Preliminary evaluation of four stocks of *Apis mellifera* on their tolerance to *Varroa jacobsoni*

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Introduction

Varroa jacobsoni Oud. is an ectoparasite of honey bees (*Apis mellifera* L.). It is regarded as a threat to world beekeeping because of the damage it inflicts on the brood and adult bees. In the United States, *V. jacobsoni* was first discovered in 1987 and no serious loss of bee colonies has yet been documented. However, Florida beekeepers believed that this parasitic mite was responsible for the mortality of several colonies during the 1991 season (Beaty per. com.).

In Europe and some parts of the U. S., chemical treatment is now routine in colony management. This approach to mite control is undesirable in the long term, since it may result in the accumulation of pesticide residues in honey and other hive products. In addition, the potential development of mites resistant to acaricides poses a long-term problem. Given these concerns, the development of honey bee stocks resistant to *V. jacobsoni* seems a more sustainable solution.

Four generations of divergent selection in a Yugoslavian *A. m. carnica* population resulted in two lines that varied in their levels of mite infection (Kulincevic *et al.*, in press). In the present study, daughters of the fourth generation of their selection were evaluated in order to determine if the level of resistance achieved exceeded that of the U. S. population and if it was sufficient to maintain commercial colonies without drug treatment.

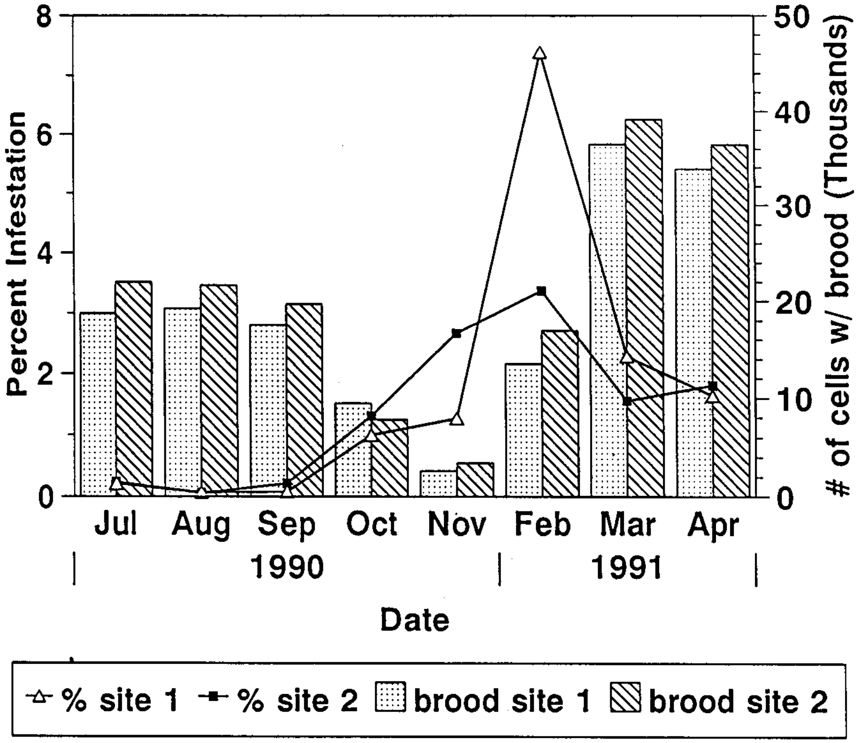

Figure 1. Percentage of brood cells infested with V. jacobsoni *and estimated number of cells occupied by immature bees regardless of bee genotype.*

Materials and Methods

The Yugoslavian stock (*A. m. carnica*) was compared to three stocks, namely: Hastings (*A. m. carnica*) from northern Saskatchewan, F_1 hybrids between Yugoslavian and Hastings stocks and a general Louisiana stock. About thirty experimental queens were reared from each stock and artificially inseminated with a mixture of semen (8 µl/queen). For the Yugoslavian stock, 15 Yugoslavian colonies were used as a sources of drones. Only one source colony was utilized for the insemination of Hastings queens. Drones for the Louisiana stock were collected using a wind-directed pheromone trap (Williams 1987) set at the Baton Rouge laboratory apiary.

