Functionality of Varroa-Resistant Honey Bees (Hymenoptera: Apidae) When Used for Western U.S. Honey Production and Almond Pollination

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J. Econ. Entomol. 107(2): 523–530 (2014); DOI: http://dx.doi.org/10.1603/EC13419

ABSTRACT Two types of honey bees, *Apis mellifera* L., bred for resistance to *Varroa destructor* Anderson & Trueman, were evaluated for performance when used for honey production in Montana, and for almond pollination the following winter. Colonies of Russian honey bees and outcrossed honey bees with Varroa-sensitive hygiene (VSH) were compared with control colonies of Italian honey bees. All colonies were managed without miticide treatments. In total, 185 and 175 colonies were established for trials in 2010–2011 and 2011–2012, respectively. Survival of colonies with original queens or with supersedure queens was similar among stocks for both years. Colony sizes of the Varroa-resistant stocks were as large as or larger than the control colonies during periods critical to honey production and almond pollination. Honey production varied among stocks. In the first year, all stocks produced similar amounts of honey. In the second year, Russian honey bees colonies produced less honey than the control colonies. *V. destructor* infestations also varied among stocks. In the first year, control colonies had more infesting mites than either of the Varroa-resistant stocks, especially later in the year. In the second year, the control and outcrossed Varroa-sensitive hygiene colonies had high and damaging levels of infestation while the Russian honey bees colonies maintained lower levels of infestation. Infestations of *Acarapis woodi* (Rennie) were generally infrequent and low. All the stocks had similarly high *Nosema ceranae* infections in the spring and following winter of both years. Overall, the two Varroa-resistant stocks functioned adequately in this model beekeeping system.

KEY WORDS *Varroa destructor*, *Apis mellifera*, genetic resistance, honey production, almond pollination

Honey bees, *Apis mellifera* L., are beset with serious health threats that have contributed to substantial year-by-year reductions in numbers of commercial colonies in the United States (National Research Council 2007, vanEngelsdorp and Meixner 2010). Parasitism by the mite *Varroa destructor* Anderson & Trueman ranks among the most consistently damaging of these threats (Guzmán-Novoa et al. 2010, Rosenkranz et al. 2010). Honey bees that have genetically based mite resistance produced by selective breeding can be used to mitigate problems from *V. destructor* in at least some beekeeping circumstances. Three types of honey bees (Minnesota hygienic bees, Russian honey bees, and bees with the trait of Varroa-sensitive hygiene [VSH]), are documented from field tests to suppress mite populations in colonies in stationary apiaries used for honey production (Ibrahim et al. 2007, Ward et al. 2008, Rinderer et al. 2010a). Colonies serving in crop pollination usually are managed differently, and often more intensively, than those used for honey production (Free 1993, Delaplane and Mayer 2000). Pollination service may stress honey bee colonies more than management for honey production because colonies are moved often and because some crops are poor sources of nectar and pollen. Despite these severe conditions, when untreated against mites, both Russian honey bees and VSH colonies performed comparably to unselected Italian colonies in a commercial beekeeping operation that focuses primarily on pollination of several major crops across the country (Danka et al. 2012).

Our earlier findings indicate little or no significant loss of beekeeping functionality of the Russian honey bees and honey bees with the VSH trait. This information may be useful in helping beekeepers decide to move away from miticides and shift toward genetic solutions to manage *V. destructor*. In this study, we sought to determine how these two types of mite-resistant honey bees fared in a third apicultural system that is used by a large proportion of U.S. beekeepers. We evaluated the performance of these two stocks in a commercial beekeeping operation that focuses primarily on summer honey production in the upper

