



Reproduction of *Varroa destructor* in worker brood of Africanized honey bees (*Apis mellifera*)

LUIS MEDINA MEDINA^{1,2,*}, STEPHEN J. MARTIN², LAURA ESPINOSA-MONTAÑO³ and FRANCIS L.W. RATNIEKS²

¹Departamento de Apicultura, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Yucatan, Apartado Postal 4-116, C.P. 97100 Merida, Yucatan, Mexico; ²Laboratory of Apiculture and Social Insects, Department of Animal and Plant Sciences, University of Sheffield, Western Bank, Sheffield, S10 2TN, UK; ³Secretaría de Producción Animal, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Circuito Exterior Cd. Universitaria, C.P. 04510, D.F., México; *Author for correspondence (e-mail: mmedina@tunku.uady.mx; phone: +52 (99) 423200; fax: +52 (99) 423205)

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Abstract. Reproduction and population growth of *Varroa destructor* was studied in ten naturally infested, Africanized honey bee (AHB) (*Apis mellifera*) colonies in Yucatan, Mexico. Between February 1997 and January 1998 monthly records of the amount of pollen, honey, sealed worker and drone brood were recorded. In addition, mite infestation levels of adult bees and worker brood and the fecundity of the mites reproducing in worker cells were determined. The mean number of sealed worker brood cells ($10,070 \pm 1,790$) remained fairly constant over the experimental period in each colony. However, the presence and amount of sealed drone brood was very variable. One colony had drone brood for 10 months and another for only 1 month. Both the mean infestation level of worker brood ($18.1 \pm 8.4\%$) and adult bees ($3.5 \pm 1.3\%$) remained fairly constant over the study period and did not increase rapidly as is normally observed in European honey bees. In fact, the estimated mean number of mites fell from 3,500 in February 1997 to 2,380 in January 1998. In May 2000 the mean mite population in the study colonies was still only 1,821 mites. The fertility level of mites in this study was much higher (83–96%) than in AHB in Brazil (25–57%), and similar to that found in EHB (76–94%). Mite fertility remained high throughout the entire study and was not influenced by the amount of pollen, honey or worker brood in the colonies.

Introduction

The newly reclassified ectoparasitic mite *Varroa destructor* (Anderson and Trueman 2000) has killed millions of *Apis mellifera* L. honey bee colonies worldwide. In European *A. mellifera* (EHB) honey bees a high proportion (76–94%) of female *V. destructor* mites are able to reproduce (reviewed by Martin et al. (1997)). This results in a yearly 12 fold increase in mite population during a 6–9 month temperate-climate brood rearing season (Martin 1998; Calis et al. 1999) and a staggering 2,248 fold increase in sub-tropical/tropical regions (Kraus and Page 1995) where brood is continuously present. EHB colony death occurs within 1–4 years depend-

ing on the brood rearing period and prevalence of secondary viral infections which are ultimately responsible for the death of the colony (Martin 2001a). However, the Africanized bee (AHB), which was derived from *A. m. scutellata* honey bees introduced into Brazil from South Africa in 1956 that hybridized with European *A. mellifera* races already present in the Americas (Moritz 1994). AHB now occurs throughout South and Central America and is tolerant to *V. destructor*. That is, mite infested colonies can survive indefinitely without assistance from beekeepers. In Brazil, where AHB has been established for over 40 years (De Jong 1996), only 25–57% of *V. destructor* mites reproduce (Camazine 1986; Message and Goncalves 1995; Ritter and De Jong 1984; Rosenkranz and Engels 1994) and this was thought to be the reason why AHB colonies survive. However, recent findings have revealed a more complex picture as *V. destructor* isolated from *A. mellifera* comprises two haplotypes. The more virulent and widespread Korea haplotype, and the less virulent Japan/Thailand haplotype which is found predominantly in South and North America (Anderson and Trueman 2000). As most mite studies on AHB were carried out in Brazil prior to 1993 when the Japan/Thailand haplotype was predominant (Anderson and Trueman 2000) this may explain the low level of mite fertility observed.

