Comparative Performance of Two Mite-Resistant Stocks of Honey Bees (Hymenoptera: Apidae) in Alabama Beekeeping Operations

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ABSTRACT The utility of USDA-developed Russian and varroa sensitive hygiene (VSH) honey bees, *Apis mellifera* L. (Hymenoptera: Apidae), was compared with that of locally produced, commercial Italian bees during 2004–2006 in beekeeping operations in Alabama, USA. Infestations of varroa mites, *Varroa destructor* Anderson & Truman (Acari: Varroidae), were measured twice each year, and colonies that reached established economic treatment thresholds (one mite per 100 adult bees in late winter; 5–10 mites per 100 adult bees in late summer) were treated with acaricides. Infestations of tracheal mites, *Acarapis woodi* (Rennie) (Acari: Tarsonemidae), were measured autumn and compared with a treatment threshold of 20% mite prevalence. Honey production was measured in 2005 and 2006 for colonies that retained original test queens. Throughout the three seasons of measurement, resistant stocks required less treatment against parasitic mites than the Italian stock. The total percentages of colonies needing treatment against varroa mites were 12% of VSH, 24% of Russian, and 40% of Italian. The total percentages requiring treatment against tracheal mites were 1% of Russian, 8% of VSH and 12% of Italian. The average honey yield of Russian and VSH colonies was comparable with that of Italian colonies each year. Beekeepers did not report any significant behavioral problems with the resistant stocks. These stocks thus have good potential for use in nonmigratory beekeeping operations in the southeastern United States.

KEY WORDS *Apis mellifera*, *Varroa destructor*, *Acarapis woodi*, Russian bees, varroa sensitive hygiene

The varroa mite, *Varroa destructor* Anderson & Truman, is recognized as the most serious threat worldwide to honey bees, *Apis mellifera* L. (Sammataro et al. 2000). The ability to manage this parasitic mite successfully is vital because of the role honey bees play as crop pollinators (National Research Council 2007). Long-term sustainability of honey bees is expected to come at least in part from bees that have reliable, economically useful genetic resistance to varroa. Minimally, resistant bees should require fewer treatments with acaricides to keep mite populations below an economic injury level. They thus could be the foundation of an integrated pest management approach to the varroa threat when combined with alternative management methods such as drone brood removal and screen bottom boards.

Research in the United States has yielded three types of bees with documented varroa resistance. One stock, MN hygienic, has been selected to have enhanced general hygiene, i.e., increased ability to remove dead brood. This has yielded moderate resistance to varroa (Spivak and Reuter 2001). Another stock originated with bees from eastern Russia, and it was developed by USDA for improved resistance to both varroa mites and tracheal mites, *Acarapis woodi* (Rennie), and to have good honey production (Rinderer et al. 2005). Russian bees have shown good mite resistance and general beekeeping traits in several field tests (Rinderer et al. 2001a, 2001b; Tarpy et al. 2007).

Less well documented is the performance of the third resistant stock, varroa sensitive hygiene (VSH) bees (formerly called suppressed mite reproduction [SMR] bees). In VSH colonies, a large percentage of the varroa population is not reproductive, and so mite infestations either decrease or increase only slowly (Harbo and Hoopingarner 1997). VSH bees showed strong varroa resistance in short-term tests during development of the trait. Selection and breeding have been focused mainly on varroa resistance, however, and the general performance of VSH bees remains unclear. Two recent tests have measured varroa-resistance traits of bees that have some VSH genes. A 2-yr field trial of Minnesota hygienic × VSH hybrid bees (colonies expected to contain on average ≈18% VSH genes) found enhanced varroa resistance in hy-

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brids, compared with standard Minnesota hygienic bees and to an unselected control stock (Ibrahim et al. 2007). These hybrid bees with low levels of VSH genes had adequate brood viability and honey production. A 1-yr field test of bees having ≈50% VSH genes showed that the bees had numerically lower varroa levels, and the colonies had a significantly greater time until an economic threshold was reached (Delaplane et al. 2005). Brood production was reduced in some of the 50% VSH colonies.

