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Original article

Honey bee colonies that have survived *Varroa destructor**

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Abstract – We document the ability of a population of honey bee colonies to survive in France without *Varroa* suppression measures. We compared the mortality of collected *Varroa* surviving bee (VSB) stock with that of miticide-treated *Varroa*-susceptible colonies. *Varroa* infestation did not induce mortality in the VSB colonies. Some of the original colonies survived more than 11 years without treatment and the average survival of the experimental colonies was 6.54 ± 0.25 years. Swarming was variable (41.50 ± 9.94%) depending on the year. Honey production was significantly higher (1.7 times) in treated than in VSB colonies. For the first time since *Varroa* invaded France, our results provide evidence that untreated local honey bee colonies can survive the mite, which may be the basis for integrated *Varroa* management.

***Apis mellifera* / honey bee / *Varroa destructor* / host-parasite relationship / natural selection / tolerance**

1. INTRODUCTION

Varroa destructor Anderson & Trueman is a major pest of the Western honey bee *Apis mellifera* L. worldwide. Under typical environmental conditions, untreated honey bee colonies survive one to four years, and the mite populations can reach up to 8000 *Varroa* mites per honey bee colony (Ritter, 1981; Korpela et al., 1992; Fries et al., 1994). Untreated *Apis mellifera* colonies infested with *Varroa destructor* were estimated to survive for one or two years in France (Robaux, 1986; Jéanne et al., 2002), and from eight months to one year in the Mediterranean climate (Branco et al., 1999).

In France, untreated and feral colonies of honey bees largely disappeared a few years after the arrival of *Varroa* in 1982. *Varroa* resistant strains of bees are of great interest for beekeeping to reduce the use of chemicals in honey bee colonies. One way to obtain resistant bees is to intensively select for single characters that decrease the growth of *Varroa* populations. This approach might force adaptations by the mite, limiting the genetic progress obtained by the breeder. Another possibility is to monitor natural selection in unmanaged populations. An advantage of natural selection is that it selects for a host-parasite equilibrium that may be more sustainable than human selection for a single characteristic. For this reason we chose this latter approach.

In 1994, a first group of 12 honey bee colonies were found surviving in two different places of France, near Le Mans and around Avignon. They were feral colonies or colonies

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in abandoned apiaries that had not been treated to control *Varroa*. In this work, we report on the ability of these colonies, some additional colonies found through a survey (Le Conte et al., 2000) and descendants of these colonies to survive in France without any form of *Varroa* mite control.

2. MATERIALS AND METHODS

2.1. Collection of candidate colonies

In 1994 a first group of 12 colonies that had not been treated for the mite for at least three years was established. Six were collected near Le Mans and 6 in Avignon. They were maintained in one apiary and in the same location where they were found.

Subsequently, based on beekeeper responses to a survey, we collected a second group of 42 colonies in 1998, and 28 colonies in 1999, from beekeepers. The colonies had not been treated for at least two years and exhibited normal development. They were added to the first group of colonies that were studied since 1994. A total of 52 colonies were placed in our apiary in Avignon, the others (30) stayed in their original area near Le Mans, western France. Thus, we had two different groups of colonies and two different locations.

The colonies were managed in standard 10 frame Dadant hives. Only healthy colonies, those showing no symptoms of diseases other than *Varroa* infestation, were selected to be included in the experiment. Observations were made on foraging activity and each frame of the colony was inspected for brood and adult worker diseases. Except in feral colonies kept in the same trees the queens were paint marked on the thorax to estimate swarming or natural queen replacement. Colonies in abandoned apiaries were transferred in new building Dadand hives when necessary.

2.2. Observations

We looked at colony survival, comparing the mortality of our collected untreated stock with control colonies that were treated with Apivar® once a year during the fall. In 1999, 36 control colonies were used in two different apiaries located at 0.5 and 2.5 kilometers from the experimental colonies in Le Mans area, and 54 control colonies were used in Avignon in three different apiaries located between 0.1 and 1.3 kilometers from the experimental ones.

Observations were made on untreated and treated colonies at least once monthly except in cold winter. We opened colonies in moveable frame hives to observe abnormal development, the presence of a queen, swarming, presence of *Varroa* mites and diseases. We also checked for colonies showing disease symptoms, like American foulbrood or heavy *Varroa* infestation.

Feral colony (n = 3) activity was observed twice a month to be sure that they survived the winter and that a swarm would not replenish the nest during the spring period. Survival data from those colonies were added to the data from in-hive colonies.

Except for monthly observations, adding supers and harvesting honey, no management was done on the colonies. Nothing was done to prevent swarming other than adding supers as needed.

