Multi-State Field Trials of ARS Russian Honey Bees

1. Responses to Varroa destructor *1999, 2000*

by T. E. RINDERER[1], L. I. DE GUZMAN[1], G. T. DELATTE[1], J. A. STELZER[1], J. L. WILLIAMS[1], L. D. BEAMAN[1], V. KUZNETSOV[2], M. BIGALK[3], S. J. BERNARD[4] and H. TUBBS[5]

SUMMARY

Field trials of Russian honey bees (ARS Primorsky stock) propagated as queen lines from queens imported from the far-eastern province of Primorsky were conducted in 1999 and 2000 in Iowa, Louisiana, and Mississippi. *Varroa destructor* populations in Primorsky colonies grew more slowly and hence, had fewer numbers than they did in domestic colonies. Colonies of six Primorsky queen-lines evaluated in 1999 averaged about half the number of mites found in domestic control colonies. In 2000, colonies of 10 Primorsky queen lines in Louisiana supported an average *V. destructor* population growth of 2.5 fold increase across 91 days, far less than the 17.3 fold increase predicted from growth models derived for domestic colonies. Most colonies of the same 10 Primorsky queen-lines in Iowa and Mississippi had no (150 colonies) to very few (48 colonies) detectable *V. destructor* three months after being inoculated with about 100 mites. Hence, in all trials, ARS Primorsky honey bees showed strong resistance to *V. destructor*. Variance within and between queen lines indicates good potential to further increase this resistance through selective breeding.

INTRODUCTION

Worldwide, the most serious biological problem in *Apis mellifera* apiculture is *Varroa destructor*, formerly *V. jacobsoni* (Anderson and Trueman, 2000). Without acaricide treatments repeated up to three times a year, colonies die. Feral and wild populations of *A. mellifera*, and the pollination afforded by them, have been virtually eliminated in North America and Europe by this mite. While some free-living honey bee colonies continue to be discovered (Kraus and Page 1995), they are probably founded by recent swarms from kept colonies that are protected by acaricides. *V. destructor* has now infested all the world's major *A. mellifera* populations except those of central Africa and Australia. Beekeepers use acaricides to maintain their colonies, but are troubled by mite populations that have developed resistance to acaricides and are burdened by increased production costs caused by this mite (Oldroyd, 1999).

One possible solution to the problems of *V. destructor* is the identification and use of resistant stocks of honey bees. The *A. mellifera* of the far-eastern Russian territory of Primorsky (Danka *et al.*, 1995) have been used to found a stock (ARS Primorsky) (Rinderer, *et al.* 1997, 1999) which has substantial resistance to *V. destructor* (Rinderer, *et al.* 2001).

We conducted field trials in order to further evaluate the resistance of Primorsky honey bees to *V. destructor* in a general way and to conduct specific progeny tests of queen lines that were previously identified as being among the best that were imported from Primorsky.

METHODS

Daughter queens of each Primorsky queen selected for progeny testing were produced using standard queen propagation procedures and mated to drones from the general pool of selected Primorsky colonies, according to a "round robin" inter-block design (Rinderer *et al.* 1999). Natural matings were facilitated on a Louisiana coastal island to assure that only drones from selected colonies mated with the queens. Daughters of six and ten queen lines were tested in 1999 and 2000, respectively. One Primorsky queen line, "Yellow 99" was used in both years and served as a control in 2000.

The trials were conducted in apiaries in Iowa, Louisiana, and Mississippi. In 1999, three apiaries were established near Cresco, Iowa, two apiaries were established near Henderson, Louisiana, and two were established near Webb, Mississippi. Forty-two Primorsky colonies and forty-two domestic colonies were studied in each state, with the colonies divided equally among the apiaries. Seven sister Primorsky queens from each of six Primorsky queen lines were evaluated in each state. Domestic colonies were of the commercial stock that has been traditionally used in each location. In Iowa and Louisiana, colonies were in hives on individual bottom boards. In Mississippi, colonies were in hives on "4- way" pallets. In Mississippi, both hives facing one direction on each pallet were the same stock to reduce effects of drift between side-by-side units. Otherwise, colony or pallet position in the apiaries was randomized.