Here, we extend information about the utility of Russian and VSH bees when used in nonmigratory beekeeping operations in the southeastern United States. This research arose from a request by Alabama beekeepers for research to address their most critical beekeeping problem: effective means to manage varroa. The primary objective of the research was to define the relative needs for mite treatments of the two resistant stocks and an unselected stock during an extended field test. We also compared honey production by the stocks, and asked beekeeper cooperators for their perceptions about significant apicultural aspects of the bees.

Materials and Methods

Bees and Test Colonies. Pure Russian stock was obtained each year from a commercial source (Harper's Honey Farm, Carencro, LA) that mated queens and drones of commercially available Russian breeding lines (Rinderer et al. 2005) at an isolated site. In 2004, VSH stock was produced by mating VSH queens from USDA research lines to presumed Italian drones in a commercial queen breeding operation (Walker Honey Co., Rogers, TX). In 2005 and 2006, we produced VSH stock by allowing VSH queens and drones of research lines to mate at an isolated site. The control stock used throughout was a commercial Italian stock from Alabama (Calvert Apiaries, Calvert, AL).

Experimental colonies were managed by cooperating beekeepers located throughout Alabama. Eight beekeepers participated in 2004, 11 in 2005, and 10 in 2006, although useful data for all parameters were not obtained from all beekeepers. The beekeepers on average had 20 yr of beekeeping experience and kept 116 colonies.

Equal numbers (5, 10, or 15) of clipped and marked queens of each stock were provided to each beekeeper. Queens were introduced in April 2004 into queenless colony divisions in hives with solid bottom boards. New queens were provided in April 2005 and 2006 for colonies that did not have original queens, to replace colonies which had died, and for colonies of new cooperators. Most beekeepers kept all experimental colonies at one location and separated colonies of different stocks within the apiary. One beekeeper kept equal numbers of test colonies at two different locations.

The status of queens was checked approximately every 6 mo, usually at times of sampling for varroa. The longevity of queens was recorded as the time of the last observation of the queen plus 3 mo. Thus, a queen installed in the spring was assumed to have lived for 3 mo if it was missing in the autumn, for 9 mo if it was missing in the following spring, etc. Each queen alive at the end of the test was assigned a longevity value equal to its observed longevity plus 3 mo.

Varroa Mites. Varroa infestations were measured twice each year at the times when acaricides could be applied properly, i.e., when colonies were not storing surplus honey. These treatment opportunities occurred in late winter (before springtime honey production), and in mid- to late summer after the major honey production from Chinese tallow, *Sapium sebiferum* (L.) Roxb. in southern Alabama and cotton, *Gossypium hirsutum* L., in central and northern Alabama. Each colony was sampled by taking ≈300 adult worker bees from the brood area, washing the bees in a detergent solution for ≈30 min to dislodge mites from bees, and counting mites and bees (Hood 2000). Standard acaricide treatments (chosen by individual beekeepers) were made to colonies whose mite infestations were ≥1% (i.e., one mite per 100 bees) in late winter, or ≥10% (2004) or ≥5% (2005, 2006) in late summer. The late summer change was made at the request of some cooperators who believed that the initial 10% treatment threshold resulted in too much mite damage. These thresholds followed current recommendations for the southeastern United States in late winter (Delaplane and Hood 1999) and late summer (5–13%) (Hood 2000, University of Georgia 2006). Similar recommendations exist for the U.S. Pacific Northwest (April, ≈1%; August, ≈5%) (Strange and Sheppard 2001) and the Canadian prairies (spring, 2%; late summer, 4%) (Currie and Gatien 2006).

Tracheal Mites. Tracheal mite infestations were assessed in colonies of some cooperators during autumn of each year of the study. Adult worker bees were taken from the lid or upper box of each hive and stored frozen. Bees were dissected and the prothoracic tracheal trunks were examined with a stereomicroscope at 30× to determine mite prevalences, i.e., the percentage of bees infested. Based on a sequential sampling protocol (Tomasko et al. 1993), the mite prevalence of a colony was declared to be <15% if the first 15 bees examined were not infested. If any of the first 15 bees was infested, a total of 40 bees were examined to determine mite prevalence. Colonies that had a tracheal mite prevalence of 20% or more were considered to have reached a treatment threshold (Nasr 2001).