The crop of honey from each *Varroa* surviving bee (VSB) colony was weighed and recorded each year from 1999 to 2005, and also in 2003 and 2004 for treated control colonies. For the other years, the average honey crop produced by the treated colonies was calculated with no indication of individual colony performance, dividing the total amount of honey produced from all the treated colonies by the number of colonies.

2.3. Population dynamics of the mite

To estimate *Varroa* populations, we followed the natural mite mortality in the collected colonies using a screened bottom board to collect the mites. Traps were continuously monitored for 12 months, and all the dead mites falling on the bottom board were counted one to three times a week depending on the amount of brood in the colonies. In Avignon, from September 2002 to August 2003, natural mortality of *Varroa* was observed in 12 VSB colonies and in 16 control colonies selected randomly from our apiaries.

3. RESULTS

3.1. Survival of the colonies

Considering the two different locations together (Le Mans and Avignon) the mortality of the untreated colonies varied between

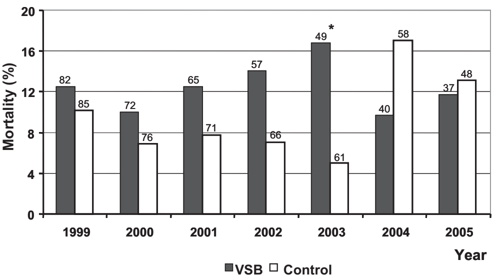

Figure 1. Mortality of untreated VSB (*Varroa* Surviving honey Bee) colonies compared to treated control colonies over a 7-year period. (* significant statistical differences, χ^2, $P < 0.05$). Numbers at the top of the bar give the number of colonies sampled per modality.

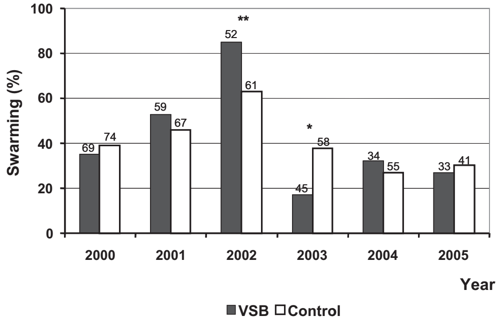

Figure 2. Percentage of swarming in the VSB colony population compared to treated control colonies between 2000 and 2005. (* and ** represent significant statistical differences, χ^2, $P < 0.05$ and $P < 0.01$ respectively). Numbers at the top of the bar give the number of colonies sampled per modality.

9.7% and 16.8% per year from 1999 to 2005 (Fig. 1). Compared to treated colonies, there was significantly higher mortality in untreated colonies only in 2003 ($\chi^2 = 4.52$, $P < 0.05$). When all 7 years (1999 to 2005) of the experiment were included, we found no significant difference between the annual mortality of VSB (12.46% ± 0.92) and treated (9.57% ± 1.59) colonies ($\chi^2 = 0.42$, $P = 0.52$).

The percentage of initial colonies surviving after the 7 year period was not statistically different between VSB (45.1%) and treated control colonies (56.5%) ($\chi^2 = 0.70$, $P = 0.40$).

In the first group of VSB colonies, 5 of the 12 original colonies survived more than 11 years without treatment and the average survival was 9.8 ± 0.7 years (mean ± SE).

In the second group of colonies, the average survival of the VSB colonies was 6.54 ± 0.25 years and 5.86 ± 0.21 years in western France (n = 30) and in Avignon (n = 52), respectively. The average survival of the treated colonies was 6.63 ± 0.3 years and 6.78 ± 0.2 in western France (n = 30) and in Avignon (n = 55), respectively. The survival times are minimum values and minimum estimates as experimental colonies were still alive in 2006 and survival times of the colonies before monitoring was not included.

During the time of the experiment, we did not observe symptoms of American foulbrood, viruses or other pathogens, and we did not exclude colonies from the experiment because of a high *Varroa* infestation. During the different beekeeping visits, the presence of *Varroa* mites was confirmed in each of the different colonies. Colony losses were due mostly to queen loss during the winter and to subsequent unsuccessful queen supersedure.

3.2. Swarming

Swarming was variable depending on the year (Fig. 2). The maximum was 85% in 2002, and the minimum 17% in 2003. Between 1999 and 2005, the average was 41.50 ± 9.94%. Except in 2002 and 2003, there was no statistical difference in the rate of swarming between VSB and treated control colonies. In 2002, significantly more colonies from VSB swarmed in comparison to the treated colonies ($\chi^2 = 7.46$, $P < 0.01$), and opposite results were obtained in 2003 ($\chi^2 = 6.03$, $P < 0.05$).

