The Breeding, Importing, Testing and General Characteristics of Yugoslavian Honey Bees Bred for Resistance to Varroa jacobsoni

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The Agricultural Research Service (ARS) of the USDA has a long standing commitment to conduct research on solving the problems caused by *Varroa jacobsoni*. Prior to the discovery of *V. jacobsoni* in the United States, the ARS began to conduct research on *V. jacobsoni* outside of the U.S. using cooperative projects. One of the cooperative projects was established in 1983, between ARS and the "Agroekonomik" Institute in Belgrade, Yugoslavia. The goal of this project was to attempt to select a stock of bees which would be resistant or tolerant to *V. jacobsoni*. Research began on the project in 1984 with Dr. Jovan Kulincevic (Fig. 2) as the Yugoslavian principal investigator and Dr. Thomas Rinderer as the lead U.S. cooperating scientist.

Three years later, in September 1987, *V. jacobsoni* was discovered in the U.S. By that time it was clear that the Yugoslavian project had produced a stock of honey bees having at least a degree of comparatively increased resistance. Perhaps the increased resistance was general enough that it would be expressed in U.S. beekeeping conditions. Perhaps other characteristics of the stock were also desirable for use as a commercial stock.

In order to explore these possibilities, the Yugoslavian honey bee stock that was bred for resistance to *V. jacobsoni* was brought to the U.S. in July, 1989. This importation was highly regulated, with oversight provided by both the State of Louisiana Department of Agriculture and Forestry and the USDA Animal and Plant Health Inspection Service. For the first six months, the stock was maintained in a quarantine apiary on Grand Terre Island off the coast of Louisiana (Fig. 1). The island has a research laboratory operated by the State of Louisiana Department of Wildlife and Fisheries, and is approved by APHIS as a quarantine station for honey bees. During the first six months, queens of the quarantined stock produced full-sized colonies that were evaluated for general beekeeping characteristics and inspected regularly for diseases and parasites, including *V. jacobsoni*. Some colonies derived from U.S. queens were kept in the apiary as an additional check against the possible transmission of a disease or pest that would not be apparent in the imported stock. No colony displayed any behavior or other trait that was objectionable, and no colony had detectable disease or parasites. Based on these inspections, state and federal regulators allowed the stock to be moved from the quarantine island to the USDA-ARS Honey-Bee Breeding, Genetics and Physiology Laboratory in Baton Rouge, Louisiana.

Once at the laboratory, the stock was propagated and placed into an intensive field trial to determine if the level of resistance achieved in breeding the Yugoslavian stock exceeded levels found in U.S. stocks and whether or not the resistance was sufficient to maintain commercial colonies without chemical treatment. This report provides information concerning the original selection program in Yugoslavia and its results, the early results from the U.S. field trials, and an evaluation of the general characteristics of the stock.

SELECTION IN YUGOSLAVIA

Two lines of honey bees (*Apis mellifera carnica)* were selectively bred through four generations. The resulting lines of bees were comparatively resistant and susceptible, respectively, to *V. jacobsoni*. Average infestation rates illustrate the differences between the two lines (Fig. 3). A base population of colonies was assembled after winter causing widespread losses of honey bee colonies. *V. jacobsoni* infestations contributed to these losses and surviving colonies were thought to have a

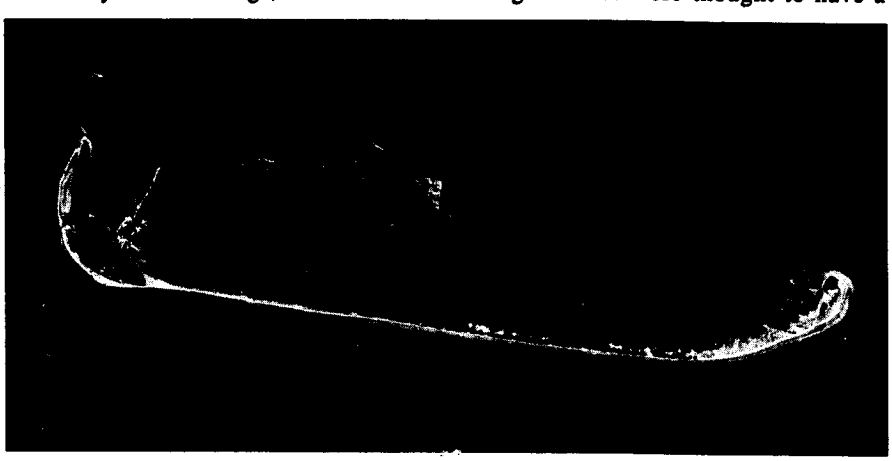

Figure 1. An aerial view of Grand Terre Island which contains the USDA Agricultural Research Service honey bee quarantine facilities.

