Evaluations of the Varroa-resistance of Honey Bees Imported from Far-Eastern Russia

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In an earlier report (ABJ 135: 11, 746-748) we described the initiation of a project to evaluate the potential for resistance to **Varroa jacobsoni** ***by honey bees from the Primorsky Territory on Russia's Pacific coast.*** **Apis mellifera** ***is not native to the area, but was first moved there in the last century. At that time, pioneers from western Russia took advantage of the completion of the Trans-Siberian Railway and moved honey bees from European western Russia to the Primorsky Territory in Asian far-eastern Russia. This far-eastern area of Russia is within the natural range of*** **Apis cerana,** ***the original host of*** **V. jacobsoni.** ***Thus,*** **A. mellifera** ***was brought into the likely range of*** **V. jacobsoni** ***even before the parasite was scientifically described in 1904. This probable long association of*** **V. jacobsoni** ***and*** **A. mellifera** ***in the region has engendered one of the best opportunities in the world for*** **A. mellifera** ***to develop genetic resistance to*** **V. jacobsoni.**

A natural history study (*ABJ* 137:11, 787-789) of Primorsky Territory honey bees showed that they had very low rates of *V. jacobsoni* infestation. Worker brood had only an average infestation of 7%, 15 months after the colonies were treated to reduce *V. jacobsoni* infestations below detectable levels. This contrasted to the results of a similar study in the United States where the worker brood in colonies reached an average of 33% infestation 12 months after miticide applications and began dying of symptoms collectively known as "parasitic mite syndrome". These and similar results for drone brood infestation rates and corresponding colony survival rates led to the importation of 100 queen honey bees from the Primorsky Territory into the United States (*ABJ* 137:11, 787-789).

The queens were brought to the USDA, ARS, Honey Bee Quarantine Station at Grand Terre Island, Louisiana on July 1, 1997 and installed into colonies prepared for them. The introduction and subsequent maintenance of colonies at the quarantine station were monitored by the USDA Animal and Plant Health Inspection Service (APHIS) and the Louisiana Department of Agriculture and Forestry. During the seven month quarantine, weekly inspections of the colonies were made by laboratory staff with regulating agencies receiving weekly reports of the condition of each colony. Early in February, 1998, the queens and their colonies were inspected by staff of the regulating agencies and found to be free of unusual diseases, parasites or behavior. Permission was granted to move the colonies to secure apiaries near the USDA, ARS, Honey Bee Breeding, Genetics and Physiology Laboratory in Baton Rouge, LA and for the unit's staff to begin research on the potential of the stock to be resistant to *V. jacobsoni.* The queens and their offspring are still under quarantine regulation. Various controls related to the security of the stock are required and, most importantly, distribution of the stock can only be made for the purpose of close-

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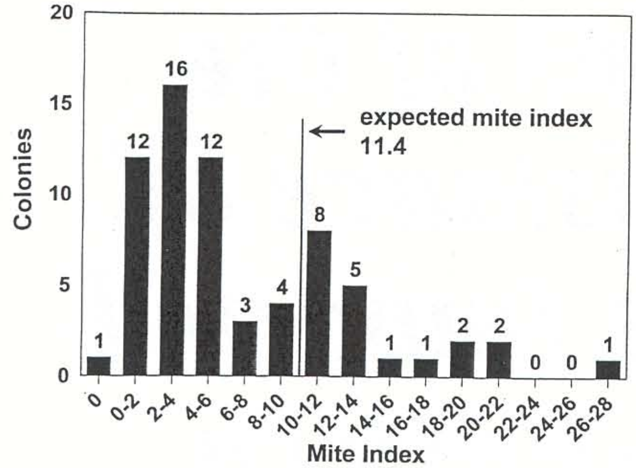

Fig. 1—The distribution of colonies produced by imported Russian queens according to their "mite index", the number of times a known population of mites grew during a nine-week field trial. "11.4" is the mite index calculated for a colony not having resistance to *Varroa jacobsoni.*