In 2000, each state was represented by three apiaries. Prior studies provided ample evidence of the general comparative resistance to *V. destructor* by Primorsky honey bees. Hence, in order to accommodate desires to conduct progeny tests on more lines, each represented by more sister queens, only Primorsky queens were established in the apiaries. Each of 9 untested queen lines and the "Yellow 99" line was represented by 36 daughters, equally divided among states and apiaries. The "Yellow 99" served as a control, but also was subject to additional selection. In Louisiana and Iowa, where colonies were on individual bottom boards, the arrangement of queen lines within apiaries was random. In Mississippi, where colonies were on 4-way pallets, each queen line was randomly assigned to one pallet in each apiary in order to reduce the drift between colonies of different queen lines.

In both years, Iowa colonies were established as queen-right 5 frame nucleus colonies (standard deep frames) in Louisiana and transported to Iowa.

[1] **USDA-ARS Honey-Bee Breeding, Genetics & Physiology Laboratory, 1157 Ben Hur Road, Baton Rouge, Louisiana 70820-5502**

[2] **Institute of Biology and Pedology, Far East Branch of the Russian Academy of Sciences Vladivostok 690022, Russia**

[3] **Golden Ridge Honey Farms, 23226 20th Street, Cresco, IA 52136**

[4] **Bernard's Apiaries, Inc., P. O. Box 615, Beaux Bridge LA 70517-0615**

[5] **Tubbs' Apiaries, P.O, Box 3, Webb, Mississippi 38966**

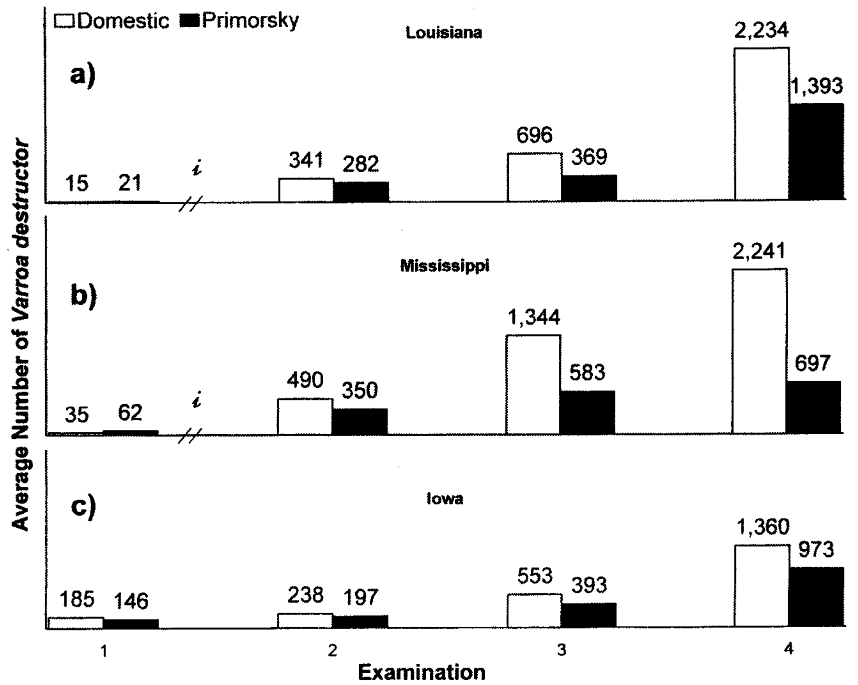

Figure 1. The average number of adult *V. destructor* mites in Primorsky (Black) and domestic (white) honey bee colonies at 4 examinations separated by 34 day intervals in three states in 1999. *i*, an inoculation of about 100 mites was given to colonies in Louisiana and Mississippi at the time indicated.

Louisiana colonies were established as queen-right five frame nucleus colonies on site during both years. In 1999, Mississippi colonies were established as queen-right five-frame nucleus colonies on site. In 2000, the Mississippi colonies were established as five-frame nucleus colonies in Louisiana and transported to Mississippi. In 1999, domestic colonies were divided to produce nucleus colonies for all locations. In 2000, Primorsky colonies were divided to produce nucleus colonies for Iowa and Mississippi, while domestic colonies were used for producing the Louisiana colonies. Subsequent management procedures differed between states. They comprised the procedures normally employed by the three cooperating beekeepers. However, both Primorsky and domestic colonies were given the same management in each state.