AHB were first reported in Yucatan, Mexico, in 1987 (Quezada-Euan et al. 1996). *V. destructor* was first found in 1994 (Medina 1998). The AHB in Mexico were also found to be tolerant to *V. destructor* (Guzman-Novoa et al. (1996, 1999); Medina and Martin 1999; Vandame et al. 1999) surviving for over five years without any apparent effect. This was surprising since the mite haplotype in Mexico was confirmed as Korea (D. Anderson, personal communication) and the high (90%) fertility rates of mites in AHB in Mexico (Medina and Martin 1999; Vandame 1996) were similar to those found in EHB in Europe. In addition, mite infested EHB colonies kept in Mexico died within 1–2 years (Vandame (1996); Medina, personal observation). Therefore, AHB tolerance to *V. destructor* appears to be independent of mite haplotype. However, there may be additional factors operating in Mexico and Brazil.

The aim of this study was to investigate why *V. destructor* has low population growth and maintains a fairly constant population size in AHB colonies in Mexico. We investigated the fertility and fecundity of *V. destructor* in AHB worker brood cells over a one year period and the effect that the amount of brood, pollen and honey stores. The role of pollen is included since, in a previous study (Moretto et al. 1997), it was suggested that pollen can affect both the fertility and fecundity of *Varroa*.

Methods and materials

The study was conducted at the Faculty of Veterinary Medicine, Universidad Autonoma de Yucatan, Merida, Mexico (89.5°W, 21.0°N). This region has a sub-humid tropical climate (Awo type; Garcia (1973)). The average temperature is 26.5

°C and rainfall is 957 mm (Duch 1988). Honey bee worker brood is reared year-round with a slight annual peak between December and June (Echazarreta and Paxton 1997).

Honey bee colonies

Fifteen full-size AHB colonies (24,000 bees) were established from captured swarms and identified morphometrically by the technique of Daly and Balling (1978) using the computer software AFUSDA7 (Rubink, unpublished). All experimental colonies became naturally infested in 1995 and no mite control measures were taken until May 2000, when the miticide Bayvarol® was used to determine the precise mite population in each colony. During the study period five of the colonies swarmed and were excluded from the study.

From February 1997 to January 1998 monthly estimates of the comb area occupied by pollen, honey, worker and drone sealed brood in each colony were made (Page 1980). The adult bee population was estimated for each colony using the method used by Calis et al. (1999) which uses the amount of worker brood registered in the previous month, the pupal development time and adult bee longevity. The longevity of AHB was assumed to have low variation, given the relatively stable tropical climate, with constant production of brood (Winston et al. 1981).

From each colony samples of worker brood (8 × 8 cm section) containing approximately 530 worker cells which had been sealed for at least 230 h (yellow thorax stage of development) and 200–350 adult bees from brood combs were collected and stored at –5 °C (brood) or in ethanol (adults).

Brood samples

In the laboratory, 100–150 worker cells were opened and the developmental stage of the bee pupae and any mite offspring were determined using the ontogenic development charts in Martin (1994). In addition, any female deutonymphs were classified in five groups (A-E) depending on their size using the photograph in Ifantidis (1983). This permits the reconstruction of each mite family in its birth order and allows the mortality rates of the mite offspring to be determined along with estimates of the number of new fertilized and unfertilized females produced by each mother mite. Each invading mother mite was classified into one of the following five categories: 1) fertilized female offspring i.e. both live mature male and female offspring produced; 2) no fertilized female offspring due to premature death of the male, i.e. male dead; 3) no fertilized female offspring due to other causes, i.e. female offspring dead; 4) only male offspring produced; 5) mother mite did not reproduce. Only cells invaded by a single mother mite were analyzed since multiple infestations can affect mite reproduction (Fuchs and Langenbach 1989).