Honey Production. Honey production was measured in 2005 and 2006 for all colonies that retained an original queen from the prior autumn. In addition, for cooperators who harvested honey from cotton, we also used data from colonies with queens installed in spring of the year of the crop because the colonies were offspring of those queens by the late-summer cotton bloom. Each beekeeper removed what he or she considered to be surplus honey, weighed supers with honey and subtracted the weight of empty equipment.

Beekeepers' Perceptions. At the end of the test, the beekeepers were asked to rate the following charac-

Table 1. Means [± SE (*n*)] for infestation by *V. destructor*, infestation by *A. woodi* and honey production for the three test stocks at sampling times during the 3-yr field test

Trait	Control	Russian	VSH	*F*	df	*P*
V. destructor, spring 2004	0.9 ± 0.3a (44)	0.8 ± 0.3a (45)	1.4 ± 0.3a (43)	1.10	2,123	0.336
V. destructor, autumn 2004	3.5 ± 0.4a (37)	2.0 ± 0.4b (43)	1.3 ± 0.4b (31)	7.10	2,103	0.001
V. destructor, spring 2005	3.7 ± 0.5a (23)	1.8 ± 0.4b (34)	1.8 ± 0.6b (19)	4.41	2,67	0.016
V. destructor, autumn 2005	6.7 ± 0.7a (29)	4.0 ± 0.7b (31)	2.1 ± 0.6b (36)	12.67	2,86	<0.001
V. destructor, spring 2006	4.0 ± 0.8a (13)	3.3 ± 0.6a (19)	1.0 ± 0.6b (21)	5.18	2,44	0.010
V. destructor, autumn 2006	9.8 ± 1.2a (24)	7.5 ± 1.3ab (20)	4.3 ± 1.1b (31)	5.94	2,65	0.004
A. woodi, autumn 2004	13.7 ± 5.9a (16)	0.1 ± 0.1b (28)	3.7 ± 4.4ab (18)	3.37	2,54	0.042
A. woodi, autumn 2005	4.8 ± 17.0a (22)	1.6 ± 6.4a (26)	3.5 ± 8.4a (27)	0.78	2,65	0.464
A. woodi, autumn 2006	1.9 ± 1.8ab (17)	0.0 ± 0.0b (19)	6.6 ± 19.2a (27)	3.62	2,54	0.034
Honey yield (kg), 2005	18.1 ± 3.2a (21)	20.9 ± 3.2a (20)	22.2 ± 2.7a (25)	0.49	2,58	0.617
Honey yield (kg), 2006	17.2 ± 3.6a (21)	21.8 ± 3.6a (19)	20.0 ± 3.2a (26)	0.35	2,57	0.706

Statistical analysis results are from ANOVA. Means within a row followed by different letters differ at $P < 0.05$ according to Tukey's HSD test.

teristics of the test stocks on a scale of 1 (very dissatisfied) to 5 (very satisfied): varroa resistance, tracheal mite resistance, honey production, gentleness, food use, overwintering ability, and overall utility. The beekeepers were asked to note any traits they felt were important throughout the study.

Statistical Analyses. Only data from colonies with original queens were used in the analyses. The experiment involved a randomized complete block design with treatment (i.e., stock) replicated across beekeepers, and beekeepers as blocks within a time period or years as blocks across time periods. Analysis of variance (ANOVA) (Analytical Software 2003) was used to evaluate effect of stocks on infestations of varroa mites, infestations of tracheal mites, and honey production. Post-ANOVA comparisons of means were made with Tukey's honestly significant difference (HSD) test. The relative numbers of colonies of the test stocks that reached thresholds for treatments against varroa and tracheal mites were compared with chi-square tests (Siegel 1956, SAS Institute 2003). Fisher exact test was used to separate means. Survival analysis (PROC LIFETEST, SAS Institute 2003) was used to estimate queen longevity and to test for effects of stock and year on longevity. Beekeepers' categorical ratings of the stocks for various traits were evaluated with likelihood ratio tests (PROC FREQ, SAS Institute 2003). Data are presented throughout as mean ± SE.