In 1999, baseline data from the Iowa colonies indicated that the colonies had an average of 165 *V. destructor* on April 14, 1999. Similar data from the Louisiana and Mississippi colonies indicated that almost none of the colonies had *V. destructor* above detection levels owing to earlier acaricide treatments. Consequently, adult *V. destructor* were added to each Louisiana and Mississippi colony on April 27, 1999 and May 6, 1999, respectively. Mites were added to the colonies by exposing a screened package containing adult bees infested with about 100 adult *V. destructor* to each colony. As the bees in the package died, the mites moved into the colony. These inoculations were done about the time Primorsky and domestic queens were introduced into the hives, which contained both adult bees and brood that were of domestic stock.

From April 14, 1999 to August 9, 1999, each apiary was inspected 4 times at intervals of 34 days. During inspections, each colony was evaluated for both its size and for the occurrence and distribution of *V. destructor*. These evaluations included: counts of mites in 200 worker brood cells (50 from each side of two combs), counts of mites in 100 drone brood cells (50 from each side of one comb), counts of mites on 300 to 600 adult bees washed in ethanol to remove mites, and comb by comb estimates (to the nearest 5%) of the number of worker and drone brood cells in the nests (sealed and potentially infested) and the number of adult bees (to the nearest 5%) comprising the colonies. These evaluations were used to estimate the number of adult female *V. destructor* in each colony. These estimates were used for general comparisons of Primorsky and domestic colonies of honey bees and for comparisons among the daughters of specific queen lines of Primorsky honey bees.

In 2000, baseline data from the Louisiana colonies indicated that they averaged 1715± 137 (mean ± standard error) *V. destructor* on May 10. Similar examinations of the Iowa and Mississippi colonies indicated that none of them had detectable numbers of *V. destructor*. Consequently, adult *V. destructor* were added to each Iowa and Mississippi colony on May 16, 2000 and May 3, 2000, respectively. Mites were added to the colonies by exposing a screened package containing adult bees infested with about 100 adult *V. destructor* to the colony. These inoculations were done about the time the Primorsky queens were introduced into the

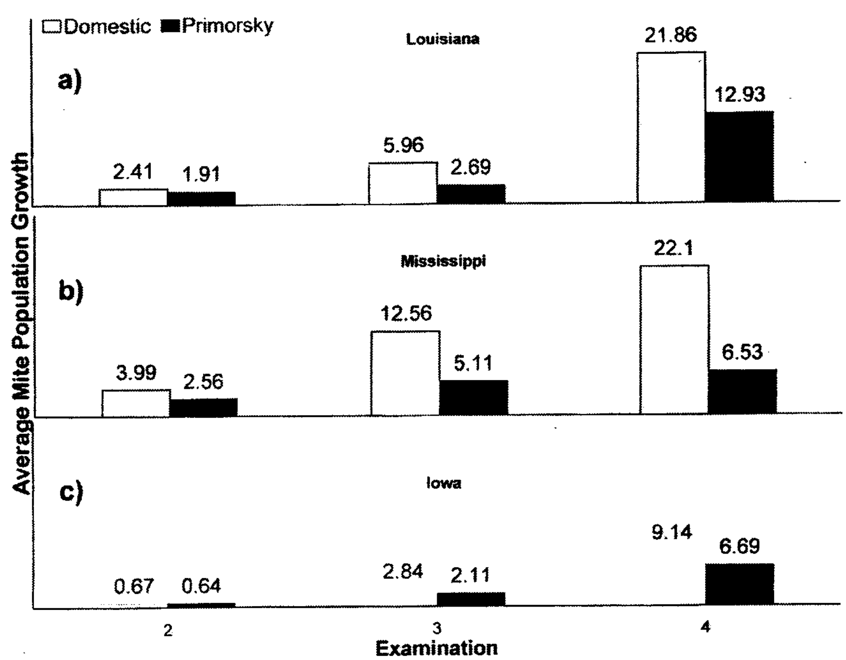

Figure 2. The average mite population growth (MPG) expressed as fold increase in *V. destructor* mite populations in Primorsky (Black) and domestic (white) honey bee colonies at 4 examinations separated by 34 day intervals in three states in 1999. Period 1 (not shown) provided base-line data.