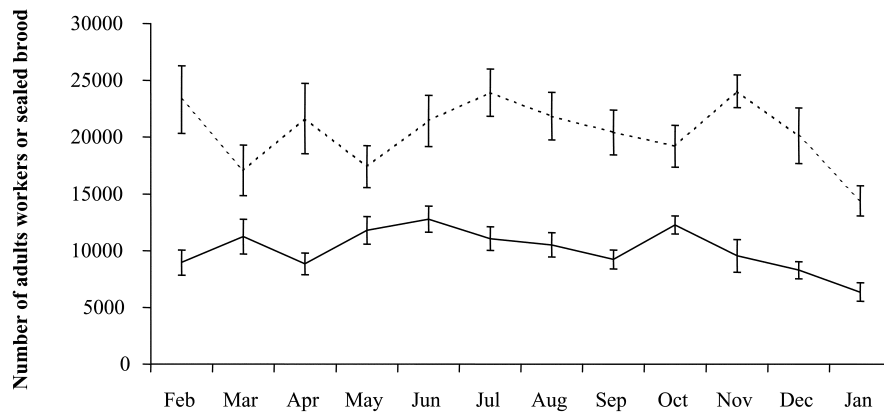


Figure 1. Numbers of sealed worker brood cells (solid line) observed and calculated number of adult bees (dotted line) from brood estimates. (n = 10 colonies).

Adult bee samples

Adult bee samples were agitated in 85% ethanol for 30 min in a circular mechanical shaker (180-rpm) and strained to separate and count mites and bees. Individual inspection of a sample of bees confirmed that this method removes 100% of the mites as was shown by De Jong et al. (1982).

Statistical analysis

To determine whether the amount of worker brood, pollen or honey affect *Varroa* reproduction, a regression analysis was performed using these variables as predictors for the five mite reproductive categories (Zar 1996). All mean values are accompanied by ± 1 standard deviation.

Results

Honey bee colonies

The amount of sealed worker brood remained fairly constant over the year (Figure 1) with an average of $10,070 \pm 1,790$ cells (n = 10) per month. This gives rise to an estimated average adult bee population of $20,560 \pm 3,330$ (Figure 1). The lowest number of sealed worker brood (6,360 cells) was recorded in January, when the honey flow is starting. The highest number (12,770 cells) was recorded in June after the two main honey flows had finished (Echazarreta et al. 1997).

Although worker brood pattern (Figure 1) was fairly constant throughout the year in each of the study colonies, the amount of sealed drone brood was highly variable (Figure 2). At one extreme, one colony (C3) had drone brood for 10

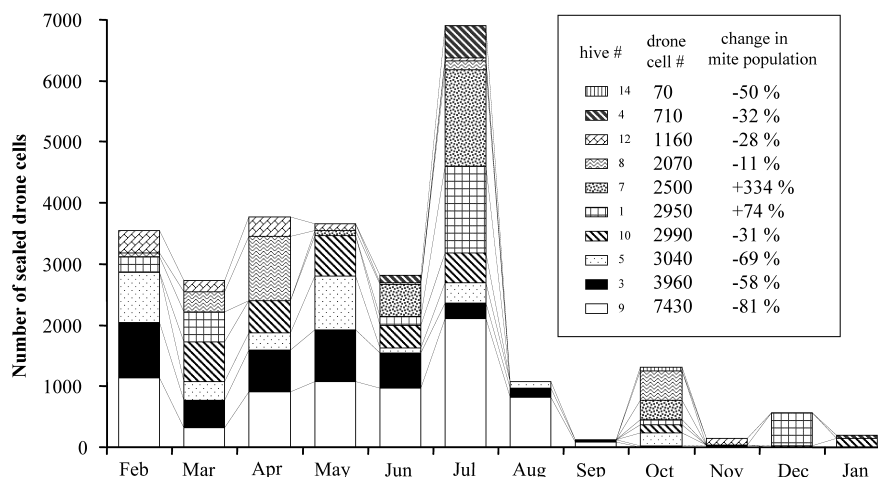


Figure 2. Monthly numbers of sealed drone brood in each of the ten study colonies. The total number of sealed drone brood recorded in each colony and the change in the mite population over the study period are also given.

months, with a monthly average of 340 ± 340 sealed cells, while another colony (C14) only had drone brood, 70 cells, during one month (Figure 2). However, there was no association between the growth or decline of the mite population and the amount of drone brood present in a colony (Figure 2).

Worker brood and adult bee infestation

The monthly mean infestation levels of the sealed worker brood cells and adult bees were $18.1 \pm 8.4\%$ and $3.5 \pm 1.3\%$ respectively. The proportion of the mite population in brood cells or on adult bees (71: $29 \pm 8\%$) varied little throughout the year (Figure 3). The presence or absence of drone brood had a small influence on the proportion of mites on adult bees, given that when drone brood was plentiful (Feb. – Jul.) 25% (± 4) of the mites were on adult bees which only increased to 34% (± 8) when drone brood was scarce (Aug. – Jan.) (Figure 2).

