Low Reproductive Success of Varroa in *Apis mellifera iberiensis*

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Sumary.

Varroa mite is a dangerous plague for the western honeybee. Treatments can produce contamination in honey and other bee products and frequently *Varroa destructor* is resistant against commercial treatments. Breeding *Varroa* tolerant honeybees is a goal for western beekeeping. For this reason, we considered 3 stocks of bees (each stock was formed by colonies with sister queens) with different parasitation levels, evaluating these by the number of parasites fallen on the bottom-boards during 4 days: A) 10.91±4.09 mites (Nº of colonies = 11), B) 20.00±4.62 mites (Nº of colonies = 13) and C) 32.65±5.07 mites (Nº of colonies = 17). From each stock we selected 2 colonies with lower *Varroa* population and 2 with higher *Varroa* population (Total 12 colonies: Six colonies with low Varroa population and 6 colonies with higher Varroa population). In these colonies we valued the suppression of mite reproduction (SMR). SMR was superior in the less infested colonies versus the more infested (39.07±6.62 and 27.41±7.62, respectively). The values of SMR for the less infested colonies among stocks were: 54.71±4.17, 37.50±4.17 and 25.56±14.44 for the stocks A, B and C respectively.

Keywords: *Varroa destructor*, Honey bee, Parasitism, Resistance, Mite reproduction, Tolerance, Population dynamic, Selection.

Introduction.

Varroa mite is an important pest for the western honey bee (*Apis mellifera*). The fight against Varroa is led fundamentally by the use of chemical treatments. Nevertheless, the parasite frequently acquires resistance to the common commercial treatments (reviewed by Milani, 1999; Martin, 2004) and the risk exists concerning the appearance of residuals in the honey and other bee products (reviewed by Wallner, 1999; Bogdanov, 2006). For these reasons an integrated pest management (IPM) is necessary for Varroa control. One important tool for IPM can be the beekeeping with varroa tolerant honeybees (Delaplane *et al*., 2005; Oliver 2007).

The biological relationship between Varroa and *A. mellifera* is unbalanced. The systematic use of synthetic treatments hinder the natural selection and the bees continue to require chemical intervention to survive. Nevertheless, it is frequent that when the colonies of honey bees remain untreated for several years, some of them survive with increased tolerance against the mite (Fries *et al.*, 2006; Oliver, 2007; Seeley, 2007).

The best examples of tolerance to Varroa are found in the original host *Apis cerana*. The first research showed a balanced relationship between *A. cerana* and *Varroa jacobsoni* based on different traits in the reproductive cycle of Varroa in worker brood cells, such as lower tendency to invade worker brood cells, limited reproduction success of Varroa, high hygienic behavior of the bees against infested worker brood, or during phoretic phase like the grooming (reviewed by Rath, 1999).

Some of these characters can also be expressed, in a natural way, in the relationship between Varroa and western honey bee, like the africanized honey bees, whose tolerance against the mite has been attributed to high hygienic behavior on the infested worker brood cells, cited in populations of bees from Mexico (Vandame *et al*., 2000), low reproductive success of the mite in Brazil (Ritter and De Jong, 1984; Rosenkranz and Engels, 1994), high grooming behaviour in the adult bees in populations from Mexico (Arechavaleta-Velasco and Guzmán-Novoa, 2001) or a pool of these factors cited (Mondragón *et al*., 2005).

Fortunately, the frecuency is increasing concerning reports of Varroa tolerant bees from European honey bees lineages, showing traits as those described in *A. cerana* or AHB (Harbor and Hoopingarner, 1997; Harbor and Harris, 1999; Le Conte, 2004; Kefuss *et al*, 2004; Rinderer *et a*l., 2001).

Low reproductive success of the mite has demonstrated to be an interesting trait that can allow an increase in the tolerance of the bees against Varroa (Harris and Harbor, 2000; Harbor and Harris, 2005; Delaplane *et al*, 2005). In agreement with Harbor and Harris (1999) Varroa has not reproductive success if: I) varroa died in the cell without reproducing, II) when the mite is infertile, III) when the offspring are only male and IV) when the offspring is too late to reach the maturity before the postcapping period is finished. Also, the reproductive success is lower and the Varroa population will grow more slowly if the ratio viable offspring/mite foundress is lower (Mondragón *et al*., 2005 and 2006).