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Figure 2. A photo of Dr. Jovan Kulencevic who developed the stock in Yugoslavia and Lillia de Guzman who evaluated the stock in the U.S. as part of her PhD work at Louisiana State University with Dr. T. Rinderer of the USDA-ARS Honey bee Genetics and Breeding Laboratory as her major advisor.

chance of having some degree of resistance. This base population was interbred to produce a parental generation of colonies. Evaluations of these parental generation colonies were founded on twice monthly examinations of the percentage of worker brood cells infested by reproducing mites during the active beekeeping season.

Parents selected from the parental generation were used to produce a first selected generation of resistant and susceptible lines. These lines differed strongly in infestation rates (Fig. 3). The second selected generation suffered severe winter mortality and was not tested. However, the survivors of both lines in the second generation were used to produce a third selected generation and, in turn, a fourth selected generation. The two lines of both the third and fourth selected generations had clearly different infestation rates (Fig. 3).

The degree to which the differences between parental and selected generations were governed by genes (heritability) was estimated. Although environmental differences between the parental and the first selected generations shifted all responses to higher levels, this effect still left a strong estimate for a genetic influence on increased resistance.

This research documents the development of lines of honey bees which are comparatively resistant and susceptible to *V. jacobsoni*. Two important conclusions are apparent. First, variation in response to *V. jacobsoni* has a genetic component. Second, much of this variation arises from genetic events which are responsive to classical selection programs. To determine to what degree the resistant stock has value for commercial application in the U.S. required that it be imported and evaluated in a U.S. commercial apiculture setting.

TESTING IN THE UNITED STATES

The Yugoslavian stock was compared to three other stocks: (1) Hastings strain of *A.m. carnica* to provide a control stock of similar subspecies origin, (2) F_1 hybrids between Yugoslavian and Hastings stocks to assess the potential combining ability of the imported stock, especially with other

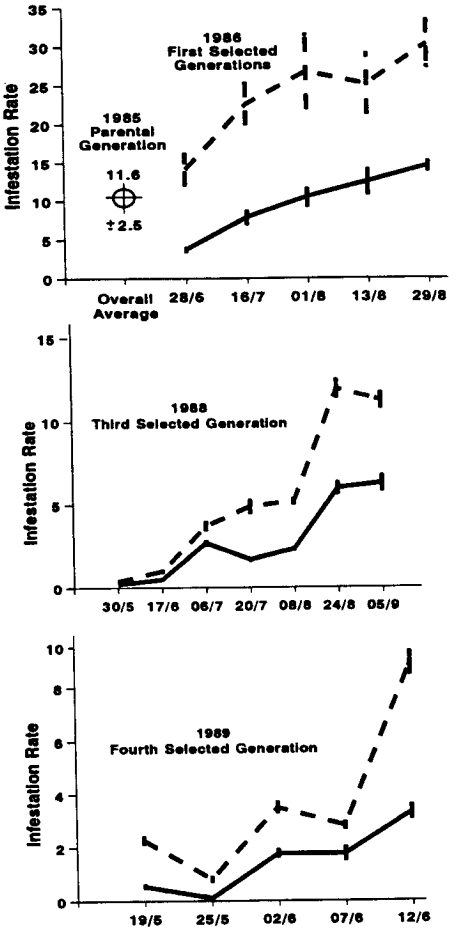

Figure 3. The average percentages (means ± standard errors of means) of cells infested with *Varroa jacobsoni* for the parental (1985) and first selected generations (1986) in colonies of resistant (——) and susceptible (-----) lines. For 1986, average infestation rates of the eight resistant and the eight susceptible colonies for 5 inspection dates are shown. The average percentages of cells infested in nine susceptible and ten resistant colonies of the third selected generation (1988) are shown as are the average percentages for the nine resistant and the eleven susceptible colonies of the fourth selected generations (1989).

carnica-type bees, and (3) a general beekeeping stock from Louisiana which represented the "U.S. Italian" bees common in commercial beekeeping.