Varroa reproduction

In total 1,073 worker cells invaded by a single mother mite were analyzed. Figure 4 shows the frequencies of the five reproductive categories during the study and shows that mite fertility rate was high (82.7–96.0%) throughout the year.

No significant correlation ($P > 0.06$ in all cases) was observed between the amount of worker brood, pollen, honey and any of the five categories of *Varroa* reproduction, indicating that these factors do not affect the fertility of *Varroa*.

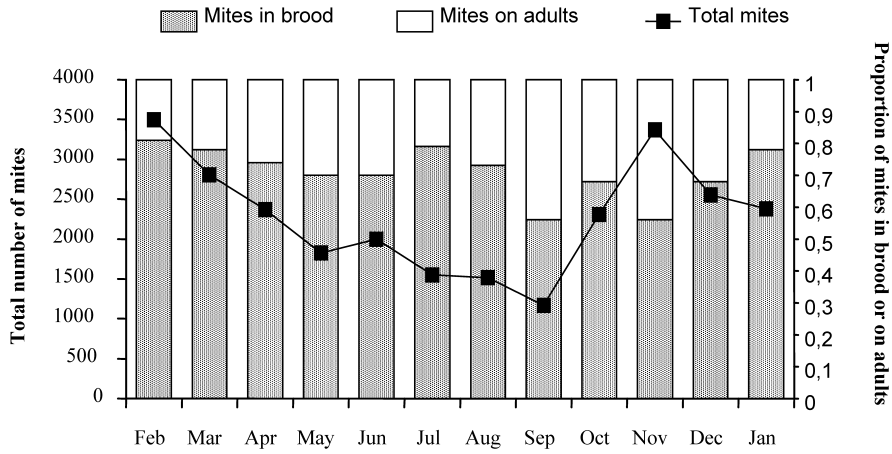


Figure 3. Total number of mites in the colony, and the proportion of mites in sealed worker brood cells and on adult bees.

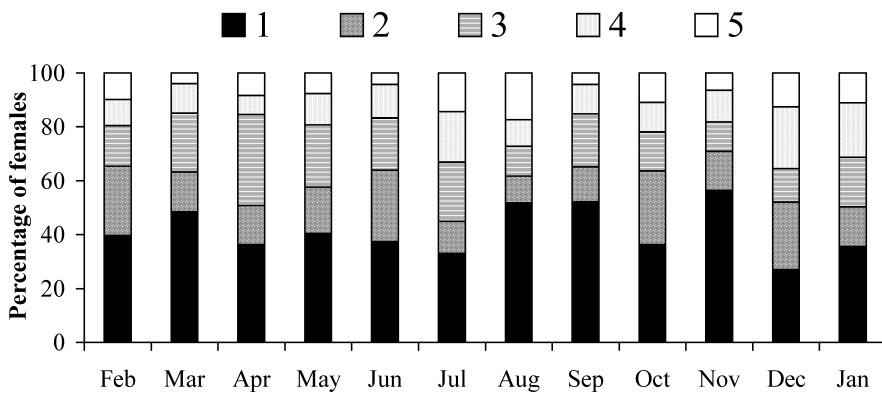


Figure 4. Fluctuations of the five reproductive categories of *V. destructor* families in Africanized worker brood during the experiment. 1 = fertilized female offspring; 2 = no fertilized female offspring due to premature death of the male; 3 = no fertilized female offspring due to other causes; 4 = only male offspring produced; 5 = mother mite did not reproduce.

Mite population

The monthly average mite population per colony was $2,280 \pm 690$ mites. The average mite population decreased from February to September, increased from September to November, and decreased again from November to January (Figure 3).

At the end of the study, January 1998, the estimated mite population per colony was lower (2,380) than 12 months previously (3,500). When the study colonies were treated in May 2000 the mean population was still only $1,821 \pm 917$ mites ranging between 246 to 3,089. This is despite colonies not been treated for five years. This demonstrates clearly that the expected rapid population growth of *V.*

destructor does not occur in AHB in Mexico despite brood being present all year and the mites being of the Korea haplotype.