Since 2001 we bred lines of Varroa tolerant honey bees from the South of Spain (*Apis mellifera iberiensis*). Each line was initiated with a tested tolerant queen from a commercial apiary, and others formed by sister queens mated naturally. From the spring to autumn season we assessed the naturally fallen mites on the debris of the botton-board and in the autumn we took advantage of the chemical treatment (Apistán® or Apivar®) to realize the study. Each year we selected in each lineage the queen (beehive) with lower Varroa population, and used this female as the mother of the next queen's generation. Our bees responded favourably to this selection, and some lines showed less mite population than unselected honey bee colonies (Flores *et al*., 2006).

The next step in our breeding process was to study which factors were related with the greater tolerance to varroa in some of our bee colonies. In this research we used 3 stocks of bees with different parasitation levels and we studied them with regard to reproductive parameters of presumable importance in the success of the fight of the bees against varroa.

Materials and Methods

The investigation was carried out in the city of Córdoba (South of Spain). The population dynamics of Varroa in this area causes that the highest parasitation (number of Varroas/number of adult bees and brood) occurs in autumn, being this an appropriate season to study the reproductive differences of varroas in several colonies (Flores *et al*., 1994).

In this study we worked with Langstroth beehives, endowed with special funds with grill (3 mm) that allow parasites to fall to the debris where they are collected, hindering the access to the bees.

In autumn of 2005 we selected 3 lineages of bees that presented different average of parasitation (table 1). The varroa population was measured by the natural dropping of parasites in the botton-boards of the beehives in a period of 4 days (Fries *et al*., 1991; Calatayud and Verdú, 1993; Flores *et al*., 2002). When the experiment was finished we treated the colonies with Apivar® and recorded the total number of parasites in each colony.

All queens were reared in the spring of 2005 and naturally mated in the same location. We selected 4 colonies from each stock (12 colonies): two with a higher degree of parasitation and two with a lower degree, with regard to the average of their lineage.

In each colony we selected a comb with sealed worker brood (white pupae with brown eyes or with the thorax lightly pigmented), that coincides with the 7-8 days after capping (Rembold *et al*., 1980). This stage is selected because varroa has already had time to generate descendants, and these descendants can be easily differentiated from the female progenitor and other offspring generated later on which will have a very low probability of surviving (Martin, 1994).

The brood combs were inspected under a binocular glass magnifying (X 20). From each colony we examined a maximum of 20 parasited cells or up to 200 cells with breeding of appropriate age, when the parasitation was lower.

As a result of this evaluation the following data was recorded:

- Percentage of parasitized brood (cells parasited with respect to the total number of examined cells).

- Number of cells infested by only one foundress.

- Number of viable reproductive parasites that haven't had reproductive success (absence of egg, the eggs were not viable, the entire descendancy was male or the varroa offspring was too late to allow the descendancy reach maturity before the birth of the bee).

- Number of deutonymph /cell with only one foundress.

To study the different factors related with the reproductive success of varroa we only consider those cells that only one foundress progenitor has entered.

The results were evaluated using descriptive parameters, variance analysis (One-Way ANOVA) and other test for means comparison (T-test) (SPSS 8.0).

Results.

The results showed significant differences among the bees lineages (One-Sample Kolmogorov-Smirnov Test: p=0.179. Test of Homogeneity of Variances: P=0.364. One-way ANOVA: p=0.01). The statistical differences were found between the lineages A and C (Tukey HSD Test: $p < 0.05$). Not significant differences were found between the lineage B and the other two.