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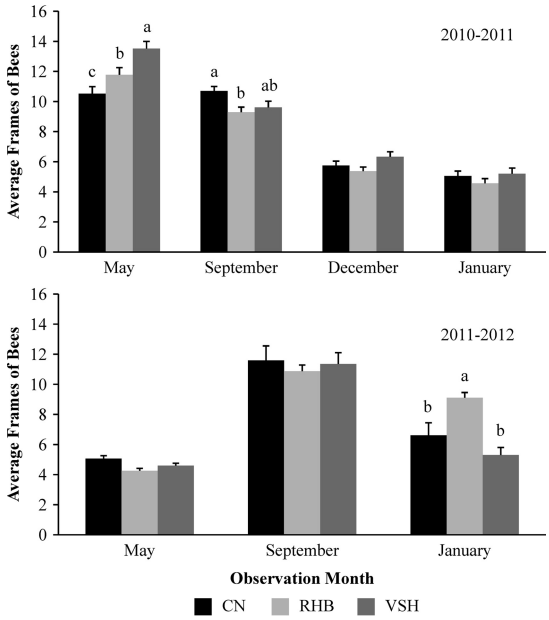

Fig. 1. Frames of bees (mean ± SE) for CN, Russian honey bee, and colonies with the VSH trait for selected months for separate trials in 2010–2011 and 2011–2012. Means that do not share a common letter differ at $P \leq 0.05$ according to ANOVA for each month.

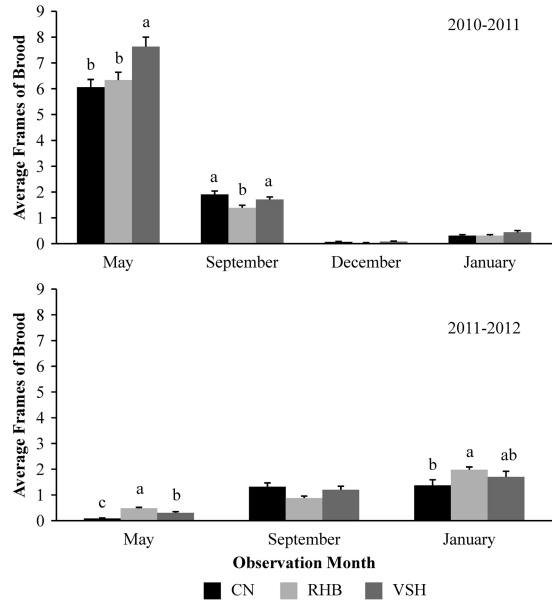

Fig. 2. Frames of brood (mean ± SE) for CN, Russian honey bee, and colonies with the VSH trait for selected months for separate trials in 2010–2011 and 2011–2012. Means that do not share a common letter differ at $P \leq 0.05$ according to ANOVA for each month.

Midwest and also moves the colonies to California in the winter for almond pollination.

Materials and Methods

Experimental Setup. In each of the two test years (2010–2011 and 2011–2012), colonies were established after the almond bloom in spring. Queens or queen cells of three types of stock (Russian honey bees, VSH outcross, and controls [CN]) were introduced each year into colony divisions created in Delano, CA, by a cooperating beekeeping company with headquarters in Montana. Colonies of Russian honey bees were established in deep (23.7 cm) 10-frame Langstroth hives with pure mated queens produced by commercial sources (Brachmann 2009). Colonies of VSH were established in five-frame hives with queen cells shipped to California after being grafted from breeder colonies maintained by a commercial breeder (2010) or us (2011). These VSH queens were allowed to mate in California with locally present drones (presumably mostly from managed colonies not containing resistant stock), and thus produced VSH outcross colonies. CN colonies were established in five-frame hives with queen cells grafted from mixed sources of "Italian" honey bees and these queens also mated with local drones. Colonies were moved to Montana in May and colonies in 5-frame hives were transferred to 10-frame hives; colonies of all stocks were of approximately equal size at this time each year (Figs. 1 and 2). Throughout the season, additional deep Langstroth hive bodies were added as necessary. For both trials, colonies were located in four apiaries, each having a proportionally equal representation of each stock. Colonies that had superseded queens were also monitored throughout the experimental period and included as supersedures of the original genotype. Superseded queens may have superseded more than once; we did not monitor queen changes after an initial supersedure occurred. We included colonies with either original or supesedure queens because they represent what a beekeeping operation typically would include.