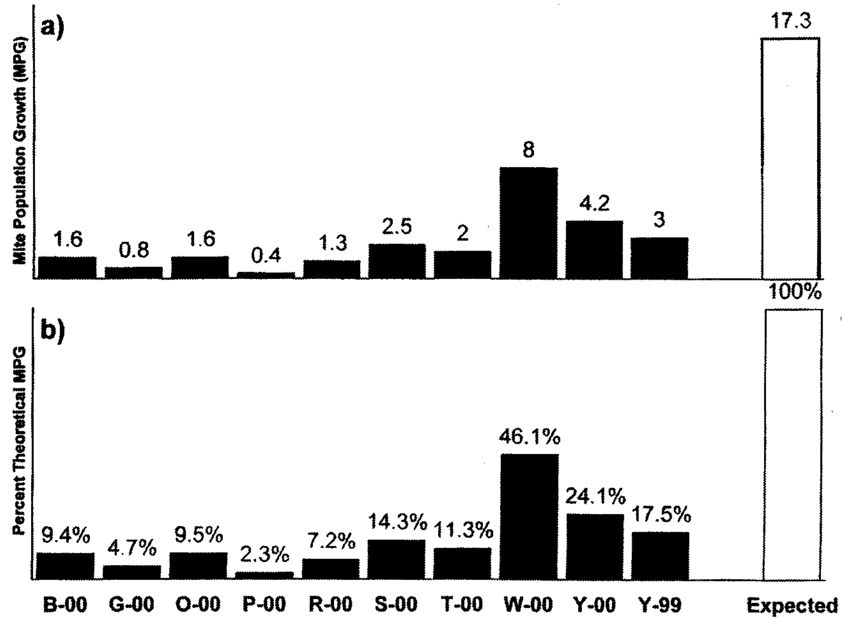

Figure 3. (a) The average mite population growth expressed as fold increase in *V. destructor* mite populations in six Primorsky queen lines and domestic control colonies, and (b) these values expressed as a percentage of the increase in *V. destructor* mite populations in domestic control colonies for trials conducted in 1999. B = blue, W = white, P = purple, G = green, Y = yellow, R = red.

hives, which contained both adult bees and brood that were of Primorsky stock.

In 2000, determinations of mite population growth (MPG) rates were made by comparing the numbers of mites in or added to colonies at the beginning of the trials to the number of mites found at the usual time of honey harvest for each location. (Mite Population Growth = (number of mites at end of trial - number of mites at beginning of trial) / number of mites at beginning of trial). The periods available for mite population growth were 91 days (Louisiana), 96 days (Iowa) and 111 days (Mississippi). Mites in each colony were estimated in the same ways as they were estimated in 1999.

RESULTS AND DISCUSSION

General 1999 Results

Overall, mite populations grew substantially less in Primorsky colonies than they did in domestic colonies. In 1999, mite populations grew during the experimental period in every apiary and state. (Fig. 1). In each case, the MPG was greater in domestic colonies than in Primorsky colonies. At the start of the experiment, average infestations were similar in Primorsky and domestic colonies (Fig. 1a, 1b, 1c; period 1) (*P* of differences = 0.31). Thereafter, the average numbers of mites in domestic colonies increased at a greater rate. At the last inspection, about 100 days after the beginning of the experiment, domestic colonies harbored about twice the number of mites found in Primorsky colonies (Figure 1a, 1b, 1c; period 4) (*P* = 0.00).

Overall, as well as state by state, MPG was less in Primorsky colonies (Fig. 2) (*P* = 0.00). Also, comparisons of MPG suggest that different beekeeping environments may enhance or impair MPG. In Iowa, where MPG is least in both Primorsky and domestic colonies, the difference between MPG in Primorsky and domestic colonies is also least (Figure 2a, 2b, 2c). In Louisiana and Mississippi, where the overall MPGs were larger, differences between MPGs in Primorsky and domestic colonies were greater (Figure 2a, 2b).