When we compared the naturally fallen parasites during a period of 4 days with the total mites fallen when the colonies were treated, the results showed a significant correlation between both values (Pearson´s correlation coefficient. p = 0.000, r = 0.971).

a. Percentage of the infested honey bee brod. The results showed that the average percentage of parasitation was lower in the 6 colonies less parasited (2 of each lineage) versus the 6 more infested (Figure 1a). When we differenciate between lineages, the discordant result was found in the 2 colonies more parasited of the lineage B, in those that the parasitation of the brood showed similar values of the less parasited colonies (Figure 2).

b. Lack of reproductive success of varroa. The mean in the lack of reproductive success was superior in the 6 colonies less parasited versus the 6 colonies more parasited (Figure 1b). When we discriminate between lineages, the data show differences between the less and more infested colonies, higher in the lineage A, followed by the B and finally C. Equally the values of lack of reproductive success of varroa decrease as a result of the increase of the degree of parasitation. These differences were less evident when we compared the infested colonies of the three lineages (Figure 3).

c. Viable offspring. The mean number of viable offspring was lower in the 6 colonies less parasited versus the 6 more infested (Figure 1c). When we discriminate between lineages, the biggest difference in the number of viable offspring among the colonies more and less parasited was found in the lineage A. In the same way, the mean number of viable offspring in the lineage A was inferior to the same values in the lineages B and C. The results showed minimal differences in the number of viable offspring in the more infested colonies in the three lineages (Figure 4).

Although the graphic representation of the results is eloquent, we only detect significant differences when we compare the degree of parasitation of the brood between the 6 less parasited colonies and the 6 with the biggest varroa population.

Discussion.

Breeding for varroa tolerant honeybees is an important question in an integral IPM to Varroa control. Different methods allow us to get tolerant bees to varroa. Frequently there are references to colonies which survived in their apiary, untreated for several years, and it is relatively frequent the recovery of the apiary from these colonies. In the same way, the selection of colonies with smaller Varroa population is another way that has been shown to be useful to get bees tolerant to the parasite (Harbor and Hoopingarner, 1997; Szabo and Szabo, 2002; Le Conte, 2004; Kefuss *et al*., 2004; Fries *et al*., 2006).

During several years we have selected colonies of bees with low varroa population (Flores *et al*., 2006). In this work we have used 3 lineages of those honey bees, and although the results show the existence of significant differences between these lineages, we also found important variations in the varroa population among the colonies of each lineage, probably because our queens were naturally mated. This also allowed us to compare the lineages, to carry out, inside each lineage, comparisons among colonies with high and low varroa population.

In agreement with other authors (Fries *et al*., 1991; Calatayud and Verdú, 1993; Flores *et al*., 2002) the selection of colonies based on the natural dropping of Varroa in botton-boards is an effective tool to discriminate between colonies with higher or lower population of parasites, like that shown in the high positive correlation between varroas colected during 4 days and the total number of varroas in colonies as a result of treatment with synthetic pesticide.

When we consider the parasitation average of the brood, the results are as expected, appearing a low average value of parasitation in the brood of the 6 colonies with smaller varroas population (Figures 1a). Nevertheless, the colonies with bigger Varroa population in the lineage B presented smaller brood infestation in comparison with the other stocks (Figures 2). This result could be due to a lower attraction of varroa to invade the brood cell, which would agree with the probable multifactorial source of the tolerance of the honey bees against varroa (Revised for Büchler, 1994).

Harbor and Hoopingarner (1997), Harbor and Harris (1999 and 2005) and Harris and Harbor (2000) demonstrated that the lack of reproductive success of Varroa could be an important traits to get European bees more tolerant to the mite. In agree with these authors, we also found differences in the lack of reproductive success of varroa among the colonies more and less infested within each stock. When we compare this factor between the colonies of the 3 stocks, the results suggest that this factor could help to a smaller grow of Varroa population in the beehives (Figures 3). Nevertheless, this analysis can only be made in an intuitive way, because the differences were not statistically ratified, probably because the number of colonies used in the experiment was not sufficiently high.