The experiment was initially located in Deland, Florida in cooperation with

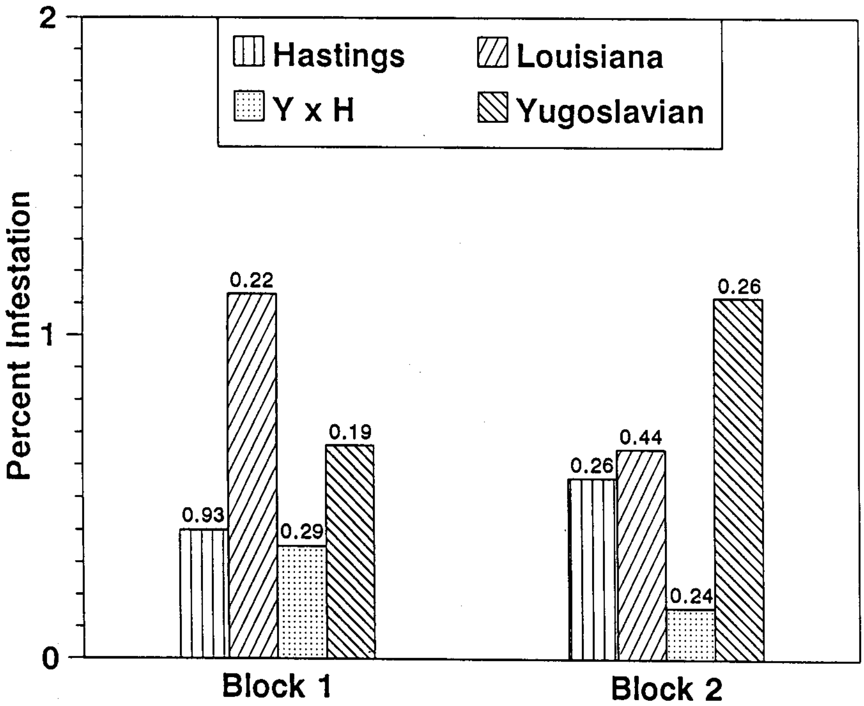

Figure 2. Levels of V. jacobsoni *infestation in the brood of four honey bee genotypes. Numbers above bars represent standard errors.*

the Bell's Honey Company. Test colonies were moved to Panama City, Florida in November 1990. Two apiary sites were used with 10 colonies/genotype/site. Nearly all colonies were initially infested with *V. jacobsoni* and tracheal mites, *Acarapis woodi* (Rennie). To suppress these mite infestations, fluvalinate fumigation (0.0024 g) and menthol (50 g) treatments were administered to all colonies before the experiment commenced. No subsequent treatments were employed after the initial chemotherapy.

All colonies were first inoculated at approximately 50 mites/colony in July 1990. Inoculation of mites was done using infested bees. The inoculum was prepared by making a large package of inoculum bees obtained from mixing worker bees from 3-5 infested colonies. The number of colonies mixed depended on the