3.3. Honey production

Between 1999 and 2005, annual mean honey production of the VSB varied between 6.9 ± 1.02 kg (in 2003) and 18.2 ± 1.23 kg (in 2004) (Fig. 3). Honey production in *Varroa* treated control colonies was significantly

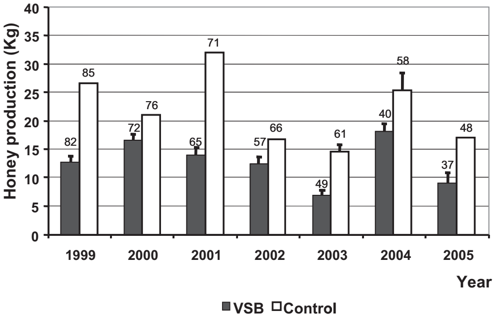

Figure 3. Honey production by the VSB colonies compared to treated control colonies over a 7-year period. (Mean ± S.E.). Bars without S.E. give the total production of the group of untreated colonies divided by the number of colonies. Bars with S.E. give means and S.E. calculated with data from individual colonies. Numbers at the top of the bar give the number of colonies sampled per modality.

higher than VSB colonies (Mann-Whitney test on average annual crop, $Z = 2.61$, $P < 0.01$) and control colonies produced 1.7 times more honey over the entire period.

3.4. Infestation rate of the surviving colonies.

The number of mites collected on the bottom boards of VSB colonies was significantly lower (3331.25 ± 465.68) than in control colonies (10278.45 ± 642.32; Mann-Whitney test, $P < 0.001$).

4. DISCUSSION

Our results clearly show that some honey bee colonies can survive without protection from *Varroa* for longer than 1 or 2 years, as previously reported (Robaux, 1986). The 12 colonies observed in the first group of colonies survived on average at least 9.8 years, and 5 of them survived more than 11 years. During the last nine-year experiment on the second group of colonies, the mortality in the VSB colonies was slightly more but not significantly different from the treated colonies. Annual colony mortality between 5 and 10 percent is considered acceptable by beekeepers in France. We can conclude that the *Varroa* infestation of untreated colonies did not cause more colony loss during that nine-year period compared to colonies treated with Apivar®.

The infestation rate of the VSB colonies, derived from mite fall on the bottom board, was especially low, three times lower, compared to control colonies. This suggests that VSB colonies have developed mechanisms to inhibit the growth of *V. destructor* populations. Thus, VSB colonies apparently have attained a host – parasite equilibrium which is not the result of beekeeper selection but due to natural selection.

For the moment, we do not know the mechanisms or causes of the more balanced host-parasite relationship. There are preliminary indications, that VHB bees might be better able to recognize the mites (Martin et al., 2001), which could enhance mite removal by grooming (Peng et al., 1987), and that they might be better able to detect and remove mites from infested sealed cells. Swarming activity did not seem to play a major role (Fries et al., 2003), as there was no marked difference between VSB and control bees in our study. Further, it seems unlikely that more benign mites strains have been selected, as after the development of molecular markers (Navajas et al., 2002; Solignac et al., 2003) we concluded that the Korean haplotype representing the *Varroa* mite population in Europe is a single invasive clone (Solignac et al., 2005), thus probably showing little genetic variation.

We however think that environment and apicultural methods could have played a part. The areas where the experiments were done are outside France's major agricultural zone and very favorable to the development of honey bee colonies. The colonies were manipulated only when necessary and were not moved or managed as professional beekeeping would recommend, and the lack of beekeeping and environmental stress might have favored bee survival.

Other honey bee populations have been reported to be naturally resistant to *Varroa* in the past. They were first restricted to South America and North Africa. The tolerance of European honey bees was reported in Uruguay by Ruttner et al. (1984) and then in Brazil by de Jong and Soares (1997). Many studies have shown the tolerance of Africanized bees to the mite (Martin and Medina, 2004) from Northern Argentina (Eguaras et al., 1995; reviewed in Rozenkranz, 1999) and Brazil to Mexico (Ritter and de Jong, 1984; Guzman-Novoa et al., 1999; Moretto and Mello, 2000; Vandame et al., 2000). More recently, Seeley (2006) found a few feral colonies in northeastern United States, and reported that they had survived for three years without treatment. Ritter (1990) described the tolerance of *Apis mellifera intermissa* to *Varroa* mites in Tunisia. Kefuss et al. (2004) reported *Varroa* tolerance in France in *Apis mellifera intermissa* imported from Tunisia. There is also evidence for mite resistance in *A. mellifera* from far-eastern Russia (Primorsky) originating from honey bees imported in the mid 1800's; those honey bee colonies have a strong, genetically based resistance to the parasite (Rinderer et al., 2001). Finally, Fries et al. (2006) using a honey bee population placed in an island of the Baltic sea, have shown that some honey bee colonies (5 of 150 initial colonies) infested by *V. destructor* may survive for over 6 years even if mite control is not practiced. While various reports provide evidence for *Varroa*-surviving honey bees in different countries, our results demonstrate, for the first time in France, the ability of untreated local honey bees to coexist with *V. destructor* for an extended time without treatment. Our observations lead us to conclude that the honey bee population we have isolated is tolerant to the mite (Rosenkranz, 1999). Indeed, though marginally less productive, our colonies may become an integral part for integrated *Varroa* management in France including soft biological or biotechnical control against the mite.

