Varroa Resistance of Hybrid ARS Russian Honey Bees

by J. W. HARRIS and T. E. RINDERER[1]

Revised manuscript received for publication July 30, 2004

SUMMARY

The varroa resistance[2] of several genetic crosses utilizing ARS Russian honey bees was tested in Alabama during 2001. Bee stocks included pure ARS Russian (Russian queens × Russian drones), commercial (commercial queens × commercial drones), Russian hybrids (commercial queens × Russian drones), and SMR-Russian hybrids [(queens bred for the suppression of mite reproduction trait) × Russian drones]. The varroa resistance of Russian hybrids was intermediate to that of pure ARS Russian and commercial stocks. This suggests that Russian hybrids may offer some varroa resistance, but pure ARS Russian stock should be used to achieve the maximum varroa resistance that is currently available in Russian bees. The lowest growth of mite populations occurred in the SMR-Russian hybrids. This may suggest that resistance genes from the two parental types combine in an additive manner, but we cannot be sure because pure SMR bees (SMR queens × SMR drones) were not included in the study.

INTRODUCTION

The varroa mite, *Varroa destructor* Anderson and Trueman, remains the most destructive parasite of the western honey bee, *Apis mellifera* L., throughout the world. Colonies of honey bees die within 1-2 years of the initial infestation. Perhaps the harmful effects of varroa mites will gradually decrease through time as natural selection changes the interaction between mites and bees. However, it is also possible that varroa mites will always kill colonies of honey bees. Even if the relationship between varroa mites and bees eventually moderates, beekeepers will need to protect their bees from varroa mites now and into the foreseeable future.

Most beekeepers use chemicals to control populations of varroa mites. These acaricides may contaminate wax and honey, and chronic use of chemicals leads to the development of acaricide-resistant mites. One solution to these problems is the development of varroa resistant stocks of honey bees. Resistant honey bees could be used alone or combined with non-chemical control methods (e.g. screened floors, or drone trapping) to minimize the need for acaricides. Our long-term goal is the release of beneficial bee stocks to the United States beekeeping industry to provide genetically based mite resistance for controlling parasitic mites. Selective breeding of honey bees for varroa resistance is imperative given that varroa mite populations in the U.S. have become resistant to one or more of the chemicals used to control them (Elzen et al. 1998, 1999, Elzen and Westervelt 2002).

Recently we released ARS Russian honey bees to the beekeeping public for use in breeding programs and production colonies (Rinderer *et al.* 2000). ARS Russian honey bees are the result of many years of selective breeding for varroa resistance in honey bees originating from far-eastern Russia. These bees exhibit significant resistance to both varroa (Rinderer *et al.* 1999, 2001) and tracheal (de Guzman et al. 2000, 2002) mites. Breeding varroa-resistant Russian honey bees is an ongoing project, and the varroa-resistance should continue to improve over the next few years.

One frequently asked question about ARS Russian honey bees is "What level of resistance can be expected from hybrid Russian bees?" This consideration is important given the increased marketing of various hybrid Russian bees as "Russian honey bees." Our perspective is that pure Russian honey bees are only those produced by our laboratory, commercial queen producers that have loyally followed our breeding protocol, or queen breeders using artificial insemination to guarantee the genetics of their stocks. Crosses of bees that are produced by mating daughter queens from selected Russian lines with commercial drones are outcrosses, or hybrids, that are not pure Russian honey bees. The levels of varroa resistance in these hybrids are unknown. Given that some queen producers market only hybrids of Russian stock, we tested the relative varroa resistance of hybrid and pure Russian stocks versus commercial controls. Another area of interest is determining the varroa resistance expected in crosses of ARS Russian with SMR bees, which were selectively bred for the ability to suppress mite reproduction at our laboratory (Harbo and Harris 2003).