Trial 1, 2010–2011. The trial began in March 2010 with 185 colonies (71 CN, 66 Russian honey bees, and 48 VSH). After the colonies were established with laying queens they were managed as usual by our beekeeping cooperator except they were not treated against *V. destructor*. In May, September, and December 2010 and in January 2011, colonies were examined to verify the presence of the original paint-marked and wing-clipped queen and to estimate populations of bees and brood which were expressed as full frames (Rinderer et al. 2001). In August and September, surplus honey was harvested. The weights of harvested boxes minus the average weight of 20 boxes with empty frames were used to determine the weight of honey harvested from each colony. In addition, in September, the weight of stored honey in frames containing brood was estimated to one-eighth of the frame, and the weight of honey was estimated based on the average weight of 120 full frames of honey. These estimates were included in total honey production.

Each time a colony was inspected, a sample of worker bees (≈300–400 bees) was taken from the brood nest, stored on ice in the field, and then frozen for transport to our laboratory. At the laboratory, samples were stored frozen (−20°C) until processed to determine the infestation rates of *V. destructor*, *Nosema ceranae* Fries et al., and *Acarapis woodi* (Rennie). *V. destructor* infestations were not sampled in December 2010.

In early December 2010, 168 colonies (68 CN, 57 Russian honey bees, and 43 VSH) were moved from Montana to California and placed in a single apiary. The value of mid-winter feeding was tested by feeding half of the colonies of each group (chosen randomly) from 7 December 2010 to 25 January 2011. Feeding consisted of ad libitum sucrose syrup (1:1, vol:vol) and a pollen substitute formulated from a dry commercial protein mix, granulated sugar, and high-fructose corn syrup.

Trial 2, 2011–2012. The trial began in March 2011 with 175 colonies (35 CN, 98 Russian honey bees, and 42 VSH). Colonies were inspected in May and September 2010 and January 2011. For each inspection, data were collected concerning the presence of queen, and populations of bees and brood as they were in Trial 1. Honey production was determined in September using the procedures noted for Trial 1. Colonies (28 CN, 67 Russian honey bees, and 28 VSH) were moved from Montana to California in early December where they were placed in a single apiary. The feeding test was repeated in Trial 2 using the same methods employed in Trial 1.

Laboratory Evaluations of Parasite Infestation Rates. Two samples of 30 bees each were removed from the samples for evaluations of *N. ceranae* and *A. woodi*, respectively. The remaining bees ($n \approx 300$) were washed with soapy water to remove *V. destructor* (Rinderer et al. 2004), and the mites and bees were counted to determine the number of mites per 100 bees.

N. ceranae infection rates were determined with a real-time quantitative PCR assay (Bourgeois et al. 2012). Briefly, midguts were removed from 30 bees of each colony and tissue was pooled and homogenized. Genomic DNA was extracted and then amplified with primers and a probe specific for *N. ceranae.* Detection and quantification were performed on a StepOnePlus Real-Time PCR System (Applied Biosystems, Carlsbad, CA). Protocols for FAST PCR and reagent specifications followed those described by Bourgeois et al. (2010) except primers and probe reagents for *N. apis* were eliminated, and distilled water was added to adjust the reaction volume to 12.5 μl. All samples were run in duplicate and were directly quantified by comparison to a standard curve of known number of copies of *N. ceranae* DNA and then converted to number of *N. ceranae* per bee (Bourgeois et al. 2010).

Infestations by *A. woodi* were determined by dissecting 30 bees per colony and examining the prothoracic tracheal trunks at 30× with a stereomicroscope. For Trial 1, a randomly selected subset of 31 CN, 31 Russian honey bees, and 29 VSH colonies were evaluated for tracheal mites in May. In September, another random subset of 31 CN, 30 Russian honey bees, and 29 VSH colonies were evaluated. In December, all surviving colonies were evaluated and in January, a randomly selected subset of 32 CN, 30 Russian honey bees, and 30 VSH colonies were evaluated. For Trial 2, a randomly selected subset of 30 CN, 31 Russian honey bees, and 30 VSH colonies were evaluated in May. In January, all surviving colonies were evaluated. We report the percentages of infested colonies per bee type and the percentages of infested bees in a colony.