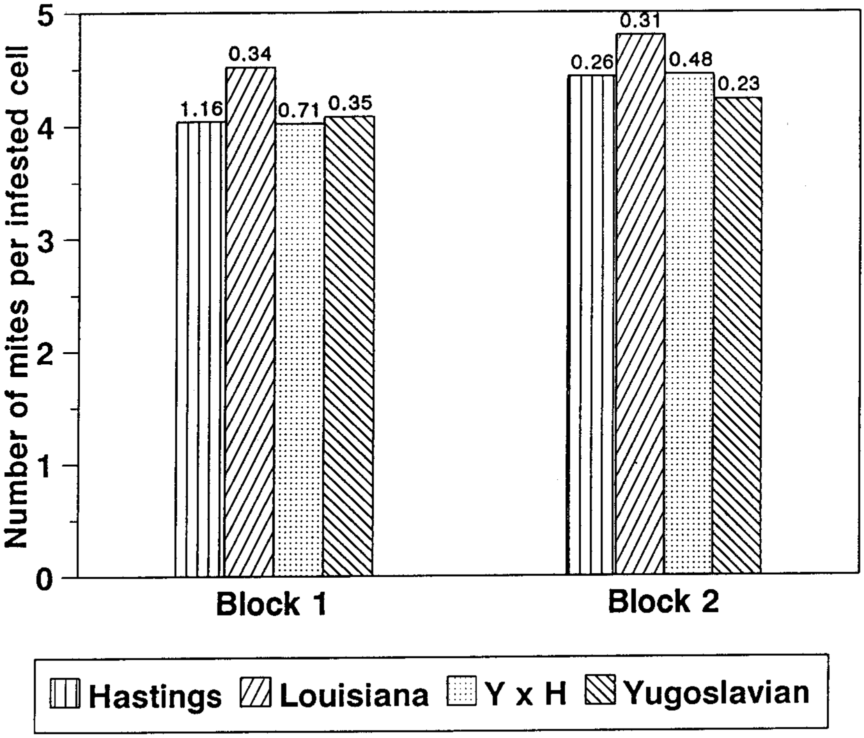

Figure 3. Number of varroa mites per infested cell in four honey bee genotypes. Numbers above bars represent standard errors.

levels of infestation of *V. jacobsoni* in the colonies. The large package of bees was sampled 5 times, one sample (approximately 50 bees/sample) in every corner of the box and one sample at the center, to get the mean infestation rate. The number of bees needed to harbor the required number of mites for inoculation was then calculated. About 300 bees were estimated to harbor the 50 mites needed. Bees in the large package were sprayed with water to prevent them from flying. Inoculum bees were placed in screen wire cages and introduced between two brood frames. All steps mentioned above were done inside a screen tent to avoid unnecessary introduction of mites into the colonies by lost or drifting bees. A second inoculation of 25 mites/colony was done during November 1990 using the same procedure.

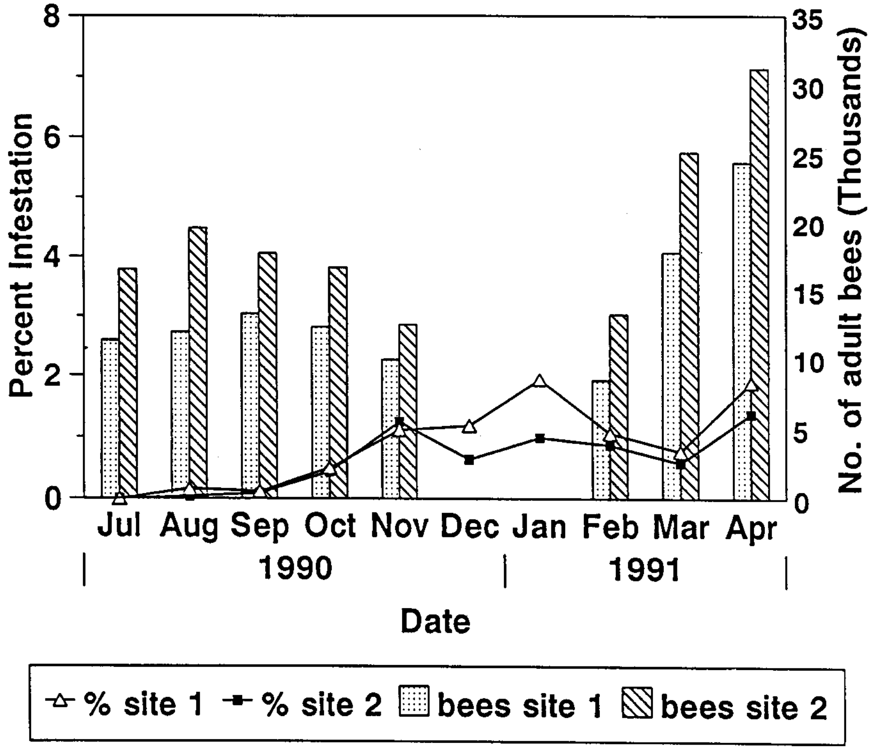

Figure 4. Levels of V. jacobsoni *infestation on adult bees and estimated number of adult bees regardless of bee genotype.*

