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The varroosis in the Mediterranean region

Zaragoza : CIHEAM
Cahiers Options Méditerranéennes; n. 21

1997
pages 59-75

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SUMMARY - In 1984, several years after *Varroa jacobsoni* was discovered in Yugoslavia and three years before this mite was identified in the United States, a joint research project was established. The goal of the project was to attempt to select a stock of honey bees tolerant or resistant to this honey bee parasite. Three queens were collected from apiaries which had extremely high losses believed to be a result of *V. jacobsoni* parasitism. Four generations of two lines of honey bees (*Apis mellifera carnica*) were selectively propagated from these queens. The two lines were selected for high and low brood infestation with *Varroa* mites. Significant differences in all generations of selection showed that this characteristic was heritable, since significant portions of this genetic variation proved to be additive. Selective breeding, if organized on a wider genetic basis, should have good promise for providing the expected long-term solution to the problem of *Varroa* mites. Because of this characteristic and other qualities, this resistant line of bees was imported into the United States for further testing and was later named ARS-Y-C-1. Using a short test for larval attractiveness, ARS-Y-C-1 was found to be less attractive to *V. jacobsoni,* having lower levels of infestation than Hastings (*A. m. carnica* from Canada), and Louisiana stocks. Infestation of a cross between ARS-Y-C-1 and Hastings stocks was intermediate. In a field experiment, results showed that all three Carniolan stocks displayed greater tolerance to *V. jacobsoni* than the Louisiana stock. These stocks survived longer with higher levels of *Varroa* infestations both in worker brood and on adult bees. The susceptible Louisiana stock died earlier with lower levels of *Varroa* infestation.

Key words: Honey bees, *Apis mellifera*, *Varroa jacobsoni*, resistance, tolerance, tolerance index, susceptibility.

RESUME - *"Sélection d'abeilles mellifères tolérantes ou résistantes à* Varroa jacobsoni*". En 1984, plusieurs années après la découverte de* Varroa jacobsoni *en Yougoslavie et trois ans avant que cet acarien soit identifié aux Etats-Unis, un projet de recherche conjoint avait été établi. Le but de ce projet était d'essayer de sélectionner des abeilles mellifères tolérantes ou résistantes à ce parasite de l'abeille mellifère. Trois reines furent collectées dans des ruchers qui avaient des pertes énormes apparemment à cause du parasitisme de* V. jacobsoni. *Quatre générations provenant de deux lignées d'abeilles mellifères (*Apis mellifera carnica*) furent propagées sélectivement à partir de ces reines. Les deux lignées étaient sélectionnées pour une infestation élevée et faible du couvain par les acariens* Varroa. *Des différences significatives dans toutes les générations de sélection montraient que cette caractéristique était héritable, car une proportion significative de cette variation génétique était additive. L'amélioration par sélection , à condition d'être organisée sur une base génétique plus large, peut offrir les solutions à long terme espérées concernant le problème des acariens* Varroa. *En raison de ces caractéristiques et d'autres qualités, cette lignée d'abeilles résistantes a été importée aux Etats-Unis* *pour faire des tests où elle a été nommée ARS-Y-C-1. En utilisant un test court pour l'attractivité des larves, on a trouvé que l'ARS-Y-C-1 était moins attractive pour* V. jacobsoni, *car elle montrait des niveaux d'infection plus bas que les abeilles de Hastings (*A.m. carnica *du Canada) et de Louisiane. L'infestation d'un croisement entre ARS-Y-C-1 et les abeilles de Hastings fut modéré. Dans une expérience de terrain, les résultats ont montré que les trois types d'abeilles de Carniolan étaient plus tolérants à* V. jacobsini *que celles de Louisiane. Ces abeilles avaient une survie plus longue avec des niveaux plus élevés d'infestation de* Varroa *dans le cas du couvain d'ouvrières et de même chez les abeilles adultes. Les abeilles susceptibles de Louisiane étaient mortes plus tôt avec des niveaux plus bas d'infestation par* Varroa.

***Mots-clés** : Abeilles mellifères,* Apis mellifera, Varroa jacobsoni, *résistance, tolérance, index de tolérance, susceptibilité.*

Introduction

Varroa jacobsoni Oudemans is an indigenous parasite of the eastern honey bees (*Apis cerana* F.) and has become a major animal health problem for beekeeping with the western honey bees (*Apis mellifera* L.) almost worldwide. If not controlled by the beekeeper, most if not all colonies would be lost due to the mite's feeding activities (Ritter, 1988).

Infestation levels of *V. jacobsoni* vary due to several factors such as the genetic make-up of the bee host, genotype of *V. jacobsoni*, and the environment where they interact. Increased resistance to *Varroa* mites has already been observed in the Eastern and Africanized honey bees with some degree of resistance observed in the European honey bees (Moritz and Hänel, 1984; Moritz, 1985; Camazine, 1986; Peng *et al.*, 1987a,b; Kulinčević and Rinderer, 1988; Moritz and Mautz, 1990; Thrybom and Fries, 1991). According to Büchler (1994) several characteristics are involved in *Varroa* tolerance of honey bees. These characters include: non reproduction of female mites, hygienic behaviour, grooming behaviour, postcapping duration and larval attractiveness.