METHODS

We conducted this experiment in cooperation with Mr. Andy Webb and family of Calvert Apiaries near Mobile, AL. Sixty-four equal-sized colonies were formed by splitting existing colonies in mid-May 2001. Colonies were placed on pallets (8 pallets per apiary) in two apiaries (32 colonies per apiary). One apiary was located on the causeway (U.S. Hwy 90/98) near the U. S. S. Alabama Memorial in Mobile, AL. The second apiary was originally located in Satsuma, AL, but was moved to Summersdale, AL a few weeks after the start of the test because of a conflict with a nearby homeowner (hundreds of bees visited her ornamental ponds for water). All harvested honey was weighed to the nearest tenth of a pound during the first week of October.

All queens used in this test were naturally mated. The queens from the commercial stock were purchased from a well known queen producer that had no resistant stocks of bees. The remaining 3 test stocks were produced by mating each type of queen (Russian, commercial and SMR) with drones from Russian queen lines that had been selected for varroa resistance during the previous year by our laboratory. All queens were free-mated to the Russian drones on a barrier island just off the coast of Louisiana where no feral or managed honey bee populations exist to insure the integrity of the crosses. The ARS Russian queens were daughters of a varroa-resistant queen that was unrelated to the Russian drones. The commercial queens were daughters of a queen randomly chosen from the pool of commercial controls that were purchased. The SMR queens were daughters of a breeder queen that had been produced by artificial insemination at our laboratory.

Queens of the four types of bees (8 pure ARS Russian queens, 8 commercial controls, 12 commercial × Russian hybrids, and 4 SMR × Russian hybrids) were randomly assigned to colonies in each apiary on June 1. All queens were released from cages on

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

[2]The term "resistance", as used in this article, is meant to mean tolerance and not the absolute absence of the parasite.

June 3, 2001. The initial adult bee population (22,108 ± 4210 bees per colony) and capped worker brood areas (319 ± 84 square inches per colony) were determined by visual estimation at this time. Colonies had been treated with an acaricide during the previous autumn, and no detectable levels of varroa mites were found in samples of adult bees (*ca.* 400 bees per sample). Each colony was inoculated with 93 ± 27 mites (mean ± SD) by placing packages of ½ lb (250 grams) infected bees on top bars of frames in the uppermost brood chamber on June 3, 2001. An empty deep rim was placed above each package to protect it while the mites transferred. The packages with dead bees were removed from all colonies about 1 week later.

The mite populations were permitted to grow for 17 weeks through the end of September. Data from 54 colonies were evaluated because 10 of the original queens were superseded before the end of the test. The final adult mite population was found in each colony by summing the total mites on adult bees with the total number of foundress female mites living in capped brood cells. First, the number of bees in each colony was determined by visual estimation of the number of deep frames of adult bees (1,215 bees per deep frame). The infestation rate of mites on adult bees (mites per bee) was determined for each colony by washing 850-1,000 bees that were shaken from 2 brood combs into an empty box and mixed before sampling. Total mites on adult bees were calculated from total numbers of bees, the number of mites in the sample, and number of bees in the sample.

The total capped worker and drone brood areas (square inches) were measured by using a 1 inch x 1 inch wire grid. Brood areas were converted to total numbers of capped worker (worker brood area x 23.6 worker cells per square inch) or drone cells (drone brood area x 16 drone cells per square inch). The infestation rate for foundress mites in capped worker brood cells was obtained by opening 200 capped worker cells from 2 brood combs. Similarly, the infestation rate for drones cells was found by inspecting 100 capped drone cells. The total number of foundress mites in each type of brood was found by multiplying the total number of capped cells by the infestation rate. The proportion of mites in each type of capped brood was the ratio of total mites in capped brood divided by the total mites in the colony of bees.