Results and Discussion

Varroa Mites. Initial infestations by varroa when the test began in spring 2004 were almost uniformly low and did not differ among groups of colonies assigned randomly to the three test stocks (Table 1). The low initial infestation presumably reflects successful prior acaricide use by the beekeepers. By autumn 2004, soon after routine treatments ceased, varroa infestations varied between stocks, and they continued to differ at all subsequent sampling periods (Fig. 1; Table 1). Varroa infestations were lower in VSH colonies than in control colonies throughout the duration of the test. Varroa infestations in Russian colonies were low and similar to those in VSH colonies during autumn 2004 through autumn 2005. In 2006, varroa infestations in Russian colonies tended to be intermediate between those in VSH and control colonies.

The percentages of colonies that reached treatment thresholds for varroa varied in concert with infestation levels (Table 2). Few colonies had infestation levels above the 10% threshold in autumn 2004. At later samplings, all of which had lower treatment thresholds, greater percentages of colonies of all three groups required treatment, but the resistant stocks needed fewer treatments than control colonies. Over the entire 3-yr study, the average percentage of colonies that reached the varroa treatment threshold at any sampling time was 12% of VSH, 24% of Russian, and 40% of controls. The treatment thresholds used in this test reflected current recommendations for the southeastern United States, and they were used to minimize the possibility of colony mortality from varroa. The data used to generate these thresholds, however, were founded on responses of bees not selected for varroa resistance, and they may be conservative for resistant bees. Establishing a higher treatment threshold for resistant bees could further reduce the amount of acaricide required to manage varroa.

Tracheal Mites. Infestations by tracheal mites generally were low throughout the test, but they differed between stocks in 2004 and 2006 (Table 1). Russian colonies had the lowest infestation in each of the 3 yr. The greatest infestations occurred in control colonies in 2004 (VSH were intermediate) and in VSH colonies in 2006 (controls were intermediate). The percentages of colonies reaching a treatment threshold of 20% mite prevalence differed only in 2004 (Table 2), with Russian bees least, VSH bees intermediate and control bees greatest. Over the entire 3-yr study, the average percentage of colonies that reached the treatment threshold was least for Russian stock (1%), intermediate for VSH stock (8%), and greatest for control stock (12%). Russian bees have been selected rigorously for resistance to tracheal mites, and only one Russian colony of 73 sampled had an infestation high enough to warrant treatment. The performance of VSH bees likely reflects that these bees have not been selected strongly for resistance to tracheal mites.