Non reproduction of foundress mites is probably considered the most important mechanism of resistance to *Varroa* mites. This characteristic has been observed in the Africanized, European and Eastern honey bees with varying ranges (Koeniger *et al.* 1981, 1983; Ritter and de Jong, 1984; Ruttner *et al.*, 1984; Camazine, 1986; Engels *et al.*, 1986; Anderson, 1994; Harbo and Hoopingarner, 1995).

In Uruguay, colonies of racial hybrids of *A. m. ligustica*, *A. m. iberica* and *A. m. carnica* maintained low levels of *Varroa* infestation due to the presence of high percentages of infertile females (60-90%) (Ruttner *et al.*, 1984). A total non reproduction of *V. jacobsoni* in *A. mellifera* colonies was observed by Anderson (1994) in Papua New Guinea and Indonesia. In a study conducted by Eguaras *et al.* (1994) in Argentina, all the *Varroa*-infested *A. mellifera* colonies survived infestations even without treatment. These colonies had at least 40% infertile female mites. Similar observation was reported by Papas (1992). Two of his *A. mellifera* colonies infested with *V. jacobsoni* for 8 years without treatments displayed about 63 and 67% non reproductive females. During the first four years, mite infestations in these two colonies remained below 10%. Tolerance to *Varroa* has also been reported in *A. m. intermissa* in Tunisia (Ritter *et al.*, 1990). Ritter found about 30-50% infertile females and thought their occurrence was one of the reasons for increased survival of the colonies. Discrepancies in non reproduction values may be due to differences in the time of observation. Otten and Fuchs (1990) found a seasonal variation. Mites stayed infertile in winter with an increase in fertility during March/April reaching a peak in May to August. Increases in non reproduction of female mites in springtime was also reported by Marcangeli *et al.* (1992) in Argentina. In Yugoslavia, a significant seasonal influence on the fertility of mites was also observed by Kulinčević *et al.* (1988) for brood samples from June to September. Different results were obtained in the following year.

When Africanized (AHB) and European honey bee (EHB) brood frames were introduced simultaneously into a *Varroa*-infested European honey bee colony, 51% of the infested brood of AHB showed no reproduction as compared to 21% in the European honey bee frame (Camazine, 1986). Similar results were obtained by Rosenkranz *et al.* (1988) having 51% and 17% non reproduction, respectively.

The causes of non reproduction of *Varroa* are unknown. However, when the trap-comb technique is employed, a significant increase in mite infertility was noted (Büchler, 1992). Fuchs (1992) assumed that the infertility was caused by differences in adult bees and not the brood. It seems that feeding on young bees is crucial in mite reproduction.

Non reproduction of *V. jacobsoni* in worker brood is very common in *A. cerana* thus, regulating *Varroa* infestations at very low levels (Koeniger *et al.*, 1981, 1983; Tewarson, 1987; de Jong, 1988; Rath and Drescher, 1990; Rath, 1991, 1993; Tewarson *et al.*, 1992; Rosenkranz *et al.*, 1993). However, de Jong (1988) observed that *V. jacobsoni* reproduced in worker brood in South Korea. In addition, effective hygienic and grooming behaviours of *A. cerana* to *Varroa* mites also played important roles.

Testing colonies of honey bees for hygienic behaviour may serve to predict their reaction to *Varroa* infestation (Spivak *et al.*, 1994). Removal of infested brood was first described by Peng *et al.* (1987a) in *A. cerana*, and later confirmed by Rath and Drescher (1990). Boecking and Drescher (1992) found a positive correlation between hygienic behaviour and infested brood removal. This behaviour is enhanced when colonies are fed sugar syrup (Boecking, 1992). When worker brood was artificially infested with *Varroa* mites from different sources, 75% of them were removed and of those left, 90% were infertile (Tewarson *et al.*, 1992). However, when the mites were collected from the same *A. cerana* colony, worker bees removed only 38% of artificially infested worker brood cells which was less than previously reported (Rosenkranz and Tewarson, 1992). Takeuchi (1993) introduced a comb of sealed *A. mellifera* worker brood having an infestation level of 68% into an *A. cerana japonica* colony. After four days, 65% of mites were on the bottom board and no mite was present after three months.

In *A. mellifera* colonies, this behaviour has also been observed. Boecking and Ritter (1993) found that *A. m. intermissa* removed up to 75% of artificially infested brood and removed up to 97-99% of freeze-killed brood in each of two trials. However, Africanized honey bees are found to be more efficient in grooming than European honey bees in Argentina. (Moretto *et al.*, 1993). Calculated heritabilities for the 20 Africanized was $h^2 = 0.71 \pm 0.41$.