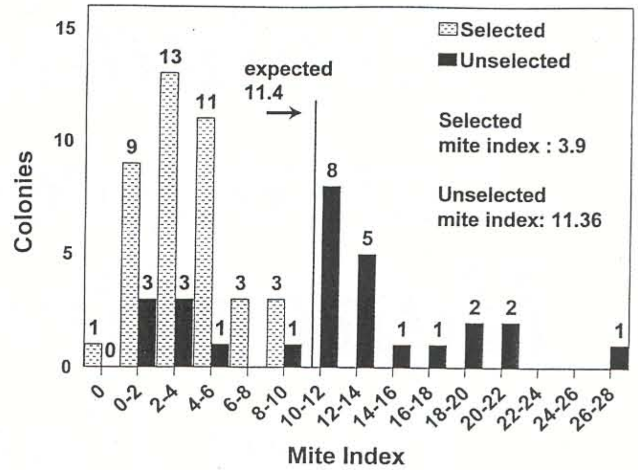

Fig. 2—The distribution according to "mite index" of colonies selected to continue a breeding program and colonies not selected for the breeding program.

ly supervised research concerning the commercial merits of the stock.

TESTING COLONIES PRODUCED BY THE IMPORTED QUEENS

The first study of the queens and their colonies concerned their relative resistance to *V. jacobsoni*. This study began with treating the colonies with Apistan® to reduce mite populations below detectable levels. During this same period, *V. jacobsoni* were being reared in domestic colonies to provide a source of inoculum mites in the test of the Russian colonies.

To begin the study, a bulk package of about 70 pounds of worker bees was taken from the domestic colonies in which *V. jacobsoni* mites were reared. Small packages averaging 1797 worker bees were taken from the bulk package, which contained an average of 1 mite per 6.9 bees.

The mite-infested bees were placed in small hardware cloth inoculation cages and inserted into the Russian colonies. The bottom board of each hive was fitted with a sticky board trap to collect mites that dropped from the colony. The inoculation cages and sticky board traps were removed from the hives after ten days. The bees in each cage were counted (average = 1797), as were the mites that remained in the cage (average = 4.03) and the mites that fell to the sticky board traps during the inoculation period (average = 69.87). These counts were used to estimate the total number of mites that successfully entered each colony from the inoculation cages (average = 187.8). (Number of infesting mites = Mites per bee in the bulk package times the number of bees in the specific package, minus the mites remaining in the package, minus the mites that fell to the trap).

After 9 weeks, the total number of adult mites in each colony was estimated from examinations of worker brood, drone brood, and adult workers. The total numbers of cells of sealed worker brood and sealed drone brood in each colony were estimated with the aid of a frame having a grid of one inch squares. Where drone cells were scattered, they were counted individually. Samples of 200 cells of sealed workers and 100 cells of sealed drones were opened and scored for the presence and numbers of adult female *V. jacobsoni*. Sampling was done on both sides of two combs (50 cells per side) for worker brood and both sides of a drone comb (50 cells per side for drone brood. The number of mites per worker cell was multiplied by the number of sealed worker cells to estimate the number of mites in the colony's worker brood. Similarly, the number of mites in the colony's drone brood was estimated. A sample of about 400 adult bees (workers and drones) was taken from the brood nest of each colony and washed in 70% alcohol to remove *V. jacobsoni* mites. Both the number of mites and the exact number of bees in the sample were counted. The number of mites per bee was multiplied by the estimated number of bees in the colony to estimate the number of mites present on the adult bees of the colony. The number of bees in the colony was estimated from estimating the number of square inches covered by bees on each frame of each colony with the aid of a 1-inch grid. Several such frame counts were followed by killing and counting all the bees on a frame to develop a standard relationship between square inches occupied and number of bees. The sum of the estimates of mites in worker brood, drone brood, and adult bees provided an estimate of the number of adult mites in the colony.

The population growth of mites in each colony was calculated by dividing the number of mites at the end of the trial by the number of mites that were introduced into the colony. The growth is termed "mite index" and expresses the number of times that the population has increased onefold. The expected growth of mite populations in the colonies if they were not resistant to *V. jacobsoni* was determined by using a mathematical model which considered the length of the trial, the proportion of mites that normally infest worker and drone brood (Fuchs, *Apidologie* (1990) 21, 193-199), and the normal reproductive rate of mites on worker and drone brood (Martin, *Journal of Apicultural Research* (1995) 34, 187-196). All colonies had ample numbers of developing drones, so the potential growth rates of mite populations was not constrained by a lack of drone brood.