Data Analyses. We assessed the main effects of bee type and sampling date on responses of parametric variables (populations of adult bees and brood, infestations of *V. destructor*, and infections with Nosema spp.) by analysis of variance (ANOVA; PROC, MIXED, SAS Institute 2009) applied to a split-plot design. Main units were the three bee types (CN, Russian honey bees, and VSH; including colonies which had superseded the original queen) arranged completely randomly with colonies as replicates within each type; subunits were repeated measured over the four sampling dates (three for *V. destructor*) in Trial 1 and three sampling dates in Trial 2. Data within each year were analyzed separately because of the different timing and frequency of colony inspections. In cases where there was a significant interaction of bee type and sampling date for a variable, means were separated (*t*-tests of least square means) within each date for which bee type had a significant effect. Frequencies of colonies infested with *A. woodi* were compared between bee types with χ^2 tests (PROC FREQ, SAS Institute 2009). Net honey production was compared between bee types with a one-way ANOVA. The effects of feeding on responses of parametric variables (populations of adult bees and brood, and infestations of *V. destructor*) on the last observation date for each year were assessed with a two-way ANOVA using bee type and feed treatment as the main effects.

We compared survivorship of colonies and of original queens among bee types within each year (logrank test in PROC LIFETEST, SAS Institute 2009). These analyses considered the last observation of a colony or an original queen as censored.

For each time period (May–September and September–January) in each of the two trials, we tested for associations between the loads of *V. destructor* and *N. ceranae* at the beginning of the time period and colony death or queen supersedure at the end of the time period. The associated risks and tests of significance used maximum likelihood estimates of the logistic function (PROC Logistic SAS Institute 2009). In addition, for each of the four time periods described above, we measured the effect of the parasite load (average between initial and final for *V. destructor* and *N. ceranae*) on the change in bee population (final–initial frames of bees in each time period) of each surviving colony. This relationship between parasite load and change in colony population was analyzed with linear regression. Possible differences between

genetic types in the effects of parasite loads were evaluated by running similar analyses for each stock type and comparing the respective risk factors (colony death and supersedure) and the regression coefficients (for changes in colony population).

Effects were considered to be significant at $P < 0.05$. Data are given as mean ± SE.

Results

Survival of Queens and Colonies. In 2010–2011, 43% of colonies had original queens and 38% of colonies had supersedure queens in January 2011, and 19% of colonies had died during the trial. The distribution of these three fates among colonies did not differ between the three bee types ($\chi^2 = 4.00$; df = 4; $P = 0.406$). The longevity of the original queens of each stock was similar: overall, 160 ± 7 d; CN, 162 ± 12 d; Russian honey bees, 147 ± 14 d; VSH, 172 ± 13 d ($\chi^2 = 1.35$; df = 2; $P = 0.510$). The longevity of colonies with either original or supersedure queens to January 2011 was also similar: overall, 222 ± 5 d; CN, 227 ± 7 d; Russian honey bees, 211 ± 10 d; VSH, 230 ± 9 d ($\chi^2 = 2.05$; df = 2; $P = 0.358$).

In 2011–2012, 35% of colonies had original queens and 23% had supersedure queens in January 2012, and 41% of colonies had died during the trial. The distribution of these three fates among colonies did not differ between the three bee types ($\chi^2 = 5.99$; df = 4; $P = 0.200$). The longevity of the original queens of each stock was similar: overall, 111 ± 9 d; CN, 93 ± 19 d; Russian honey bees, 123 ± 12 d; VSH, 96 ± 16 d ($\chi^2 = 4.51$; df = 2; $P = 0.105$). Colony longevity with original or supersedure queens was similar with no differences among stocks: overall, 170 ± 8 d; CN, 175 ± 17 d; Russian honey bees, 166 ± 12 d; VSH, 176 ± 15 d ($\chi^2 = 0.38$; df = 2; $P = 0.827$).

