings, bees crawling in front of the hive and unusually severe adult bee mortality were observed in Africanized honey bee colonies in Costa Rica. A large proportion of the adult bee population in most of these colonies was infested with *Varroa destructor*. Adult bee samples were sent to the Department of Entomology, Penn State University, for virus analysis. This analysis showed the presence of deformed wing virus and Kashmir bee virus. The occurrence of adult bee mortality and the detection of these viruses in Africanized colonies in Costa Rica infested with *V. destructor*, should be considered as a cause of concern.

Introduction

*Varroa destructor*² (fig. 1) is a dangerous ectoparasite of worldwide economic importance for honey bees (*Apis mellifera*) and a serious threat to beekeeping. Most honey bee colonies do not survive infestation by this mite if left untreated.³

Varroa was first detected in Costa Rica on 26 September 1997¹⁵ in brood and adult bee samples collected in the Los Santos area (Central Valley) of Costa Rica, and it has quickly spread over the country.⁶ Adult female mites collected in Costa Rica and sent to the Bee Research Center in Baton Rouge, LA, USA, for mtDNA sequence analysis in 1999, were confirmed as the

Russian type, nowadays referred to as the Korean haplotype of *V. destructor*.²

A year after *V. destructor* was discovered, the widely used acaricide Apistan (tau-fluvalinate) was officially approved for the treatment of infested colonies in Costa Rica. More recently, some experimental trials with formic acid treatment have been undertaken in order that this treatment can be incorporated into mite management strategies.⁷

It is reported that colonies infested with *V. destructor* appear restless, the brood is neglected and parasitized bees have a lower birth weight, and may have malformations.⁸ Shimanuki *et al.*¹⁴ have suggested using the terminology 'parasitic mite syndrome' for



FIG. 1. Adult female *Varroa destructor*.

colonies that are infested with *V. destructor*, and sometimes with the tracheal mite, *Acarapis woodi*. Amongst the range of symptoms described are a reduced adult bee population, crawling bees in front of the hive and queen supersedure.⁹ According to Ball,⁵ much of the damage caused by varroa infestations is due to secondary viral infections. Bees from dying infested colonies have been found to be infected with a number of different honey bee viruses. It is known that acute paralysis and deformed wing viruses are transmitted by the mite.⁴ Furthermore, *V. destructor* can act as a vector for sacbrood and black queen cell viruses.³

There are no reports concerning the presence of deformed wing virus and Kashmir bee virus in Africanized honey bee (AHB) colonies in Costa Rica.^{1,10,11} Because the eventual collapse of colonies infested with *V. destructor* may be due to secondary infections by viruses, the occurrence of adult bee mortality and the detection of deformed

wing virus and Kashmir bee virus in infested AHB colonies in Costa Rica, is officially reported.

Detection of damaged bees

A commercial apiary of 35 AHB colonies, located in the Los Santos area (the south of the Central Valley) of Costa Rica, was visited during September 2002 (rainy season). All colonies were housed in Langstroth hives, fed alternatively with dry sugar and sugar syrup (1 : 1) during the dearth season and requeened with a young marked queen. Newly emerged bees with deformed and shortened wings (poorly developed wings), or even without any wings, crawling in front of their hive unusually accompanied by severe adult bee mortality was observed in more than 12 colonies. No brood mortality was evident in the inspected colonies.

Mite infestation

Levels of mite infestation in adult bees were determined from eight colonies. Adult worker bees (about 100–200 bees) taken from the brood combs of each colony were

brushed into glass jars using a cardboard funnel. Soap powder and water (5% soap) was added to the bees and the mixture was shaken for one minute. The solution and bees were poured into a double sieve and then washed with a strong spray of water

TABLE 1. Virus analysis for deformed wing virus and Kashmir bee virus by ELISA in adult bees of AHB colonies in Costa Rica infested with *Varroa destructor*.

Sample collection	Sample ID	Kashmir bee virus	Deformed wing virus
Outside the colony	F1	0.345*	0.208*
	F2	0.236	0.115
	F3	0.353*	0.171
	F4	0.496*	0.332*
	F5	0.252	0.157
	F6	0.214	0.101
	F7	0.287*	0.159
	F8	0.245	0.146
	F9	0.228	0.110
	F10	0.372*	0.178
	F11	0.246	0.078
	F12	0.319*	0.183
	Negative	0.133	0.096
	Positive (*)	0.554	0.558
Inside the colony	D1	0.411*	0.201*
	D2	0.367*	0.235*
	D3	0.334*	0.261*
	D4	0.370*	0.215*
	D5	0.524*	0.283*
	D6	0.385*	0.223*
	D7	0.420*	0.288*
	D8	0.462*	0.348*
	D9	0.381*	0.267*
	D10	0.314*	0.192
	D11	0.414*	0.280*
	D12	0.214	0.098
	Negative	0.129	0.101
	Positive (*)	0.536	0.592

(running water) to wash all mites through the coarse sieve. Bees remained in the coarse sieve and mites were collected in the fine sieve.

The coarse sieve was a 2-mm wire screen and the fine was 0.05-mm wire mesh to guarantee proper separation of bees and mites. The number of mites and bees were counted to determine the levels of infestation on adult bees (number of mites/number of bees \times 100). The number of mites found in these samples of adult bees ranged from eight to 15 (averaging up to 10% of mite infestation).

Virus identification

Emerging bees with deformed wings, crawling bees in front of the hive, dead bees and adult bees taken from the brood combs were sent to the Department of Entomology, Penn State University, for virus detection. In total, 12 adult bee samples taken from the brood frames (inside the colony) and 12 taken from crawling bees and dead bees (outside the colony) were analysed (table 1). The virus analysis for deformed wing virus and Kashmir bee virus by ELISA (direct-ELISA using horseradish peroxidase-conjugated protein-A as secondary antibody; 33'55' tetramethylbenzidine as substrate (terminated with H_2SO_4), read at 450 nm) indicated the presence of both. Most of the adult bee samples taken from the brood nest were infected with deformed wing virus and Kashmir bee virus. Meanwhile, most of the bee samples taken from outside of the colony were negative for both viruses.

Prospects

Since *V. destructor* populations increase when brood is present, it would be expected that in tropical climates, where brood rearing takes place year-round, the effect of the mite

would be even more devastating, because reproduction never ceases.

In Costa Rica, some beekeepers have reported colony losses, reduced honey production and pollination efficiency, as a consequence of *V. destructor* introduction. Furthermore, natural *V. destructor* mortality up to 191 mites per day collected from the floor of the colony, and infestation rates of up to 22.0% found in adult bees in AHB colonies during a 170-day research study (from February to July 2001) (unpublished data), indicates that *V. destructor* is a significant problem in Costa Rica. These large numbers of mites are higher than those reported for AHB in Brazil. Mite infestation rates in adult bees on AHB colonies in Brazil are mainly below 5%; higher infestation levels were detected only during the first years of occurrence of *V. destructor* in this country.¹³ This decrease in infestation rates shows that an adaptive process has occurred in this host-parasite relationship.¹²

As the eventual collapse of colonies infested with *V. destructor* may be due to secondary infections by viruses, the occurrence of adult bee mortality, and the detection of deformed wing virus and Kashmir bee virus in AHB colonies in Costa Rica infested with *V. destructor*, should be considered as a cause of concern to us all.

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