Damaged *Varroa* mites by bees were first observed by Peng *et al.* (1987a,b). Chmielewski (1992) also described damage to dead mites collected from bottom boards during winter. Rath (1991) claimed that the grooming success of *A. cerana* workers in relation to *V. jacobsoni* was 62% during the first 48 hours and decreased to 18% after 48-96 hours. Büchler (1993) found that numbers of damaged mites that dropped naturally varied. Buchler observed about 10% in early March and 64% in mid June. Büchler *et al.* (1992) described grooming and catching mites. European bees were less effective groomers than *A. cerana*. Ruttner and Hänel (1992) selected 12 honey bee colonies from 700 European honey bee colonies. The selected colonies showed slower *Varroa* population growth, high mite mortality and good colony overwintering even without treatment. These colonies displayed high proportions of damaged mites ranging from 30% to 50%.

For several researchers (Moritz and Hänel, 1984; Ritter and de Jong, 1984, Büchler and Drescher, 1990; Schousboe, 1986) decreasing the average length of the capped brood stage is one of the factors of *Varroa* tolerance. Büchler and Drescher (1990) indicated that with a 1-hour reduction in the capped period of honey bees, there is a corresponding 8.7% decline in *Varroa* populations. Likewise, all immatures are believed to die after bee emergence. However, some deutonymphs were able to survive to adulthood when inoculated into newly capped larvae (de Guzman, 1994). Rosenkranz and Engels (1994) do not support the suggestion that the tolerance observed in Africanized bees is due to a shorter postcapping period than the European worker bees. This observation is based on experiments conducted in Brazil. Similar non supporting results for short postcapping are given by Wilde (1994).

According to Moritz (1994) hygienic behaviour, grooming behaviour and postcapping duration as tolerance mechanisms are under genetic control, however they are difficult to select for in a practical breeding programme.

To investigate intensity of *Varroa* infestation in different European strains of bees, Büchler (1990) started with the same number of mites under standardized conditions. Differences in rates of infestation up to seven fold were observed between honey bees of different origins after test periods of 1 to 1.5 years. When colonies are inoculated with the same number of 100 female mites, significant differences in susceptibility between races of European honey bees were also established (Büchler, 1992). Another method of evaluating tolerance is to test for brood attractiveness to *Varroa* mites. Büchler (1988) introduced combined brood sections from several different strains of bees and found that one *A. m. mellifera* colony was less attractive to *V. jacobsoni* than the other strains tested. Two strains of Buckfast bees exhibited greater attractiveness in this study.

In this review, we attempt to provide the most recent knowledge relating to *Varroa* resistance which may be a long term solution to mite problems. More detailed reviews on *Varroa* tolerance in honey bees are published by Büchler (1994), and Boecking and Ritter (1994).

Breeding honey bees for resistance and susceptibility to *Varroa* mites in Yugoslavia

When we started with the joint research programme in 1984, *V. jacobsoni* had been present in Yugoslavia for over a decade. It took some time for the parasite to be detected. At that time, as it is today, chemical treatments were the only means to save infested colonies.

Koeniger and Fuchs (1988) described both the histories of the development of chemical controls and the predictable long-term difficulties resulting from their frequent use. The expectation that *V. jacobsoni,* similar to other mites and insects, will rapidly develop acaricide resistance is the main problem with chemical treatments. The combinations of a constant use of one chemical and the fast reproduction of mites make acaricide resistance likely to develop quickly. Most recent reports from Italy (Accorti and Luti, 1994) are confirming this assumption.

Our five-year experimental selection of honey bees for resistance and susceptibility to *V. jacobsoni* lasted from 1984 to 1989. Two lines of honey bees were selectively bred through four generations. The resulting lines of bees were comparatively resistant and susceptible (Kulinčević *et al.*, 1992).

Throughout the experimental period we used honey bee colonies of *A. m. carnica* in standard Langstroth hives. All the colonies studied, within each year, were exposed to the same general environment and consequently could be expected to have been subject to random and relatively similar *Varroa* infestation rates.

The original population containing three egg laying queens, was assembled from three different bee yards, after severe winter losses presumably caused by *V. jacobsoni*. Those colonies that survived, from which we took the three queens, were thought to have some degree of resistance. From this base population, eleven open mated queens were produced and introduced into the full-sized colonies.

Evaluation of the parental colonies was based on the presence of reproducing mites during the active beekeeping season (Kulinčević and Rinderer, 1988; Kulinčević *et al.*, 1988). A wide variation of infestation which ranged from 3.8 to 32.5% served to select parents to produce a first selected generation of supposedly more resistant and more susceptible lines. Infestation rates of the resistant parent colonies averaged 5.6% and the susceptible colonies 14.8%.