The experiment was initiated in DeLand, Florida in June 1990 as part of the PhD dissertation work of Lillia de Guzman (Fig. 2). The work was done in cooperation with Bell's Honey Company. They provided equipment, bee feed, and 100 colonies. We requeened the colonies with 20 queens of each stock that had been instrumentally inseminated using colonies in two apiary sites. The remaining colonies were held in reserve to provide replacement colonies early in the experiment. In November, 1990, the experiment was relocated to the Florida panhandle.

Nearly all colonies were infested at the time of requeening with *V. jacobsoni* and tracheal mites, *Acarapis woodi*. Before queens were introduced and the colony populations were of the desired genotype, it was necessary to suppress the mite infestations to start the experiment as equally as possible for all the stocks. To achieve this, colonies were treated with both fluvalinate and menthol. Thereafter, no chemicals were applied. Having treated the colonies, it was then necessary to uniformly inoculate them with *V. jacobsoni* adults. This was done by adding infested bees from other colonies.

Resistance to *V. jacobsoni* was evaluated using monthly estimates of the percentage of brood cells infested with mites. This report is based on data collected from two replications of the experiment. The first replication was conducted from July 1990 to August 1992 and the second from June 1991 to September 1992.

Levels of infestation were generally low during the first experimental period but varied through time. The lowest levels were noted during the first three months (July, August and September) of observations. By October, infestations started to increase with the highest infestations occurring in February. This increase coincided with the decrease in brood rearing. In February, which sees the onset of brood rearing in Florida, a large increase in the levels of infestation was observed. During March and April when brood rearing peaked, the rates of infestation decreased. Analysis showed that the percentage of infestation was not correlated with the amount of brood. However, the decrease during spring was probably due to the presence of drone brood. *V. jacobsoni* is known to prefer drone brood to worker brood. 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 the growth of spring-time colonies.

Nonetheless, the proportion of brood cells infested strongly suggested the presence of resistance to *V. jacobsoni* among the bees in the experiment. In the first apiary, Louisiana stock had a significantly higher infestation rate while Yugoslavian,

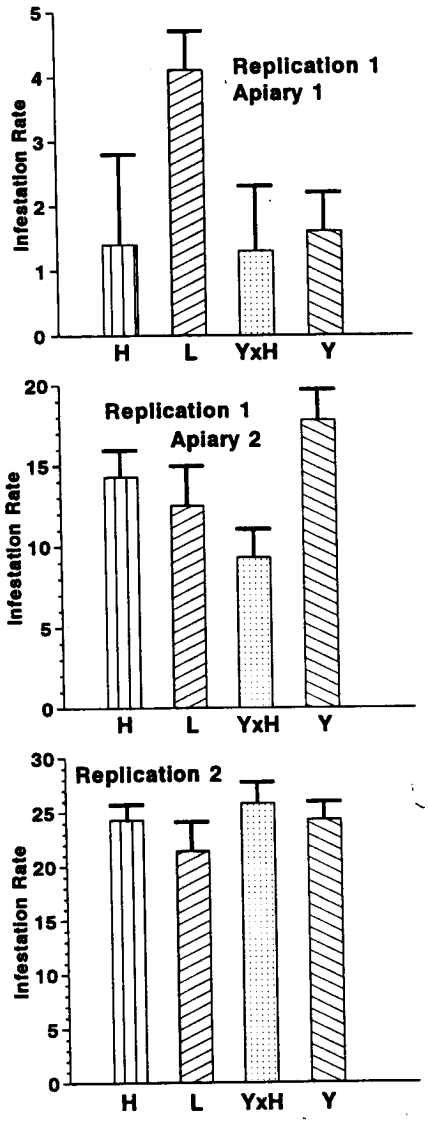

Figure 4. Average percentage (means ± standard errors of means) of Varroa jacobsoni infestation in the brood of four honey bee stocks for the two experimental apiaries of Replicate 1 (July 1990 to August 1992), and for the combined data from Replicate 2 (June 1991 to September 1992).