Discussion

Honey bee colonies

The small fluctuations in the amount of worker brood observed in this study were caused by seasonal change in flowering. However, unlike Echazarreta and Paxton (1997) or Vandame (1996), no single peak of worker brood was observed which is probably due the particular conditions of that year. Like worker brood, drone brood showed no single peak during the year, unlike EHB colonies under temperate conditions which have a distinct brood rearing peak in summer. The amount of drone brood in this study was less than that produced by AHB from the same region in another study (Echazarreta and Paxton 1997). However, in the latter study two additional drone combs were introduced to each colony to stimulate drone production.

Although 3,000+ mites in association with deformed winged virus are sufficient to kill a colony under temperate conditions (Martin 2001a) colony collapse in AHB has not been observed despite the presence of deformed winged virus in Mexico AHB colonies (Martin and Ball, unpublished data). The constant production of worker brood and reduced adult longevity of AHB under tropical conditions (Winston et al. 1981) reduce the transmission of the virus between adult bees and brood. Therefore the size of the mite population needed to kill an AHB colony will be greater than that required to kill an EHB colony. However, the presence of other diseases such as chalkbrood (*Ascosphaera apis*), a fungal pathogen, may alter the balance between the birth and death of bees sufficiently to cause *Varroa* infested AHB colonies to collapse (Medina and Mejia 1999).

Worker brood and adult bee infestation levels

The fluctuation of brood infestation levels corresponds with the fluctuations in the amount of worker brood. This agrees with Cabrera (1998) data from the same locality which showed that a reduction in worker brood resulted in a significant increase in the proportion of cells containing *Varroa*. Both studies, therefore, suggest a dilution effect so that when colonies had more brood the infestation level was lower.

Infestation levels of adult bees were normally low (c. 3.5%) and similar to levels reported for AHB in Brazil (Moretto et al. 1991). Low infestation levels on adult bees were influenced by the fairly constant number of adult bees throughout the study.

Varroa reproduction

The fertility of female *Varroa* mites invading cells in this study (83–96%) was similar to that reported in other studies in Mexico with AHB and EHB (52–69%, Guzman-Novoa et al. (1996); 82–97%, Vandame et al. (1999)), and EHB in Europe (76–94%, Martin et al. (1997)), but much higher than in AHB colonies in Brazil (25–57%, Martin et al. (1997)). This supports the findings that the two different haplotypes of *V. destructor* have different fertilities which are independent of their host honey bee.

There was no effect of food stores on the relative proportion of *Varroa* fertility or fecundity in any of the five categories of *Varroa* reproduction. This is contrary to the findings of Moretto et al. (1997) who suggested that in AHB colonies in Brazil the lack of pollen affects both the fertility and fecundity of *Varroa*. However, our findings are consistent with those of Janmaat and Winston (2000), who found no difference in the proportion of fertile *Varroa* mites reared in EHB colonies in British Columbia with high or low pollen stores, and with Blum (1989) who reported that the proportions of fertile *Varroa* mites in Germany were the same when colonies were given high or low pollen supplement. In both studies the proportions of fertile females varied throughout the year and decreased during late summer (Janmaat and Winston 2000), or was low during the winter (Blum 1989). The low fertility of *Varroa* during the broodless winter period is a common phenomena in temperate regions (Martin 2001b) but was not observed in this study, probably because of near constant availability of worker brood.

Mite population

The observation that the mite populations did not increase during the study were unexpected, especially because year round brood production allows the mites to breed without interruption. Vandame (1996) observed a very similar pattern in mite population growth in AHB at another locality in Mexico where worker brood were also present year round. The average number of mites per colony estimated by Vandame (1996) was also similar (1,513 mites) to this study (2,380 mites in 1998 and 1,821 in 2000). Although mites in this study can only produce 0.7 fertilized female offspring per reproductive cycle in worker brood due to higher offspring mortality (Medina and Martin 1999), they can reproduce several times so growth of the population is still expected, but clearly does not occur. This is illustrated by the initial (3,500) and final (2,380) mean number of mites found in the study colonies. We are still unable to explain the fairly constant pattern of mite numbers observed in this and other studies. We now know that it is not due to variation in mite fertility or fecundity during the year (Figure 4) or variation in drone brood. Although *Varroa* reproduction is more successful in drone than worker brood the mite population was lower during the period when drone brood production was at its peak. Other factors such as postcapping duration and grooming behaviour are also unable to explain tolerance (Vandame et al. 1999). It is possible that the interactions of many factors, such as increased offspring mortality, increased phoretic

mortality, increase removal behaviour and currently unknown factors all combined to produce the observed population dynamics of the mites. It is still important that the tolerance mechanism which is operating in AHB be fully understood, since this is the only race of *A. mellifera* known to be tolerant to *Varroa* which has had such a devastating affect on all other races of *A. mellifera*.