The seasonal responses of the two lines in the first generation were significantly different ($P < 0.05$). As the season progressed, numbers of infested cells increased in both lines (Fig. 1). A similar trend was apparent in natural mortality of mites.

The second generation of both lines was not tested because of heavy winter losses. The surviving colonies were used to produce the third generation. Because of the winter losses, we decided to apply fluvalinate as an acaricide. Results of the third and the fourth generation indicated continued differences between the two selected lines of bees ($P < 0.004$ and $P < 0.044$). The infestation rates were lower because of fluvalinate treatments. More resistant colonies had less than half the numbers of mites than the susceptible line (Fig. 2, $P < 0.002$), nonetheless resistant colonies had less than half the numbers of mites which were counted in the susceptible line.

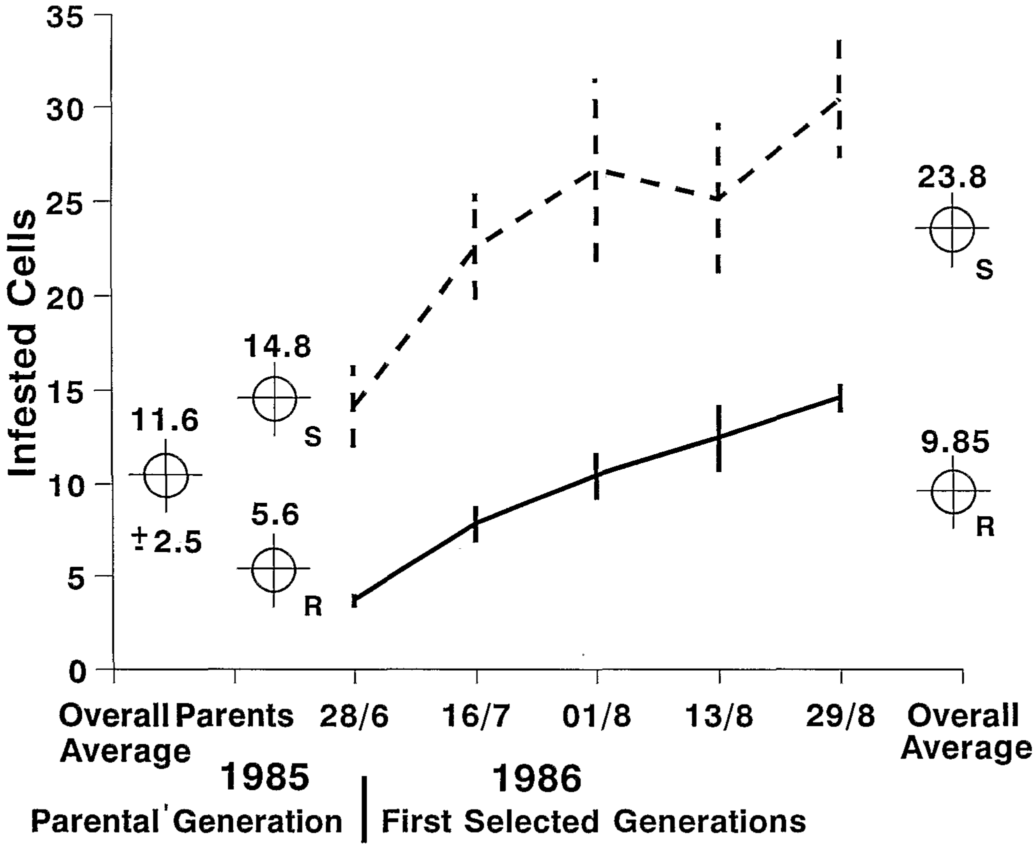

Fig. 1. The average infestation levels of *Varroa jacobsoni* in colonies of the parental and first selected generations in resistant (R and ———) and susceptible (S and ---------) lines.

Calculated heritabilities suggest that resistance and susceptibility in this breeding programme are of polygenic determination. We employed Moran's (1984) approach to calculate heritability ($h^2 = R/S \times 3/2$) which adjusts for the application of open mating in selection program. Using this approach the realized heritabilities are 0.3 for increased resistance and 4.2 for decreased resistance. Environmental differences between the 1985 and 1986 shifted all responses to higher levels and inflated the h^2 estimate of decreased resistance. The continuity of similar responses to *V. jacobsoni* in the third and fourth generation is further proof that variation is of a genetic nature that can be utilized in selective breeding.

In this investigation, we did not determine the precise nature of the mechanisms for increased tolerance. The measurement of percentage of brood cells infested was chosen because it was presumed to cover several possible mechanisms of resistance. Likewise, we did not compare the resistant and the susceptible line with a non-selected common stock of bees, because our goal was limited to finding out whether or not it was possible to do such breeding in beekeeping. We really wanted to split a population of bees and form two new statistically different populations.