General 2000 Results

Studies in 2000 produced similar results. In Louisiana, the Primorsky queen line evaluations had colonies which started the trial with an average of 1715 mites. This occurred since domestic colonies infested with *V. destructor* were used as sources for nucleus colonies receiving Primorsky queens. The time between making the splits and the honey flow was too short to permit abating the mites chemically and then inoculating the colonies with a standard number of mites, so the evaluations were conducted with more than the usual 100 adult mites.

Because the colonies started with varied numbers of mites, we calculated MPGs for each colony in order to compare lines. The overall average MPG of Primorsky colonies in Louisiana was 2.5 fold, considerably smaller than the 1999 Primorsky colony average of 9.84 fold. Either the Primorsky queen lines evaluated in 2000 were more resistant to *V. destructor* than the queen lines evaluated in 1999 or environmental differences between the two years in Louisiana caused a comparative reduction in MPGs in 2000. One possible cause of the difference is the lack of large numbers of domestic colonies with large numbers of mites in the apiaries in 2000. This would reduce the invasion pressure of mites from outside sources which tends to inflate MPGs. The queen lines evaluated in 2000 may have initially been more resistant, the additional year of selection may have improved their degree of resistance, or some environmental factor may have enhanced the effects of genetic resistance in 2000.

Results in 2000 from Mississippi and Iowa provided further evidence that Primorsky honey bees resist *V. destructor*. Most of the colonies in Iowa and Mississippi had no (150 colonies) to very few (48 colonies) detectable *V. destructor* about 100 days after being inoculated with about 100 mites. Only two colonies had enough mites to permit an accurate estimation of their population size. These colonies were side-by-side on the same pallet. Some unknown environmental event such as an invading swarm might have caused these colonies to be outliers.

Since the mite inoculation technique was effective for introducing *V. destructor* to field colonies in 1999 and in other experiments, we examined our use of the technique in 2000 for differences from prior successful uses of the technique. There were two notable differences. First, prior to being infested with mites, the colonies had been treated with coumaphos. Two weeks or more intervened between removal of chemical treatments and the insertion of inoculation packets of infested bees. Nonetheless, it may be that residual coumaphos killed the mites in the inoculum packages. Second, in the past we have inoculated domestic colonies at about the same time that they were given a Primorsky queen. In 2000, we inoculated Primorsky colonies. It may be that Primorsky worker bees are more able to detect mites with foreign odors and remove them from the hive.

Queen Line Comparisons

We also made comparisons between Primorsky queen lines based on MPGs. All of the six Primorsky queen lines evaluated in 1999 had average MPGs that fell below the observed MPG for susceptible colonies (Figure 3a), while the domestic control colonies had an average MPG (18.4), which was very similar to the theoretically expected MPG (19.9) (Fig 3). The six queen lines had MPGs which averaged 9.5 fold (Figure 3a) and ranged from 5.1 to 13.5 fold. All of these MPGs compare favorably to both the observed MPG of 18.4 (statistically lower, *P* = 0.00) and the-