On the other hand, a low average of viable offspring can be another factor that can reduce the varroas population growth in the colonies (Ibrahim and Spivak, 2006). A typical reproductive cycle of Varroa in worker brood cell of european honey bee produces one male and up to 3 viable females. Although, the second and third female descendants contribute to an inferior quantity in the populations increase of the parasites. Lower fecundity of the Varroa progenitor or a delay in the egg laying or in the development of the descendants, will contribute to a slower mite population growth, so that in the phase of pupa with brown eyes or with the thorax lightly pigmented only the varroa descendants in the phase of deutonymph reach maturity before the end of the worker brood capped period. (Martin, 1994).

The results of our investigation also show a low number of varroa descendancy with possibilities of arriving to the mature state and to be mated in the 6 colonies less parasited (Figures 1c). In the same way, this also happened in the beehives less parasited of the lineage A with respect to the rest of experimental colonies (Figures 4), allowing us to suspect that this characteristic could also have some influence in the levels of parasitation at the end of the experiment.

In conclusion we think that the lack of reproductive success of varroa can be one cause of the smaller parasitation degree in our colonies, as indicated the results, while all the reproductive parameters studied are favourable in the less infected colonies.

Acknowledgements.

The research was supported by “Programa Nacional de Medidas de Ayuda a la Apicultura” grant API06-010. Special thanks to the “Diputación de Córdoba” for their continued encouragement and support.

Lineage	**Average of Varroa natural fallen in 4 days and number of colonies in each lineage**	**Colony number**	**Number of mites natural fallen in 4 days**	**Number of mitess register when we used Apivar®**	**Relative parasitation degree**
A	10,91±4,09 (n= 11 colonies)	1	5	177	Low
		2	6	175	Low
		3	27	Died	High
		4	38	1127	High
B	20,00±4,62 (n= 13 colonies)	5	9	454	Low
		6	9	686	Low
		7	22	614	High
		8	25	849	High
C	32,65±5.07 (n= 17 colonies)	9	5	360	Low
		10	9	482	Low
		11	64	2448	High
		12	72	2176	High

Table 1. The study was carry out with 3 honey bee lineages (A, B and C). In each lineage we evaluated the average number of naturally fallen mites in the debris of the botton-boards in 4 days. In each lineage we selected 4 colonies: 2 less parasited and 2 more parasited (total number of colonies = 12). At the end of the study we treated the colonies with Apivar ® and recorded the total number of parasites.

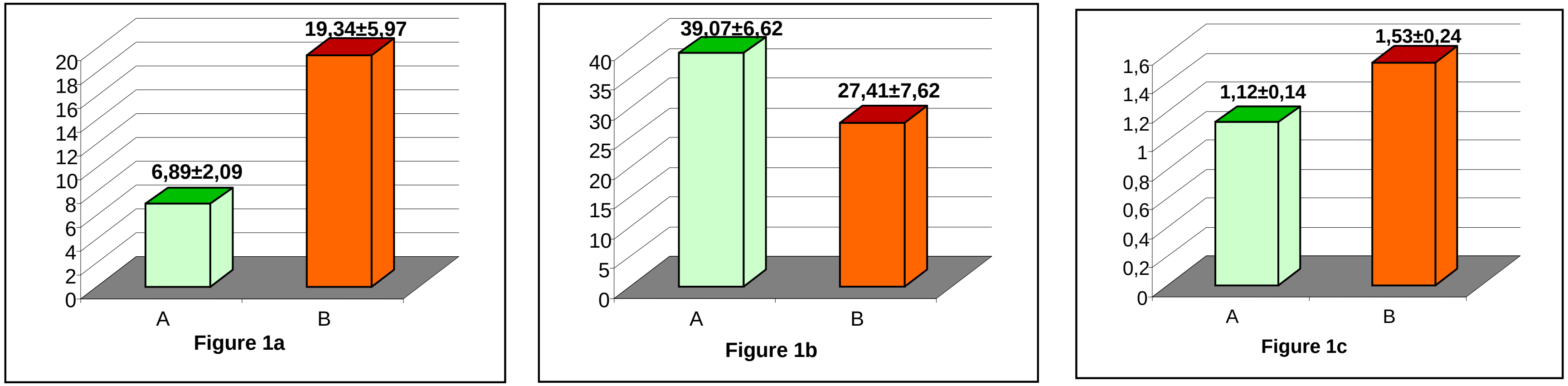

Figure 1. Means ± s.e. percentage of infestation on the worker brood cells (1a), percentage of Varroa without reproductive success (1b), average deutonymph/ cells parasited by one foundress (1c) in 6 less infested colonies versus 6 more infested, without discriminating between the three lineages.