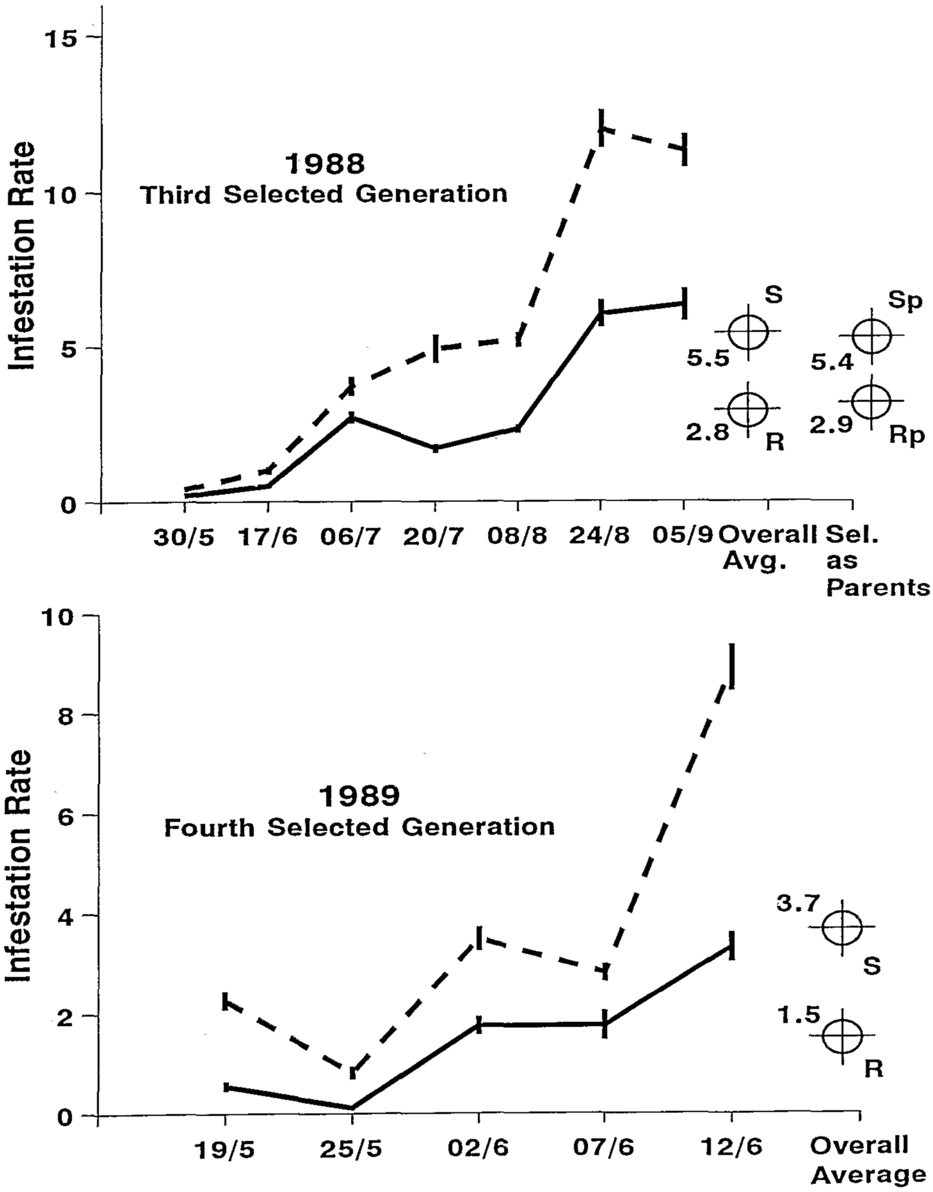

Fig. 2. The average infestation levels of *Varroa jacobsoni* in colonies of the third and fourth selected generations in resistant (R and ———) and susceptible (S and ---------) lines.

Testing of Yugoslavian honey bees in the United States

To investigate its degree of tolerance to *V. jacobsoni*, the Yugoslavian honey bee was brought into the United States in 1989 and later called ARS-Y-C-1. This importation was highly regulated (de Guzman *et al.*, 1990; Rinderer *et al.*, 1993).

Using both a short test and a field evaluation, the ARS-Y-C-1 was compared to three other stocks: Hastings strain of *A. m. carnica* (from Canada), F_1 hybrids between ARS-Y-C-1 and Hastings stocks, and a general Louisiana stock (Rinderer *et al.*, 1993; de Guzman *et al.*, 1995, de Guzman *et al.*, in press).

Since most damage to honey bees by *V. jacobsoni* occurs during the developmental stages of the host bee, reduced attractiveness of larvae is considered to be one of the factors in selecting stocks for mite resistance. So with our short test, we compared the larval attractiveness of these four stocks to *V. jacobsoni* using the grafting technique (de Guzman *et al.*, 1995). Newly hatched larvae of these stocks were grafted in to an area at the centre of a brood frame occupying 8 rows of 20 cells. After larval transfer, the brood frame was introduced into *Varroa* infested colony. After two weeks, we found that ARS-Y-C-1 had a significantly lower infestation level than Hastings and Louisiana brood (20% vs. 36% and 40%). Hybrids were intermediate having 29% *Varroa* infestation. This observation suggests that larvae of the ARS-Y-C-1 stock, which has been selected for resistance to *V. jacobsoni,* are less attractive to foundress mites than the larvae of the other stocks.