Resistance to *V. jacobsoni* was evaluated on the basis of: 1) the percentage of brood cells infested with the mites (Kulincevic and Rinderer 1988), [Sampling was done every month (from July 1990 to present) by examining 100 worker cells containing dark-eyed pupae with yellowish abdomens (approximately 16-17 days old) (Ifantidis 1984)], 2) the number of mites (including progeny) per infested cell, 3) levels of infestation on adult bees, [This was determined by sampling at least 100 adult worker bees every month. Adult bees were collected into specimen jars from a brood frame preferably containing old larvae. Bees were then frozen and later washed with 70% ethyl alcohol], and 4) the number of mites collected on bottom board traps [Colonies were excluded from the whole experiment when superseded].

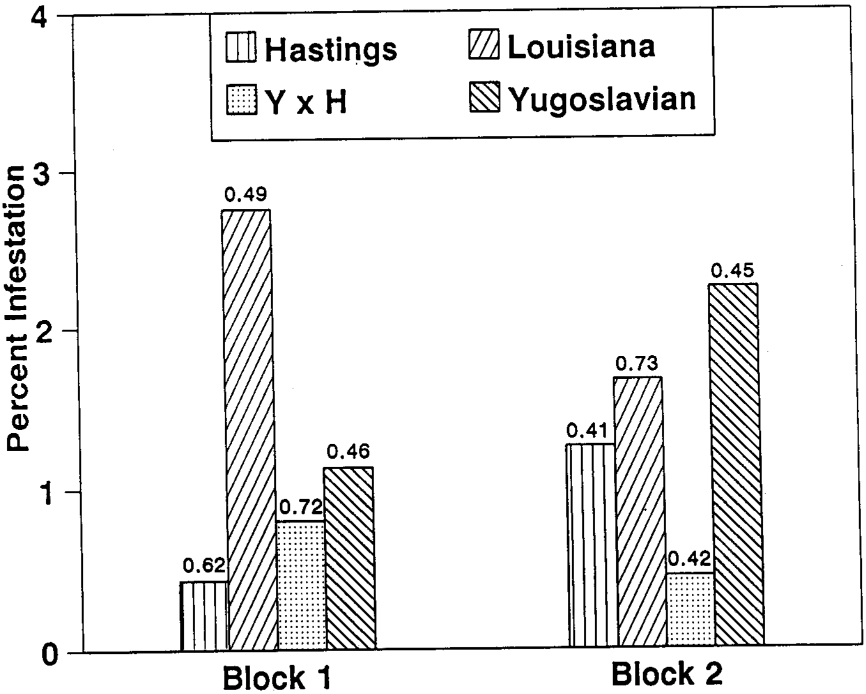

Figure 5. Levels of V. jacobsoni *infestation on adult bees of four genotypes. Numbers above bars represent standard errors.*

The amount of brood and the number of adult bees in the test colonies were estimated as described by Rogers *et al.* (1983) and Burgett and Burikam (1985), respectively.

This paper reports data from July 1990 to April 1991. Data were analyzed as a split plot in a randomized block design. Means were compared using multiple t-tests.

Results and Discussion

Infestation levels of V. jacobsoni *in the brood*

Although levels of infestation were generally low during the experimental period, they varied significantly (P> 0.0001) through time (Figure 1). The lowest