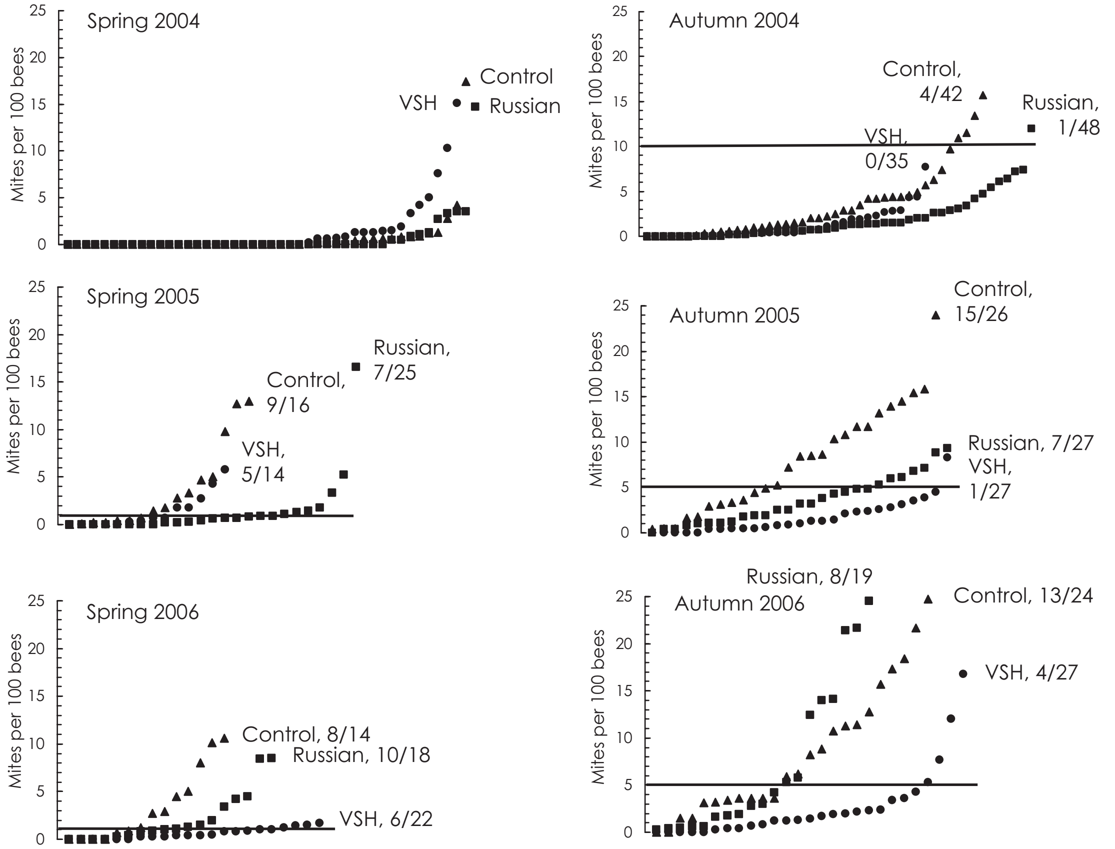

Fig. 1. Infestations by *V. destructor* of individual colonies of the three test stocks of honey bees at each of six sampling times. Within each sampling time, each point represents a colony and colonies are arranged in ascending order of infestation. The horizontal line indicates the treatment threshold at each sampling time (no treatments were made in spring 2004). The numbers with each stock type give the number of colonies above the treatment threshold and the total number of colonies sampled. Triangles are control stock colonies, squares are Russian stock colonies, and circles are VSH stock colonies.

Honey Production. Honey production did not differ among the three stocks either in 2005 or 2006 (Table 1). The 2-yr average honey production of Russian (21.8 ± 2.7 kg [48 ± 6 lb]) and VSH (21.8 ± 2.3 kg [48 ± 5]) bees was comparable to that of control bees (18.1 ± 2.3 kg [40 ± 5 lb]), suggesting adequate

Table 2. Percentages of colonies of the three stocks that reached acaricide treatment thresholds for *V. destructor* or *A. woodi* at individual and combined sampling times

Sampling time (n)	Stock			χ^2	df	P
	Control	Russian	VSH			
V. destructor						
Autumn 2004 (130)	9a	2a	0a	5.21	2	0.074
Spring 2005 (55)	6a	28a	36a	3.35	2	0.188
Autumn 2005 (80)	58a	26b	4c	19.01	2	<0.001
Spring 2006 (54)	57a	56a	23b	6.00	2	0.050
Autumn 2006 (70)	54a	42a	14b	9.03	2	0.011
All autumn (280)	34a	17b	5c	25.30	2	<0.001
All spring (109)	57a	40a	28a	5.69	2	0.058
Overall (389)	40a	24b	12c	26.32	2	<0.001
A. woodi						
Autumn 2004 (65)	28a	0b	5ab	10.59	2	0.020
Autumn 2005 (84)	8a	4a	9a	0.62	2	0.733
Autumn 2006 (63)	6a	0a	7a	1.41	2	0.493
Overall (212)	12a	1b	8ab	7.41	2	0.050

Statistical analysis results are from 3 by 2 chi-square tests of numbers of treated versus untreated colonies within "sampling time." Means within a row followed by different letters differ at $P < 0.05$ according to 2 by 2 Fisher exact tests.