The range of mite indexes for the Russian honey bee colonies was large (Fig. 1). Twenty of the colonies had indexes higher than the expected 11.4 fold mite population increase (mite index) for colonies that were not resistant. However, 48 colonies had mite indexes that were below the expected index of 11.4. Many of these colonies were well below this expectation for nonresistant bees, indicating that they did have resistance to *V. jacobsoni.*

Forty of the queens that were imported from Russia were chosen as "breeders" to found a stock of resistant honey bees and to produce offspring for further tests (Fig.2). While the test of the colonies produced by the imported queens was being

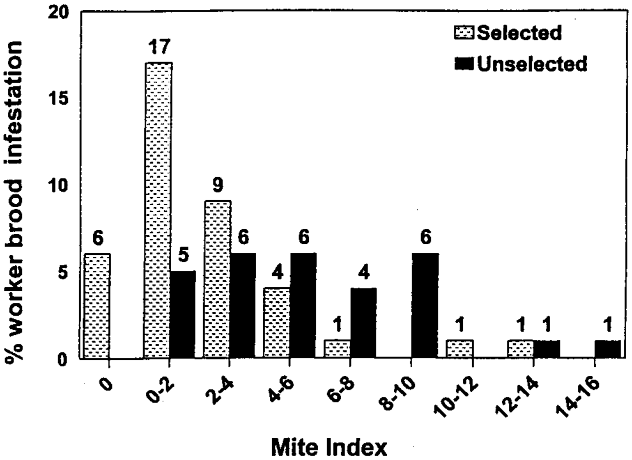

Fig. 3—The relationship between the "mite index" of colonies selected and not selected for continued breeding to the percentage of worker brood infestation at the end of the trial.

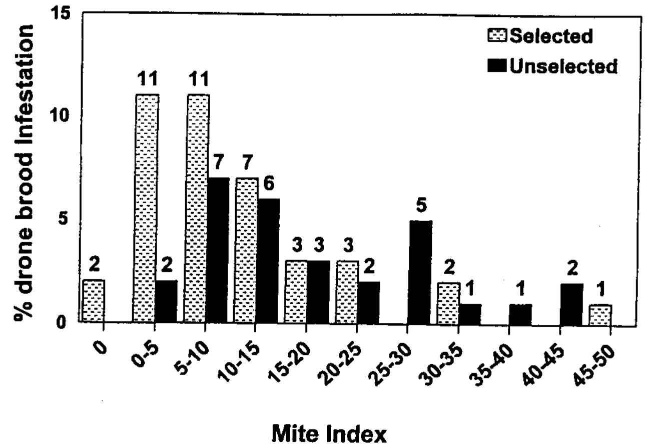

Fig. 4—The relationship between the "mite index" of colonies selected and not selected for continued breeding to the percentage of drone brood infestation at the end of the trial.

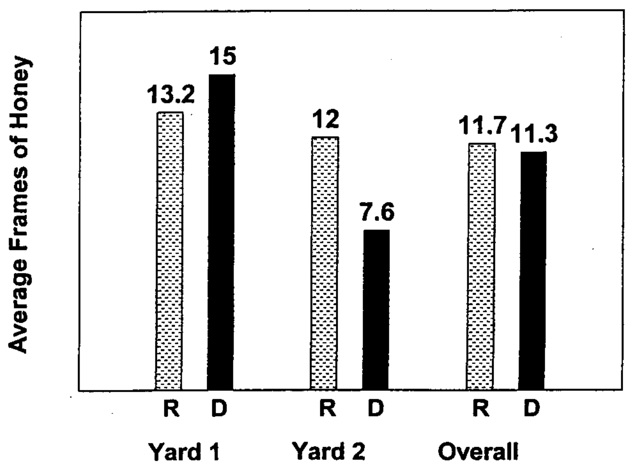

Fig. 5—The average frames of honey produced by Russian (R) colonies and domestic (D) colonies in two apiaries and the overall average production in both apiaries.

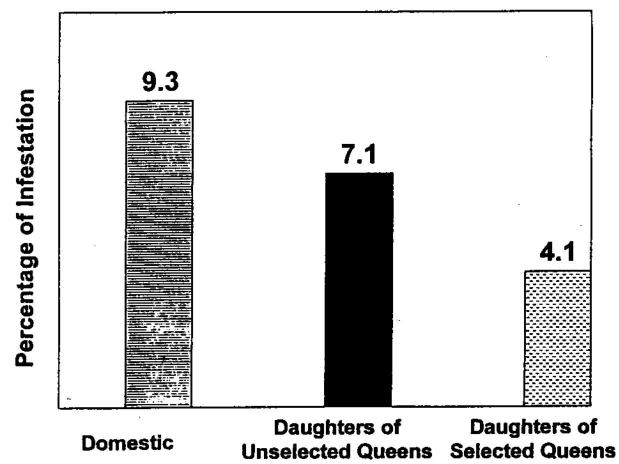

Fig. 6—The percentage of worker brood infestation for domestic colonies, colonies produced by daughters of imported Russian queens that were not selected for future breeding because of comparatively poor resistance to *Varroa jacobsoni*, and daughters of imported Russian queens that were selected for future breeding because of comparatively good resistance to *Varroa jacobsoni*.