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References

- Anderson D.L. and Trueman J.W.H. 2000. *Varroa jacobsoni* is more than one species. *Exp. Appl. Acarol.* 24: 165–189.
- Blum R. 1989. Reproduction of *Varroa* in relation to protein supply of the honey bee colonies. *Apidologie* 20: 509–512.
- Cabrera D.A.C. 1998. Infestation levels of the mite *Varroa jacobsoni* in honey bee (*Apis mellifera* L.) in Yucatan, Mexico. MSc Dissertation, Universidad Autónoma de Yucatán, Yucatán, México.
- Calis J.N.N., Fries I. and Ryrle S.C. 1999. Population modelling of *Varroa jacobsoni* Oud. *Apidologie* 30: 11–124.
- Camazine S. 1986. Differential reproduction of the mite *Varroa jacobsoni* (Mesostigmata: Varroidae), on Africanized and European honey bees (Hymenoptera: Apidae). *Ann. Entomol. Soc. Am.* 79: 801–803.
- Daly H.V. and Balling S.S. 1978. Identification of Africanized honey bees in the Western Hemisphere by discriminant analysis. *J. Kans. Entomol. Soc.* 51: 857–869.
- De Jong D. 1996. Africanized honey bees in Brazil: forty years of adaptation and success. *Bee World* 77: 67–70.
- De Jong D., Roma D.A. and Goncalves L.S. 1982. A comparative analysis of shaking solutions for the detection of *Varroa jacobsoni* on adult honey bees. *Apidologie* 13: 297–306.
- De Jong D. and Goncalves L.S. 1998. The africanized bees of Brazil have become tolerant to *Varroa*. *Apiacta* 33: 65–70.
- Duch J.G. 1988. La conformación territorial del estado de Yucatán. Universidad Autónoma de Chapingo, 427 pp.
- Echazarreta C.M. and Paxton R.J. 1997. Comparative colony development of Africanized and European honey bees (*Apis mellifera*) in lowland neotropical Yucatan, Mexico. *J. Apic. Res.* 36: 89–103.
- Echazarreta C.M., Quezada-Euan J.J.G., Medina L.M. and Pasteur K.L. 1997. Beekeeping in the Yucatan peninsula: development and current status. *Bee World* 78: 115–127.
- Fuchs S. and Langenbach K. 1989. Multiple infestation of *Apis mellifera* L. brood cells and reproduction in *Varroa jacobsoni* Oud. *Apidologie* 20: 257–266.
- Garcia E. 1973. Modificación al sistema climático de Köppen. Instituto Nacional de Geografía, UNAM, Mexico, D.F.
- Guzman-Novoa E., Sanchez A., Page R.E. Jr and Garcia T. 1996. Susceptibility of European and Africanized honey bees (*Apis mellifera* L.) and their hybrids to *Varroa jacobsoni*. *Apidologie* 27: 93–103.