Table 3. Ratings (mean ± SE) given by cooperating beekeepers about characteristics of the test stocks

Trait	Control	Russian	VSH	Likelihood ratio	df	*P*
Varroa resistance	3.2 ± 0.4	4.0 ± 0.0	4.6 ± 0.2	15.05	6	0.020
Tracheal mite resistance	4.0 ± 0.4	4.0 ± 0.4	4.0 ± 0.4	0.00	4	1.000
Honey yield	3.7 ± 0.6	3.5 ± 0.4	3.7 ± 0.6	5.27	6	0.510
Gentleness	3.2 ± 0.4	2.7 ± 0.4	3.7 ± 0.6	7.81	6	0.253
Food use	3.4 ± 0.4	4.4 ± 0.2	3.8 ± 0.2	8.41	6	0.210
Overwintering ability	4.2 ± 0.2	4.8 ± 0.2	3.8 ± 0.5	10.74	6	0.098
Overall utility	3.5 ± 0.4	3.8 ± 0.4	3.7 ± 0.6	6.28	6	0.393

Ratings ranged from 1 (very dissatisfied) to 5 (very satisfied). Statistical analysis results are from likelihood ratio tests.

productivity by the resistant stocks. Honey yields by all stocks used in the test were somewhat lower than the official state averages reported for Alabama of 29.9 kg (66 lb) per colony in 2005 and 32.7 kg (72 lb) per colony in 2006 (NASS 2007).

Queen Survival. The longevity of queens of the three stocks did not differ (log-rank $\chi^2 = 3.61$, df = 2, $P = 0.164$). The estimated average life span was 10.6 ± 1.0 mo ($n = 65$) for Russian queens, 9.6 ± 0.8 mo ($n = 74$) for VSH queens, and 8.4 ± 0.8 mo ($n = 67$) for control queens. Beekeepers commented on the difficulty in introducing VSH queens initially in 2004. We are not able to ascribe this difficulty to genetics versus environmental factors, such as poor matings. We suspect that at least some VSH queens in 2004 were poorly mated based on reports from the commercial queen producer who provided the queens. Beekeepers also commented that the supersedure rate of test queens of all stocks was greater than they had experienced previously with their own bees. However, this rate of replacement of queens may reflect a normal situation that typically is unknown because beekeepers do not closely monitor queen changes, as was done in this study.

Beekeepers' Ratings of Stocks. Perceptions about stock performance were similar for most traits (Table 3). The only characteristic viewed to differ among the stocks was varroa resistance, for which mean beekeeper ratings were 4.6 for VSH, 4.0 for Russian and 3.2 for control stock. The overall perceptions of the resistant bees (Russian rating, 3.8; VSH rating, 3.7) were at least as good as that of the control stock (rating 3.5).

In conclusion, both Russian and VSH stocks performed relatively well in resisting infestation by varroa and tracheal mites, and having honey production comparable to a standard commercial Italian stock used in Alabama. Participating beekeepers rated the resistant stocks at least as good as the control stock in 11 of 14 pairwise comparisons.

Of particular interest was the performance of VSH bees relative to resistant Russian bees and to commonly used Italian bees. VSH bees showed excellent resistance to varroa. For the first 12 mo of the test, hybrid VSH colonies from outcrossed VSH queens performed about as well as pure Russian colonies and better than control colonies. Later, pure VSH colonies had lower varroa infestations and lower percentages of colonies that needed treatment than both Russian and control colonies. Overall, the need for treatment of VSH colonies was 50% that of Russian colonies and 30% that of control colonies. VSH bees had some resistance to tracheal mites, with performance that generally was intermediate between resistant Russian and susceptible control bees. Honey production by VSH equaled that of the other two stocks, suggesting that at least this aspect of beekeeping utility was not hampered by fitness costs associated with high varroa resistance, e.g., reduced brood production.

These data showed the worth of resistant stocks in reducing the need to treat for varroa when colonies were kept in small-scale, stationary honey production operations in the southeastern United States. Remaining to be tested is how mite resistant stocks respond when challenged with higher varroa levels such as in migratory operations where colonies often are exposed to an influx of mites as they are mixed with other colonies brought to crop pollination sites.

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