For the field experiment, resistance to *V. jacobsoni* was evaluated using several predictors, and one of them was monthly estimates of the percentage of brood cells infested with *Varroa* mites. A significant interaction between stock and sampling month was observed for the proportion of worker pupae infested in 1990 and 1991 but not in 1992 (Fig. 3). The growth of *Varroa* populations in our test colonies varied through time. Levels of *Varroa* infestation in all stocks were low from July 1990 to May 1991 with a growth trend emerging by November 1990. *Varroa* infestation in the Louisiana stock started to increase at this time with a significant increase observed in February. This increase in infestation coincided with the decrease in the amount of brood in the colonies. However, when brood rearing peaked in March and April 1991, the levels of *Varroa* infestation in worker brood decreased. This decrease in infestation was probably due to the presence of drone brood during these months. *V. jacobsoni* is known to prefer drone brood more than worker brood (Schulz, 1984; Fuchs, 1990). This phenomenon may have contributed to the sudden increase in the level of infestation in June 1991 observed for all stocks with more increase in the Louisiana, ARS-Y-C-1 and Hastings stocks. The F_1 hybrid colonies had the lowest infestation, with the mean infestation significantly lower than the means of Louisiana and ARS-Y-C-1 stocks but not significantly lower than the mean of the Hastings stock. A similar trend was observed in July 1991. During the autumn months (August to October 1991) comparably high levels of *Varroa* infestation were recorded in all the stocks. The last colony of the Louisiana stock died at the end of 1991. By March only a few colonies were still alive: one colony of Hastings stock, two colonies of ARS-Y-C-1, and three F_1 hybrid colonies. In April and May, Hastings and F_1 colonies developed numerically higher levels of infestation compared to ARS-Y-C-1. *Varroa* infestation of the Hastings stock continued to increase until all Hastings colonies perished. There were two ARS-Y-C-1 colonies and one F_1 hybrid colony surviving at the end of the experiment in June 1992.

The survival of the queens or colonies did not differ significantly among the four genotypes of honey bees (Log-rank χ^2 = 2.1; df = 3; P< 0.5). Hastings stock survived mite infestations for 10.90 ± 0.89 months, Louisiana stock 9.05 ± 0.94, F_1 hybrid colonies 9.46 ± 1.03 months and ARS-Y-C-1 11.04 ± 1.22 months. However, a highly significant association between the infestation of *Varroa* in the brood cells and queen or colony mortality (Log-rank χ^2 = 24.12; df = 1; P< 0.0001) was observed. This result suggests that further inspection of the relationship using the tolerance index may be useful. However, the tolerance index devised to fully explore our data is not a technique for selecting bees resistant to *Varroa*. Further studies should be done to determine its limitations.