Adult female varroa mites can be found in 3 areas of the honey bee nest: (a) on adult bees, (b) in capped worker brood and (c) in capped drone brood. The percentages of the mites found in these portions of the nest can be calculated. Instead of analyzing each of these percentages (percentage of mites on adult bees, percentage of mites in capped worker brood, and percentage of mites in capped drone brood) separately, we chose to analyze a single variable that summarized the distribution of mites in the entire colony. For this purpose, we defined the distribution of mites as the following ratio: (percentage mites in capped worker brood) ÷ [(percentage mites on adult bees) + (percentage mites in drone brood)]. A value of 1 for this ratio indicates that half the mites were in capped worker brood and the other half were either on adult bees or in capped drone brood. Values > 1 indicate that more than 50 % of the mite population occurred in capped worker brood. Values < 1 indicate that less than 50 % of the mites occurred in capped worker brood.

RESULTS AND DISCUSSION

Both apiaries provide very similar patterns among the genetic crosses that were tested. Hybrids of Russian bees (commercial queens × Russian drones) had final mite populations intermediate to those of commercial stocks (highest populations) and pure ARS Russian stocks (low populations) (Figure). SMR-Russian hybrids had the lowest final mite populations, which may suggest additive genetic resistance. However, we cannot be sure because honey bees bred pure for the SMR trait were not included for comparison. Although pure Russian bees had fewer mites than commercial stocks, the differences in final mite populations among the 4 stocks were weak (P=0.14) (Table 1). The reasons for not being able to detect stronger statistical differences relate to two things: (a) too few colonies of each stock type were tested in each apiary, and (b) the differences in final mite population were significantly and strongly different between the two apiary locations (Figure and Table 1). Overall, apiary **A** had nearly three times more mites than apiary **B** after 17 weeks of growth. We cannot explain this difference between apiaries. All colonies were inoculated with mites obtained from the same source, a package of 35 lbs of infected bees that had been thoroughly mixed before being subdivided into 64 uniform packages. Both apiaries were located in full sun with only partial shade during the late afternoon. The only obvious clear difference in the management of the two apiaries is that apiary B was moved a few weeks after the start of the experiment.

The distribution of mites in colonies was the only variable that differed significantly among the 4 types of colonies (Tables 1 and 2). Pure ARS Russian stock had a significantly lower value than either commercial stocks or SMR-Russian hybrids (Table 2). Pure Russian bees had a value for the distribution of mites < 1, which indicates that less than 50 % of the total mites were in capped worker brood (ca. 48 %). The values for commercial stocks and SMR-Russian hybrids were > 1, which indicates that > 50 % of the mites were in capped worker brood (ca. 58-60 %). Commercial × Russian hybrids had a value intermediate to the pure Russian and commercial stocks (ca. 53 % of mites in capped worker brood).

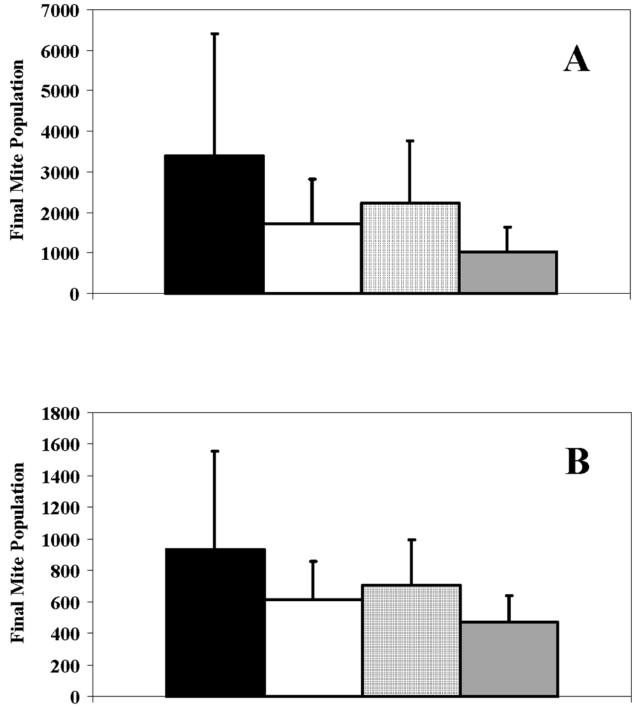

Figure – Comparison of final mite populations among 4 bee stocks after four months of growth in 2001. Colonies were located in two apiaries, one in Mobile, AL (A) and the other in Summersdale, AL (B). Colonies were inoculated with 180-220 mites at the start (avg. 93 mites). Black bars = commercial stocks; clear bars = Russian stocks; dotted bars = commercial queens mated to Russian drones; and gray bars = SMR queens mated to Russian drones.