conducted, general observations were made concerning the honey production, general vigor, and disease status of the colonies during weekly inspections. Although the mite index for each colony was an important factor determining whether or not a colony would be used in stock formation, these other factors also contributed. Hence, some colonies with a low mite index were not included because of the presence of chalk brood, comparatively poor honey production, or a general lack of colony quality. Likewise, a few colonies with a mite index of higher than 6 were selected because they showed comparatively good honey production, no evidence of disease, and displayed excellent general quality.

For the most part, selected colonies also had low percentages of worker infestation by *V. jacobsoni* (Fig. 3). This is one component of the mite index. However, two of the selected colonies had relatively high percentages of their worker brood infested. In these colonies, very few mites were found on adult worker bees and their drone brood was not heavily infested.

Likewise, selected colonies usually also had low infestation rates in their drone brood (Fig.4). However, in a few colonies the drone brood infection rates were much higher than they were for unselected colonies. In these cases, almost all of the mites in the colonies were found infesting drone brood.

The data suggested at least one mechanism of resistance that was expressed by Russian honey bees. In infestations of non-resistant honey bees about 65-75% of the mites in a colony are found infesting brood at any given time (Martin, *Journal of Apicultural Research* (1995) 34, 187-196). The colonies produced by Russian colonies which were chosen as "breeders" had an average of 48.1% of their infesting mites in brood cells. Colonies produced by Russian colonies which were not chosen as "breeders" had an average of 57.3% of their infesting mites in brood cells. Hence, overall, the Russian colonies had a reduced proportion of the infesting mites actively reproducing at any given time. Lower rates of brood infestation will lead to overall lower rates of population increase for mites.

AN EARLY PROGENY TEST

The first step after the colonies were moved from island quarantine was to propagate additional Russian queens. Daughter queens were raised from each surviving queen and naturally mated to a mixture of drones from all the imported queens. Mating was done on a coastal Louisiana island free of any other honey bees. This propagation was essential since propagation was not permitted during island quarantine and we were unsure of the age of the imported queens. Some of the daughters of this propagation were used in an early trial to evaluate the honey production of the Russian honey bees. This test was started March 1998 with packages during the main honey flow. Two apiaries each had 20 colonies headed by Russian queens and 10 colonies headed by domestic queens. The domestic queens were from a widely used commercial stock that is considered an excellent producer of honey by many beekeepers.

Honey production for the two types of honey bees was nearly identical (Fig. 5). In apiary one, domestic colonies produced somewhat more honey than did the Russian colonies. In apiary two, Russian colonies produced somewhat more honey than did the domestic colonies. Overall, Russian and domestic colonies had very similar average honey production.

Ten of the Russian queens in this test were daughters of queens that were eventually selected as breeders and some were daughters of queens that were eventually not selected as breeders. When honey production data were collected in the test, we also evaluated the percentage of worker brood cells that were infested by *V. jacobsoni* by examining 200 cells of sealed worker brood in each colony and determining if they were infested. Domestic colonies had the highest rates of infestation (Fig. 6). Daughters of unselected Russian queens had intermediate rates of infestation and daughters of selected Russian queens had the lowest rates of infestation. Of course, all of the Russian queens were mated to drones from both selected and unselected Russian queens. The difference between the mite infestation rates of daughters of unselected Russian queens and the mite infestation rates of selected Russian queens indicates that the resistance to *V. jacobsoni* found in the selected Russian queens is an inherited genetic trait.

Future research with the Russian stock will focus on large multi-state field trials. These field trials are intended to supply information concerning the comparative *V. jacobsoni* resistance and comparative honey production of the Russian stock in commercial beekeeping. Also, the field trials are designed to compare the relative merit of different Russian queens as queen-mothers for further stock breeding. Favorable results from these trials will lead to a general release of the stock to the beekeeping industry.

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