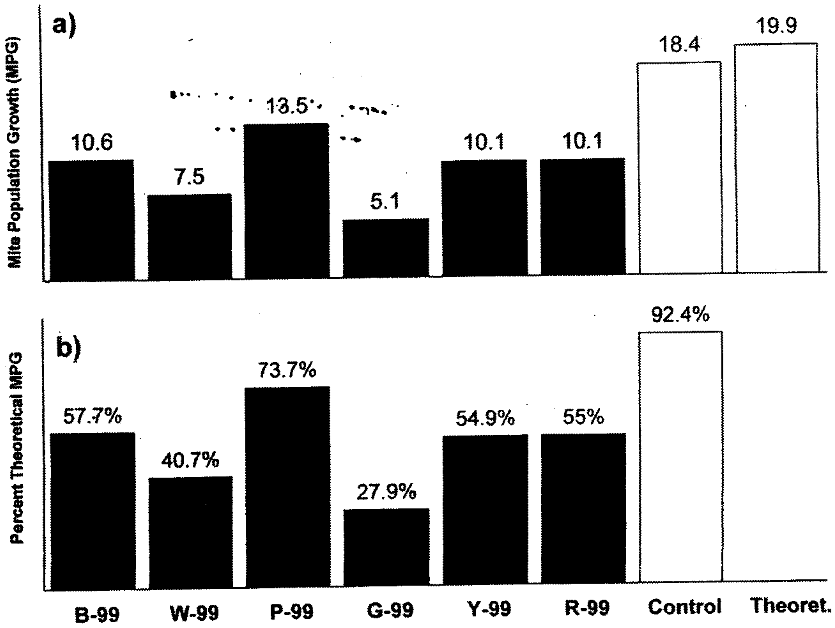

Figure 4. (a) The average mite population growth (MPG) expressed as fold increase in *V. destructor* mite in ten Primorsky queen lines and expected MPG for domestic colonies, and (b) these values expressed as a percentage of the expected increase in *V. destructor* mite populations in domestic colonies for trials conducted in Louisiana in 2000. B = blue, W = white, P = purple, G = green, Y = yellow, R = red, S= silver, T= tan, O = orange.

oretical MPG of 19.9 for susceptible colonies, indicating that all of them had resistance to *V. destructor* and also varied in their levels of resistance.

The 10 queen lines evaluated in 2000 showed similar patterns (Figure 4a, 4b). The 10 queen lines allowed MPGs which averaged 2.5 fold (Fig. 4a) and ranged from 2.3% to 46.1% (Fig. 4b) of the expected MPG for susceptible colonies.

Every ARS Primorsky queen line tested was more resistant to *V. destructor* than the commercial control stocks. Hence, the ARS Primorsky queen lines reported on here which have been excluded from the breeding and selection program because of their relatively poor resistance to *V. destructor* are only considered poor in comparison to more highly resistant ARS Primorsky queen lines. The queen lines retained in the program still show variation in resistance to *V. destructor,* suggesting that future selective breeding will further enhance the resistance in the overall stock.

CONCLUSIONS

A strong degree of resistance to *V. destructor* was observed for ARS Primorsky honey bees in both 1999 and 2000.

Although the expression of resistance to *V. destructor* varied between locations and is probably dependent to some degree on environmental factors, it can be expected to occur in a wide variety of beekeeping settings.

In order to assure that the study was completed in one beekeeping season, mites were added or not suppressed at the beginning of the experiments. Hence, these evaluations cannot provide information regarding treatment schedules to control *V. destructor.*

Overall, the variation between the ARS Primorsky queen lines retained for a breeding and selection program suggests that future selective breeding will further improve the resistance in the overall ARS Primorsky stock.

ACKNOWLEDGMENTS

We thank Rachel Watts and Warren Kelly for their very able assistance in data collection.

REFERENCES

Anderson, D. L., & Trueman, J. W. H., 2000. *Varroa jacobsoni* (Acari: Varroidae) is more than one species. *Exper. & Applied Acar.* 24: 165-189.

Danka, R.G., Rinderer, T.E., Kuznetsov, V.N., & Delatte, G. T. 1995. A USDA-ARS project to evaluate resistance to *Varroa jacobsoni* by honey bees of Far-Eastern Russia. *Am. Bee J.* 135: 746-748.

Kraus, B. & Page, R.E. Jr., 1995. Effect of *Varroa jacobsoni* (Mesostigmata: Varoidae) on feral *Apis mellifera* (Hymenoptera: Apidae) in California. *Environ. Entomol.* 24: 1473-1480.

Oldroyd, B.P., 1999. Coevolution while you wait: *Varroa jacobsoni*, a new parasite of western honeybees. *Trends Ecol. Evol.* 14: 312-315.

Rinderer, T.E., Kuznetsov, V.N., Danka, R.G., & Delatte, G.T., 1997. An importation of potentially Varroa-resistant honey bees from Far-Eastern Russia. *Am. Bee J.* 137: 787-789.

Rinderer, T.E., Delatte, G.T., de Guzman, L.I., Williams, J., Stelzer, J.A., & Kuznetsov, V.N., 1999. Evaluations of the varroa-resistance of honey bees imported from Far-Eastern Russia. *Am. Bee J.* 139: 287-290.

Rinderer, T. E., deGuzman, L. I., Delatte, G. T., Stelzer, J. A., Lancaster, V. A., Kuznetsov, V., Beaman, L., Watts, R., & Harris, J. W., 2001. Resistance to the Parasitic Mite *Varroa destructor* in Honey Bees from Far-Eastern Russia. *Apidologie. In Press.*