These results support a commonly reported characteristic of pure ARS Russian bees having a lower proportion of mites in capped worker brood. A chronically lower proportion of mites in capped worker brood can be an important varroa resistance factor for several reasons. First, most of the harm caused to a colony of bees by varroa mites results from infestation of capped worker brood. Therefore, a lower infestation of worker brood will reduce the numbers of workers ultimately affected by the feeding behavior of mites and their progeny. Secondly, a lower proportion of mites in brood may reflect a longer phoretic period on adult bees where mites are more vulnerable to grooming. With a longer phoretic period the average number of reproductive cycles

Variable	Source of Variation		
	Stock	Yard	Stock x Yard interaction
Final mite population	>0.14	<0.002*	>0.4
Final capped worker brood	>0.3	>0.8	0.4
Final capped drone brood	>0.6	>0.7	>0.9
No. adult bees at end	>0.3	>0.9	>0.4
Distribution of mites	0.038*	>0.13	>0.25
Cropped honey	>0.3	>.0.5	>.0.5

Table 1 – Summary of statistical analyses for all variables that were measured in the study. Significant sources of variation are indicated by an asterisk.

attempted per mite during a lifetime is greatly reduced because mites spend a longer period on adult bees between reproductive cycles.

Alternatively, a higher percentage of mites in drone brood will also lower the percentage of mites in capped worker brood. Varroa mites prefer drone brood to worker brood by a 9:1 ratio, and some drone brood was available in all colonies at the end of the test when colonies were evaluated. While infestation of drone cells reduces the harm to developing worker bees, the growth of the mite population is higher in drone brood because varroa mites can produce more female offspring in drone cells. We did not measure mite populations when drone brood was unavailable to see if the distribution of mites in capped worker brood was significantly lower in the pure Russian stock; however, this has been the case in other studies with pure Russian honey bees (Rinderer et al. 2001).

There were no significant differences among stock types or between the two apiaries for variables related to colony size (number of adult bees, amount of capped worker brood, and amount of capped drone brood) (Tables 1 and 2). Honey yield did not differ between apiaries or among stocks (Tables 1 and 2).

CONCLUSIONS

In general, there were no significant differences among the different genetic crosses in this study for honey production, mite growth, and growth of the bee populations. Given the level of variation in final mite population among colonies and between apiaries, this experiment or a similar one should be repeated with fewer stock types and more colonies per stock type to verify the current trends. Such an experiment began in Louisiana during 2003.

Beekeepers wanting resistance to varroa mites should insist upon pure ARS Russian honey bees produced by reputable queen breeders with a well-established Russian honey bee breeding program or those that use instrumental insemination to produce pure Russian queens. The current study suggests an intermediate level of resistance for Russian hybrids when compared to commercial and pure Russian stocks. In particular, the percentage of mites infesting capped worker brood was significantly lower in pure ARS Russian bees than in commercial bees, and Russian hybrids had a level that was intermediate. Thus, it appears that Russian hybrids are likely to have less resistance than ARS Russian bees, but more varroa resistance than commercial stock.

Another issue to consider is that not all Russian hybrid crosses will have equal varroa resistance. Some commercial stocks may be more varroa resistant than others when crossed with ARS Russian bees. In addition, beekeepers produce Russian hybrids by free-mating ARS Russian queens to unselected drones. These Russian hybrids are reciprocals of the kind used in this study. Although we do not think that it would make a difference, we cannot be sure that reciprocal hybrids (e.g. ARS Russian queen × commercial drones versus commercial queens × ARS Russian drones) will have similar varroa resistance. Finally, although the SMR-Russian hybrids had the lowest mite populations in the study, limitations of this study (too few colonies tested; high variation between apiaries) prevent us from saying that additive resistance occurs when crossing ARS Russian bees with SMR bees. However, the prospect of additive resistance between these two types of honey bees merits further investigation.