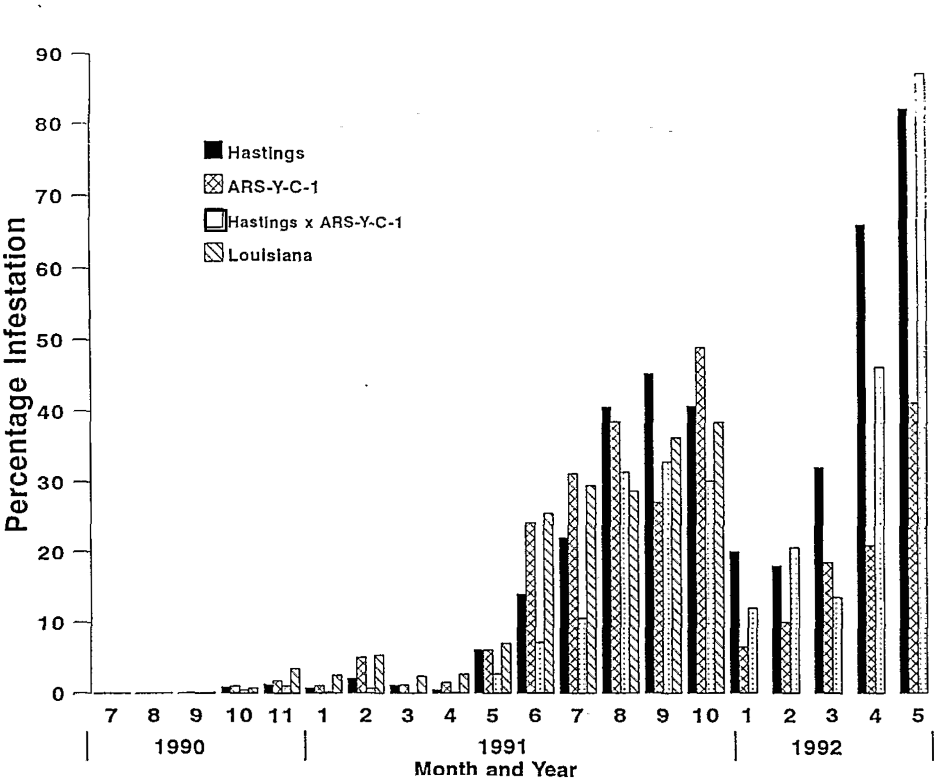

Fig. 3. Infestation levels of *Varroa jacobsoni* in four selected stocks of *Apis mellifera* through time.

A tolerance index value was derived for each colony by multiplying the rate of brood infestation during the colony's last month of survival times the number of months the colony survived. The Kruskal-Wallis χ^2 approximation analysis of the rank-sum scores showed significant differences among the four stocks (χ^2 = 8.98; df=3; P< 0.05) (Fig. 4). Hastings had the highest tolerance level to *Varroa* mites followed by F_1 hybrid and ARS-Y-C-1 stocks. Louisiana stock was more sensitive to *Varroa* infestation, and exhibited a lower tolerance index. We infer from this study that Louisiana stock is more susceptible to *Varroa* infestation due to their earlier death with lower levels of *Varroa* infestation in the brood cells than Hastings, ARS-Y-C-1 and F_1 hybrid colonies. This observation suggests that the Louisiana stock was less able to survive moderate levels of infestation. This finding underlines one chief conclusion of this study: resistance or tolerance as measured by a single variable has a limited chance to be expressed in field conditions as resistance or tolerance. A variety of interacting factors determine the expression of resistance or tolerance in field conditions. Stocks with traits that apparently would produce resistance such as the support of only low levels of infestation rates are not necessarily expressed in concert with other genetic and environmental conditions in ways that result in resistance in field conditions.

In the course of this investigation, we also discovered that ARS-Y-C-1 showed strong evidence of economic resistance to tracheal mites (Fig. 5) (Rinderer *et al.*, 1993; de Guzman, 1994). A similar observation was reported by Danka *et al.* (1994) and de Guzman *et al.* (in press). This observation suggests the possibility that the genes which produce tracheal mite resistance to ARS-Y-C-1 and their hybrids may have dominance at least in this cross and that the selection for resistance to *Varroa* increased the resistance to *Acarapis woodi.* It must be emphasized that tracheal mites were never detected in Yugoslavia where the selection and breeding of the ARS-Y-C-1 occurred. Because of the indications of tracheal mite resistance, and several other beekeeping characteristics of high qualities, USDA-ARS had released this stock in 1993.

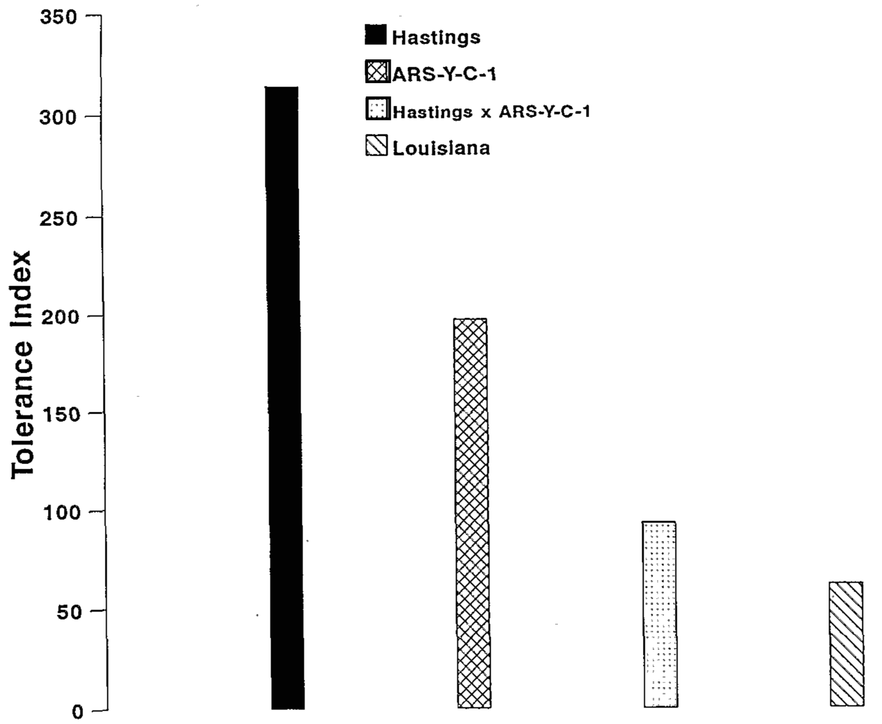

Fig. 4. Tolerance index scores evaluating the relative tolerance of four selected stocks of *Apis mellifera*.