Hastings, and F_1 stocks had lower rates (Fig. 4). In apiary 2 of the first replication, hybrid colonies had an average infestation rate which was significantly lower than the average rate of colonies of the other stock bees (Fig. 4). In this apiary the Louisiana, Hastings and Yugoslavian stocks were not statistically different. The reduction in infestation observed in the F_1 stock may arise from hybrid vigor derived from epistatic and additive resistance present in Hastings or Yugoslavian stocks.

Colonies of all stocks died in the second replication of the experiment in just over one year, compared to the two years required for the death of colonies in the first replication. These accelerated increases resulted in much higher infestation rates overall and our procedures did not detect differences among stocks (Fig. 4), since the steep growth in rate of infestation may have required more frequent inspection of colonies to reveal differences.

GENERAL CHARACTERISTICS

Additional data were collected concerning the monthly numbers of mites (including offspring) per infested cell, the monthly levels of infestation on adult bees, the monthly number of mites collected on bottom board traps, the dates of death of experimental colonies or the dates when queens were superseded, the monthly proportions of bees having tracheal mites, the monthly amounts of brood, the monthly numbers of adult bees, and the seasonal honey production. Some of these results still are being analyzed for the second replication of the experiment and comments in this section regarding collected data are based on the first replication of the experiment. Observations were also made concerning colony temper, supersedure and swarming tendencies, occurrences of disease, wax working, propolis use, wintering ability, and spring development patterns.

Mite load

A strong trend suggested that the Louisiana stock had the highest number of mites per infested cell. Additional study is necessary to confirm this result.

Levels of infestation on adult bees

The levels of infestation on adult bees for all bee stocks were generally low throughout the first replication of the experiment. Lowest infestations were recorded during the first three months. Rates of infestation increased in October with peaks observed during winter and spring. The winter increase in adult infestation rates may be due to a reduction in numbers of brood cells. The spring increase may indicate that a large population of mites had already been established at this time which produced a substantial rise in the numbers of mites.

Natural mortality of V. jacobsoni

No trends or differences were found in rates of natural mortality of *V. jacobsoni*.

Mortality of queens

Yugoslavian queens lived numerically longer than the queens of the other stocks but the difference observed was weak.

Resistance to tracheal mites

The Yugoslavian bees showed strong evidence of economically valuable resistance to tracheal mites (Fig. 5). Data were collected on rates of tracheal mite infestations during the course of the first replication of the experiment. The Yugoslavian colonies and the hybrid colonies both had strong tendencies to remain tracheal mite free and had significantly fewer infested bees. Since the Hastings stock did not show any level of resistance beyond that of the Louisiana stock, the resistant performance of the hybrid colonies suggests that