ACKNOWLEDGMENTS

We thank Mr. Andy Webb and his family for providing the bee colonies and apiary locations used in this study. David Dodge helped with all field and laboratory measurements.

REFERENCES

de Guzman, L. I., Rinderer, T. E., Delatte, G. T., Stelzer, J. A., Williams, J. L., Beaman, L. D., Kuznetsov, V., Bernard, S. J., Bigalk, M. and H. Tubbs. 2001. Multi-state field trials of ARS Russian honey bees. 3. Responses to *Acarapis woodi* 1999, 2000. *Am. Bee J.* 141 (11): 810-812.

de Guzman, L. I., Rinderer, T. E., Delatte, G. T., Stelzer, J. A.,

Stock	No. Colonies	Capped Worker Brood (sq. inches)	Capped Drone Brood (sq. inches)	No. Adult Bees	Distribution of mites[a]	Weight of Honey (lbs)
Commercial	14	454 ± 161	31 ± 24	26,113 ± 6,909	1.43 ± 0.55 a	78 ± 16
Rus x Rus	13	354 ± 142	33 ± 32	26,060 ± 5,860	0.91 ± 0.42 b	85 ± 19
Com x Rus	21	439 ± 162	25 ± 23	26,232 ± 6,232	1.14 ± 0.51 ab	78 ± 26
SMR x Rus	6	425 ± 112	43 ± 51	24,931 ± 4,875	1.51 ± 0.77 a	65 ± 16

[a]The distribution of mites was defined as (mites in worker brood) ÷ [(mites in drone brood & mites on adult bees)].

Table 2 – Comparison of colony size, distribution of mites and honey yield among the 4 stocks. Only the distribution of mites within colonies was significantly different among stocks (means having the same letter are not significantly different).

Beaman, L. and V. Kuznetsov. 2002. Resistance to *Acarapis woodi* by honey bees from far-eastern Russia. *Apidologie* 33: 411-415.

Elzen, P. J., Eischen F. A., J. B. Baxter, J. Pettis, G. W. Elzen and W. T. Wilson. 1998. Fluvalinate resistance in Varroa jacobsoni from several geographical locations. *Am. Bee J.* 138: 674-676.

Elzen, P. J., J. R. Baxter, M. Spivak and W. T. Wilson. 1999. Amitraz resistance in Varroa: a new discovery in North America. *Am. Bee J.* 139: 362.

Elzen, P. J. and D. Westervelt. 2002. Detection of coumaphos resistance in *Varroa destructor* in Florida. *Am. Bee J.* 142: 291-292.

Harbo J. R. and J. W. Harris. 2003. An evaluation of commercially-produced queens that have the SMR trait. *Am. Bee J.* 143: 213-216.

Rinderer, T. E., Delatte, G. T., de Guzman, L. I., Williams, J., Stelzer, J. A. and V. N. Kuznetsov. 1999. Evaluations of the Varroa-resistance of honey bees imported from far-eastern Russia. *Am. Bee J.* 139 (4): 287-290.

Rinderer, T. E., de Guzman, L. I., Harris, J. W., Kuznetsov, V., Delatte, G. T., Stelzer, J. A. and L. Beaman. 2000. The release of ARS Russian honey bees. *Am. Bee J.* 140 (4): 305-307.

Rinderer, T. E., de Guzman, L. I., Delatte, G.T., Stelzer, J. A., Lancaster, V. A., Kuznetsov, V., Beaman, L., Watts, R. and J. W. Harris. 2001. Resistance to the parasitic mite *Varroa destructor* in honey bees from far-eastern Russia. *Apidologie* 32: 381-394.