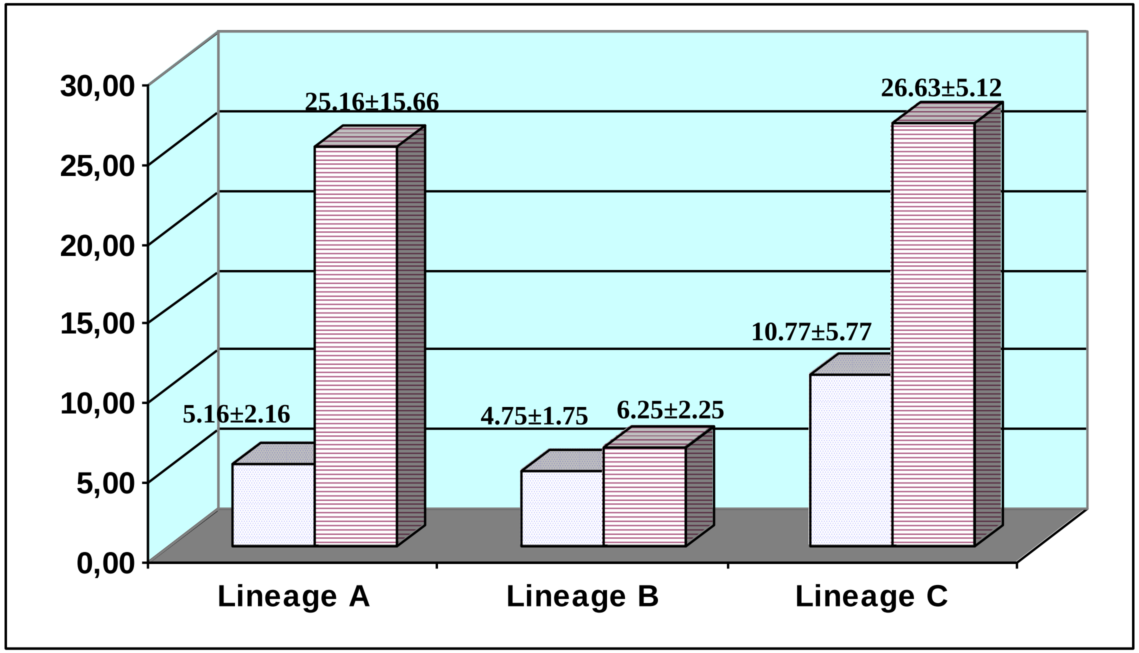

Figure 2. Means ± s.e. percentage of parasitation of the worker brood cells from 3 lineages of bees. From every lineage 2 colonies less parasited and 2 colonies more parasited are selected.