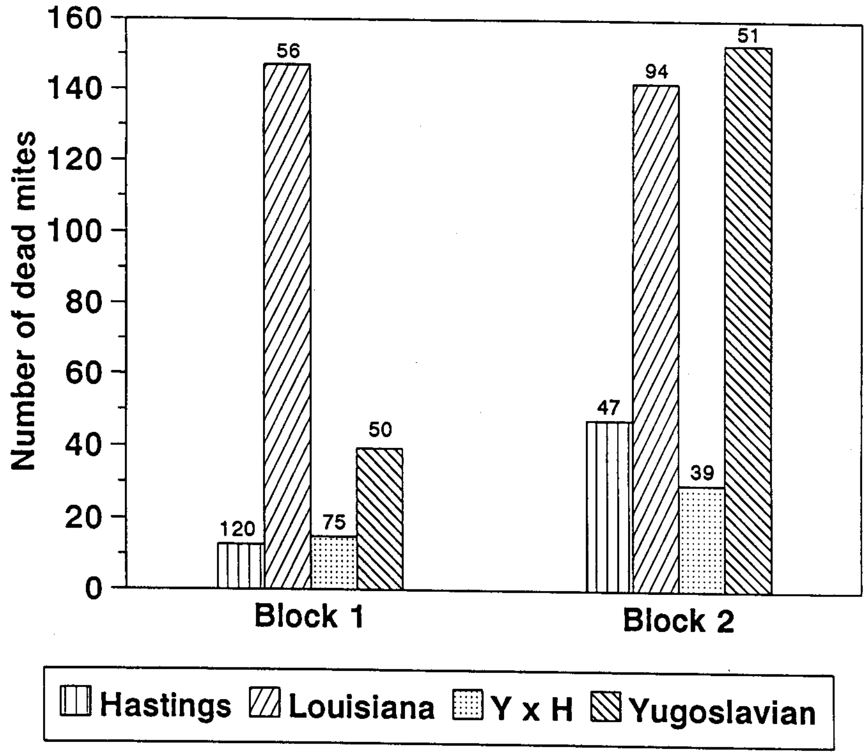

Figure 6. Natural mortality of V. jacobsoni *collected from bottom board traps. Numbers above bars represent standard errors.*

levels of infestation were observed during the first three months (July, August and September) of observations both for site 1 and site 2. By October, infestations started to increase with the highest infestations occuring in February. This increase coincided with a decrease in brood rearing (Figure 1). In February, which sees the onset of brood rearing in Florida, a large increase in the levels of infestation was observed, especially at site 1. During March and April, when brood rearing peaked, the rate of varroa infestation decreased. The reason for this fluctuation in mite infestation is unclear. Analysis showed that percent brood infestation was not correlated with the amount of brood present in the hives [r=0.02, P>0.83 (site 1); r=-0.09, P>0.49 (site 2)]. However, the decrease in varroa infestation during March-April was probably due to the presence of drone brood during these months. *V. jacobsoni* is known to prefer drone brood by a factor of 3.1-8.6 more than worker brood (Schulz 1984, Woyke 1987, Fuchs 1990). It is also possible that the proportion of uninfested brood increased during this period since the mite population does not increase at the same rate as brood rearing.

Bee genotype significantly influenced the levels of infestation in the brood at both sites (Figure 2). Louisiana stock had the highest infestation rate (P>0.009) among the four stocks used in site 1. Yugoslavian, F1 hybrids and Hastings stocks had lower infestation rates and were not significantly different from each other (P>0.76). At site 2, the highest infestation was observed in the Yugoslavian stock but not significantly different from Louisiana and Hastings stocks. Hybrid colonies had the lowest level of infestation but showed no significant differences from Hastings and Louisiana stocks.

Mite Load

The highest number of mites per infested cell for site 1 was observed in the Louisiana stock with the lowest mite load recorded in the hybrid colonies (Figure 3). For site 2, Louisiana also had the highest mite load per infested cell. No significant differences among the stocks were noted for either site. Whether or not this observation is an indication of similarity in the degree of attractiveness for varroa reproduction cannot be discerned from our available data. No significant variation in the number of mites per infested cell was observed through time.