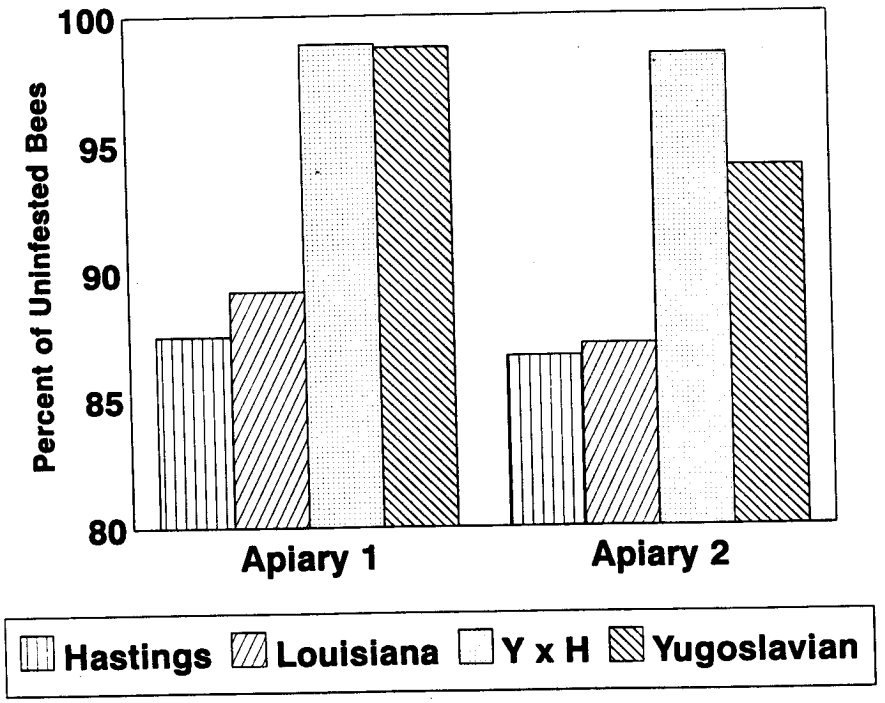

Figure 5. Percentage of honey bees which remained uninfested with tracheal mites from colonies of four honey bee stocks in two experimental blocks.

the genes which impart tracheal mite resistance to Yugoslavian honey bees and their hybrids may be dominant, at least in this specific cross.

Numbers of brood and adult worker bees

Yugoslavian bees were as good or better than the other stocks in respect to colony size. However, their brood nests developed later as is typical of *A.m. carnica*. In response to the presence of floral resources, the Yugoslavian colonies developed good populations of brood and adults.

Honey production

All stocks were similar in honey production.

Temper

The Yugoslavian colonies are very gentle. They remain calm on the comb during colony inspection, are easy to manipulate without excessive runniness.

Supersedure and Swarming

The Yugoslavian bees had only a weak tendency to swarm, and as evidenced by the good queen longevity, had very acceptable supersedure rates.

Disease

During the course of the experiment, four diseases were detected: chalkbrood, AFB, EFB, and purple brood, a problem caused by summer titi forage in the Florida panhandle. There was no indication that the Yugoslavian stock was either more resistant or susceptible to most of these problems than the other stocks in the trial. Although no precise measurements were made, the Yugoslavian stock did seem to show less of the purple brood syndrome than the Louisiana stock.

Wax working

The Yugoslavian bees draw foundation rapidly and evenly. Honey cappings are white.

Propolis

A.m. carnica stocks have a reputation for using so much propolis that hives become difficult to manipulate (personal observation, J.M. Kulencevic). This is not the case for Yugoslavian honey bees which use very little propolis.

Wintering ability

Unlike the Louisiana stock, the Yugoslavian colonies showed excellent temperate climate overwintering traits. They reduced brood rearing early and stopped brood rearing entirely during the winter. They reorganized the stores in the combs in Autumn by bringing honey lower to just above where clusters later formed. Even small clusters had excellent survival. Similar small clusters of all the other stocks died.

Spring development

Spring buildup for the Yugoslavian stock was very gradual. Resources seemed to be the primary trigger for brood rearing. These bees may be especially good where nectar flows are late in the season.

CONCLUSIONS

The indications of resistance to *V. jacobsoni* found in Yugoslavia were also apparent in the data from the U.S. study. In one instance the Carniolian stocks (Yugoslavian and Hastings) and their hybrids, and in a second instance, their hybrids showed tolerance to *V. jacobsoni* based on the proportion of brood cells infested with mites. Also, there was considerable evidence of stock by apiary interactions for several of the other measurements in the study such as numbers of dead mites on bottom board traps. These observations suggest that both genetics and environment are important aspects of resistance of tolerance to *V. jacobsoni*. It also suggests that careful continued breeding of the Carniolian stocks in this study may yet produce stocks of bees which will have economic levels of resistance to *V. jacobsoni*.

The Yugoslavian stock showed several beekeeping characteristics of high quality. Strong evidence of potentially economic levels or resistance to tracheal mites was found. In addition, the overwintering abilities, frugal use of stores, and gradual spring buildup are potentially very useful for beekeepers in areas experiencing harsh winters.

This information served as the basis for a decision to release the Yugoslavian stock to the beekeeping industry by ARS and the Stock Release Panel.

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