- Guzman-Novoa E., Vandame R. and Arechavaleta M.E. 1999. Susceptibility of European and Africanized honey bees (*Apis mellifera* L.) to *Varroa jacobsoni* Oud. in Mexico. *Apidologie* 30: 173–182.
- Ifantidis M.D. 1983. Ontogenesis of the mite *Varroa jacobsoni* in worker and drone brood cells. *J. Apic. Res.* 23: 227–233.
- Janmaat A.F. and Winston M.L. 2000. Removal of *Varroa jacobsoni* infested brood in honey bee colonies with different pollen stores. *Apidologie* 31: 377–385.
- Kraus B. and Page R.E. Jr 1995. Population growth of *Varroa jacobsoni* Oud. in Mediterranean climates of California. *Apidologie* 26: 149–157.
- Martin S.J. 1994. Ontogeny of the mite *Varroa jacobsoni* Oud. in worker brood of the honeybee *Apis mellifera* L. under natural conditions. *Exp. Appl. Acarol.* 18: 87–100.
- Martin S.J. 1998. A population model for the ectoparasitic mite *Varroa jacobsoni* in honey bee (*Apis mellifera*) colonies. *Ecological Modelling* 109: 267–281.
- Martin S.J., Holland K. and Murray M. 1997. Non-reproduction in the honeybee mite *Varroa jacobsoni*. *Exp. Appl. Acarol.* 21: 539–549.
- Martin S.J. 2001a. The role of *Varroa* and viral pathogens in the collapse of honeybee colonies: a modelling approach. *J. App. Ecol.* 38: 1082–1093.
- Martin S.J. 2001b. *Varroa destructor* reproduction during the winter in *Apis mellifera* colonies in UK. *Exp. Appl. Acarol.* 25: 321–325.
- Medina L.M. 1998. Frequency and infestation levels of the mite *Varroa jacobsoni* Oud. in managed honey bee (*Apis mellifera* L.) colonies in Yucatan, Mexico. *Am. Bee J.* 138: 125–127.
- Medina L.M. and Martin S. 1999. A comparative study of *Varroa jacobsoni* reproduction in worker cells of honey bees (*Apis mellifera*) in England and Africanized bees in Yucatan, Mexico. *Exp. Appl. Acarol.* 23: 659–667.
- Medina L.M. and Mejia E.V. 1999. The presence of *Varroa jacobsoni* mite and *Ascospaera apis* fungi in collapsing and normal honey bee (*Apis mellifera* L.) colonies in Yucatan, Mexico. *Am. Bee J.* 139: 794–796.
- Message D. and Goncalves L.S. 1995. Effect of the size of worker brood cells of Africanized honey bees on infestation and reproduction of the ectoparasitic mite *Varroa jacobsoni* Oud. *Apidologie* 26: 381–386.
- Moretto G., Gonçalves L.S. and De Jong D. 1997. Relationship between food availability and the reproductive ability of the mite *Varroa jacobsoni* in Africanized bee colonies. *Am. Bee J.* 137: 67–69.
- Moretto G., Gonçalves L.S., De Jong D. and Bichuette M.Z. 1991. The effects of climate and bee race on *Varroa jacobsoni* Oud infestation in Brazil. *Apidologie* 22: 197–203.
- Moritz R.F.A. 1994. Molecular biology of the honey bee. *Adv. Insect Physiol.* 25: 105–149.
- Page R.E. Jr 1980. New photographic method for estimating numbers of brood cells. *J. Apic. Res.* 19: 202–204.
- Quezada-Euan J.J.G., Echazarreta C.M. and Paxton R.J. 1996. The distribution and range expansion of Africanized honey bees (*Apis mellifera*) in the state of Yucatan, Mexico. *J. Apic. Res.* 35: 85–95.
- Ritter W. and De Jong D. 1984. Reproduction of *Varroa jacobsoni* in Europe, Middle East and tropical South America. *Z. Angew. Entomol.* 98: 55–57.
- Rosenkranz P. and Engels W. 1994. Infertility of *Varroa jacobsoni* females after invasion into *Apis mellifera* worker brood as tolerance factor against varroaosis. *Apidologie* 25: 402–411.
- Vandame R. 1996. Importance of host hybridization in the tolerance to a parasite. Example of the parasitic mite *Varroa jacobsoni*, in colonies of European and Africanized honey bees *Apis mellifera*, in humid tropical climate of Mexico. Ph.D., Université Claude Bernard, Lyon, France.
- Vandame R., Colin M.E. and Otero-Colina G. 1999. Africanized honeybees tolerance to *Varroa* in Mexico: mite infertility is not the main tolerance factor. *Apiacta* 34: 12–20.
- Winston M.L., Dropkin J.A. and Taylor O.R. 1981. Demography and life history characteristics of two honey bee races (*Apis mellifera*). *Oecologia* 48: 407–413.
- Zar J.H. 1996. *Biostatistical Analysis*. Prentice Hall, Inc., New Jersey, USA, 662 pp.