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Des colonies d'abeilles (*Apis mellifera*) qui survivent à *Varroa destructor* en France.

***Apis mellifera* / *Varroa destructor* / relation hôte-parasite / sélection naturelle / sensibilité-résistance / tolérance**

Zusammenfassung – Honigbienenvölker, die *Varroa destructor* in Frankreich überlebten. Die Überlebensdauer von mit *Varroa destructor* befallenen *Apis mellifera* Völkern ohne Behandlung wurde bisher auf ein oder zwei Jahre geschätzt. 1994 wurden an zwei verschiedenen Standorten 12 Honigbienenvölker gefunden, die ohne Behandlung gegen *Varroa* überlebt hatten. Wir stellten zusätzliche potentiell resistente Bienenvölker von Imkern zusammen und verstärkten diese Gruppe damit. In der vorliegenden Arbeit berichten wir über die Fähigkeit dieser Bienen in Frankreich, ohne jede Form der Varroabehandlung zu überleben. Wir verglichen die Sterblichkeit unserer Versuchsgruppe mit Kontrollvölkern, die mit Akariziden behandelt wurden. Außer monatlichen Beobachtungen, dem Zufügen von Magazinen und der Ernte des Honigs, wurden die Völker nicht bewirtschaftet, es fand auch keine Schwarmkontrolle statt. Die Honigernte wurde für jedes Volk bestimmt und mit der der Kontrollvölker verglichen.
Die Sterblichkeit der Völker mit varroaüberlebenden Bienen (VSB) schwankte in der Zeit zwischen 1999 und 2005 zwischen 9,7 % und 16,8 % pro Jahr. Fünf der 12 Originalvölker überlebten mehr als 11 Jahre ohne Behandlung, die Überlebensdauer in dieser Gruppe betrug im Schnitt 9,8 ± 0,7 (Mittelwert und Standardabweichung) je nach Jahr, und wir fanden keine signifikanten Unterschiede zwischen der Sterblichkeit der VSB (12,46 ± 0,92) und der behandelten Kontrollvölker (9,57 ± 1,59). Die Schwarmtätigkeit war in den verschiedenen Jahren sehr variabel (Mittelwert und Standardabweichung: $41,50 \pm 9,94$ %) und konnte das unterschiedliche Überleben der Völker nicht erklären. Die Honigproduktion der gegen *Varroa* behandelten Kontrollgruppe war gegenüber den VSB Völkern um einen Faktor von 1,7 signifikant erhöht.
Unsere Ergebnisse zeigen klar, dass einige Honigbienenvölker auch ohne Schutzmaßnahmen gegen

Varroa wesentlich längere Zeiten überleben können als dies zuvor angenommen worden war. Der Befall mit *Varroa* verursachte während der 9jährigen Untersuchungszeit bei den VSB Völkern keinen signifikanten Tod. Wir diskutieren verschiedene Hypothesen, die das Phänomen erklären könnten. Es ist naheliegend anzunehmen, dass die Resistenz der Bienenvölker, aber auch die Virulenz von *Varroa* und das Vorkommen von Virusinfektionen unter beständigem Selektionsdruck auf das Überleben von beiden, dem Wirt und dem Parasiten, stehen. Das erste Mal seit der Einwanderung von *Varroa* nach Frankreich zeigen unsere Ergebnisse die Fähigkeit unbehandelter lokaler Honigbienen den Befall mit den Milben zu überleben. Diese Bienen könnten im Rahmen des integrierten Bienenmanagements in Frankreich von hohem Nutzen werden.

***Apis mellifera* / Honigbienen / *Varroa destructor* / Wirt-Parasit-Beziehung / natürliche Selektion / Toleranz**

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