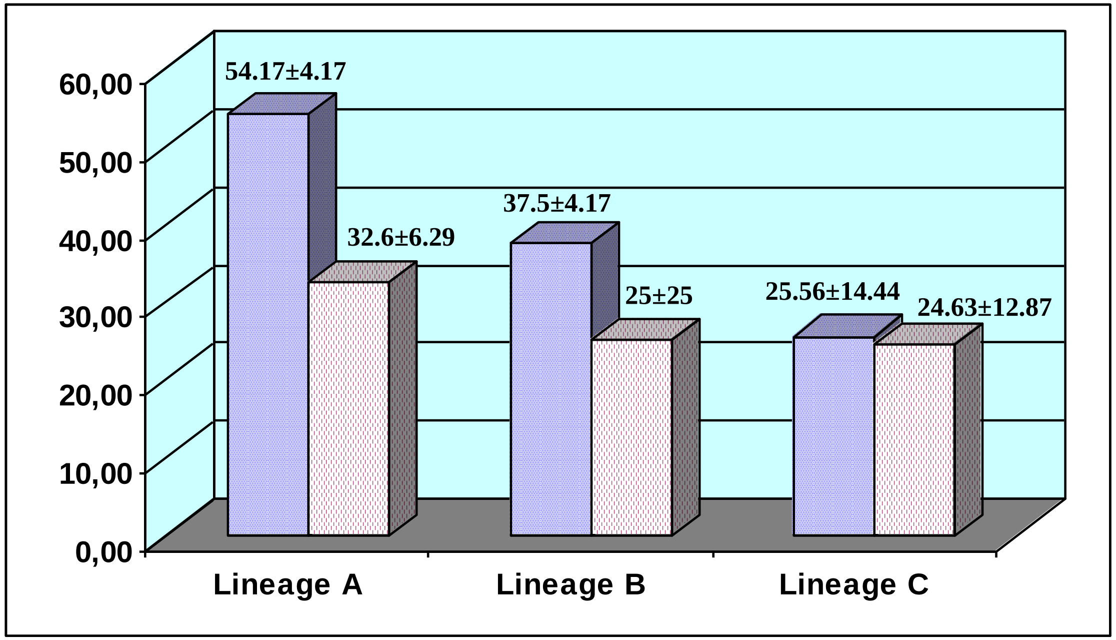

Figure 3. Means ± s.e. percentage of Varroa without reproductive success in worker brood cells in 3 lineages of bees. From each lineage 2 colonies less parasited and 2 colonies more parasited are selected.

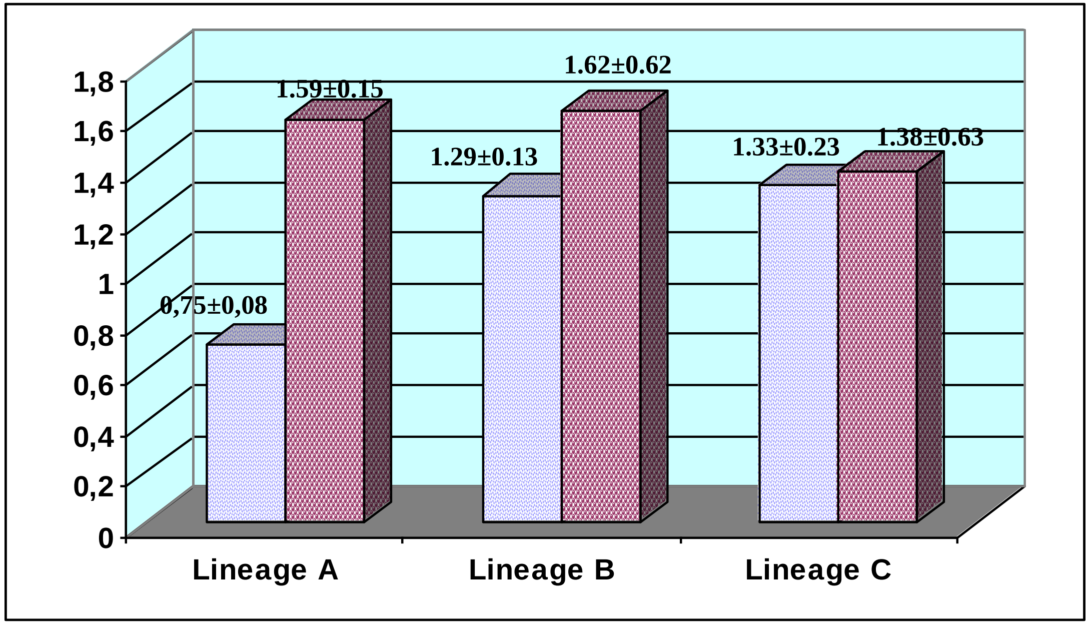

Figure 4. Means ± s.e. of deutonymphs/number of cells with only one foundress in 3 lines of honey bees. From each lineage 2 colonies less parasited and 2 more parasited are selected.

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