Infestation levels of V. jacobsoni *on adult bees*

The levels of varroa infestation on adult bees of the four bee genotypes were also low throughout the experimental period (Figure 4). Lowest infestations were recorded during the first three months of examination. Rates of infestation increased in October with three peaks observed during November, January and April. November's increase in adult infestation may be due to the reduction of available brood for the mites. According to Schulz (1984) and Woyke (1987), *V. jacobsoni* are phoretic on adult bees as they seek new hosts. This mite species can survive outside brood cells for 10-13 days when suitable brood is not available. The increased infestation in January may be due to the scarcity of brood during this month. Some colonies had small patches of brood while others had none during this winter month. The cause of the peak of infestation in April is unclear. However, it is possible that a large population of mites had already been established at this time and thus, a high proportion of invading/phoretic mites was therefore present. Adult samples were usually collected from brood frames containing old larvae. Adult bee population was also not correlated with varroa parasitism on adult bees [r=0.02, P>0.76 (site 1); r=-0.02, P>0.90 (site 2)].

At site 1, Louisiana and Yugoslavian stocks had the highest rates of infestation on adult bees (Figure 5). The lowest infestations were observed on the Hastings and hybrid colonies. At site 2, Yugoslavian stock had the highest infestation rate, which was not significantly different from that of Louisiana and Hastings stocks. Hybrid colonies had the lowest level of infestation but it was comparable with the infestation rates of Louisiana and Hastings stocks. However, no significant differences among the stocks were observed at both sites.

Natural mortality of V. jacobsoni

For site 1, Louisiana stock had the highest number of dead mites collected from the bottom board traps (Figure 6). At site 2, Yugoslavian and Louisiana had the highest number of dead mites recovered. However, ANOVA revealed no significant differences among the stocks at either site. Whether or not Louisiana and Yugoslavian stocks possess increased grooming behavior, which may have caused the increased number of dead mites collected from these stocks, has yet to be determined. A separate experiment will be done to clarify this matter.

Overall, only the F_1 hybrids demonstrated considerable tolerance to *V. jacobsoni* based on the proportion of brood cells infested with mites. Reduction in varroa infestation in this genotype may reflect epistatic and additive resistance derived from Yugoslavian or Hastings stocks.

The decreased resistance displayed by the Yugoslavian stock may be a consequence of how some of the lines were selected (Kulincevic *et al.* 1992). There were 15 lines of this stock that were used as sources of queens and drones and some of these lines were selected by fluvalinate fumigation. Therefore, it is possible that the colonies will still do better when treated chemically. Further, this experiment was conducted in Florida, which has different environmental conditions than Yugoslavia where the stock was selected. Thus, it is possible that temperature and other important environmental factors may have influenced the ability of the mites to reproduce on the stock. Genotype by environment interaction for levels of brood infestation is suggested by the different levels of brood infestation measured on the same genotypes at different sites. If this is indeed true, then breeding may yet produce stocks of bees with economic levels of resistance to *V. jacobsoni.*

Acknowledgments

We thank Bell's Honey Company for donating honey bee colonies and bee equipment. We also thank Lorraine Beaman, Daniel Pursifull, James Pursifull, Anthony Stelzer and Dan Winfrey for their technical help.

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Abstract

Four genotypes of honey bees were evaluated for potential tolerance to *Varroa jacobsoni* in northern Florida, USA. Levels of varroa infestation in colonies of the four stocks were low throughout the experimental period. Louisiana, Yugoslavian and Hastings genotypes had the highest proportion of infested cells while the hybrid colonies (Yugoslavian x Hastings) maintained the lowest level of infestation. Brood and adult infestations increased in October as brood rearing declined. The highest brood infestation occured in February, corresponding with the onset of brood rearing. Levels of varroa infestation on adult bees had three peaks: during November when brood areas declined, in January when no or little brood was available and in April when the mite population was experimentally established. The lowest level of infestation was observed two months (August and September) after mite inoculation and during March-April when brood rearing peaked. The

number of mites per infested cell and level of infestation on adult bees were not influenced by bee genotype. Mite load ranged from 4-4.8 mites per infested cell. No significant differences in the number of dead mites collected from the bottom board traps of the four bee genotypes were observed.

Significant differences in levels of brood infestation were observed among the genotypes studied. However, the ranking of genotypes was influenced by site, indicating strong genotype by environment interactions.

Key Words:

Varroa jacobsoni, mite, tolerance, *Apis mellifera*, selection, resistance, U.S.A.

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