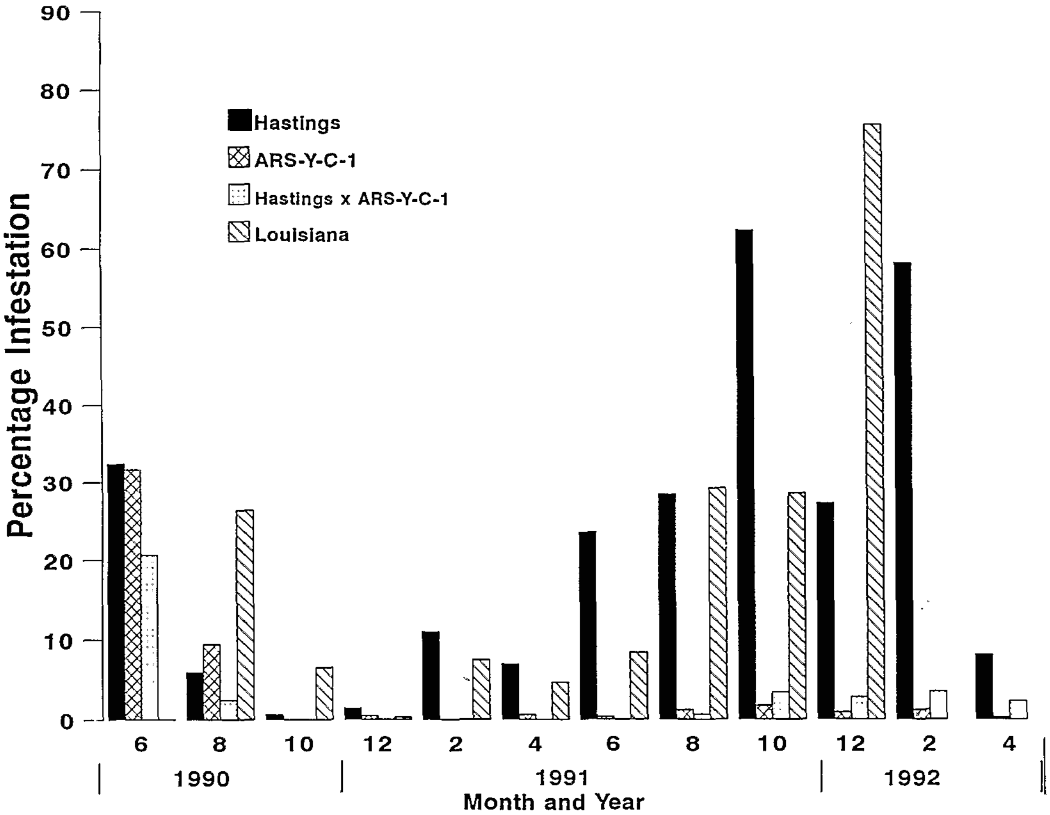

Fig. 5. Infestation levels of *Acarapis woodi* in four selected stocks of *Apis mellifera* through time.

Discussion

Research efforts to discover possible mechanisms of *Varroa* tolerance in western honey bees have been rather fruitful. The only problem is to find out the most efficient way to apply that knowledge in practical breeding programmes.

Selection for a single characteristic, if it is genetically determined, is much easier and faster than a complex breeding for several of the beekeeping industries. Important characteristics such as honey production, mite resistance, disease resistance, temper, and other desirable traits considered as a group are difficult challenges in breeding.

We agree that permanent progress in selection can only be achieved in large populations (Büchler, 1994). Hundreds if not thousands of honey bee colonies should be used. Possibly, such programmes are already taking place in Austria, Germany and the Czech Republic (Cobey, 1996). The most crucial problem in such large cooperative projects involving several breeders and breeding associations is how to secure uniformity of test conditions.

The breeding value of individual queens would be estimated on the bases of the test data for different characteristics. The genetic relationships must be considered between all colonies of the entire population before decisions are made on queen reproduction.

Bienefeld and Pritsch (1992) have taken a first approach in this direction. A large data set is a basic requirement for estimating heritabilities and genetic correlations with satisfactory precision (Büchler, 1994). This German approach might work with hobby beekeepers, however it is hard to believe that commercial beekeepers would be ready to become involved in a complicated, labour intensive and expensive programme of testing and data collecting. Even smaller beekeepers, for example in Yugoslavia, would not agree to do more than to estimate or to count the number of dead mites that dropped on the bottom boards after a chemical treatment.

The point of all these considerations is to define the simplest techniques in finding *Varroa* most tolerant colonies, or to use just a single test like the larval attractiveness described by de Guzman *et al.* (1995).

There is no doubt about the need for international cooperation in honey bee selection for *Varroa* tolerance and productivity. This must have broader financial support and to go across geographical and political borders.

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