Population of Adult Bees. In 2010–2011, the analysis of population size identified an interaction between bee type and sampling date ($F = 4.16$; df = 6, 639; $P < 0.001$) (Fig. 1). In May, bee types differed in size with VSH (13.52 ± 0.48 frames of bees) > Russian honey bees (11.78 ± 0.48) > CN (10.52 ± 0.47). In September, CN (10.71 ± 0.29) > Russian honey bees (9.29 ± 0.34), with VSH (9.61 ± 0.41) not differing from either CN or Russian honey bees. Generally, all colonies were smaller in December (≈5.8–6.3 frames of bees) and January (≈4.8–5.2 frames of bees) than they were in May and September.

In 2011–2012, the analysis of population size again identified an interaction between bee type and sampling date ($F = 8.99$; df = 4, 397; $P < 0.001$; Fig. 1). Colonies of all types were similar in May (≈4.3–5.1 frames of bees), but were much smaller than in the first year. In September, colonies of all stocks remained similar in size (10.9–11.6) and were about the same size as in September in Trial 1. By January, all colonies had decreased in size and sizes differed among stocks with Russian honey bees (9.11 ± 0.35) being larger than CN (6.61 ± 0.84) and VSH colonies (5.30 ± 0.51; $F = 17.31$, df = 2, 397, $P < 0.001$).

Population of Brood. In 2010–2011, the main factors of bee type and sampling date interacted ($F = 2.51$, df = 6, 637, $P = 0.021$; Fig. 2). In May, VSH colonies had more frames of brood (7.63 ± 0.37) than either CN (6.06 ± 0.30) or Russian honey bee colonies (6.34 ± 0.30) ($F = 9.52$; df = 2, 637; $P < 0.001$). By September, Russian honey bee colonies had less brood (1.39 ± 0.10) than either CN (1.91 ± 0.13) or VSH colonies (1.71 ± 0.10; $F = 4.34$, df = 2, 637, $P = 0.014$). In December (≈0.03–0.08) and January (≈0.3–0.4), the bee types had similar brood populations ($F = 0.77$; df = 2, 637; $P = 0.463$; and $F = 0.76$; df = 2, 637; $P = 0.469$, respectively).

In 2011–2012, the main factors of bee type and sampling date interacted ($F = 7.50$, df = 4, 393, $P < 0.001$; Fig. 2). In May, colonies had many fewer frames of brood than they did in the prior year. Also, all three stocks differed ($F = 13.57$; df = 2, 393; $P < 0.001$) in the number of frames of brood (Russian honey bees [0.48 ± 0.04] > VSH [0.30 ± 0.05] > CN [0.09 ± 0.02]). In September, colonies of all three stocks had similar ($F = 2.81$; df = 2, 393; $P = 0.062$) amounts of brood (≈0.9−1.4) that were somewhat smaller than observed the prior year (≈1.4–1.9). In January, stocks differed ($F = 5.36$; df = 2, 393; $P = 0.005$) in the number of frames of brood (Russian honey bees [1.98 ± 0.11] > CN [1.37 ± 0.22], with VSH [1.70 ± 0.22]) not differing from either. However, the frames of brood in January were much larger in the second year (≈1.4–2.0) than they were in the first (≈0.3–0.4).

Infestation With *V. destructor*. The analysis for the number of *V. destructor* per 100 bees in 2010–2011 showed an interaction between bee type and sampling date ($F = 4.46$, df = 4, 398, $P = 0.002$; Fig. 3). In May, no mites were detected in any of the bee types. However, by September, significant differences ($F = 18.23$; df = 2, 398; $P < 0.001$) were found among stocks (CN [4.6 ± 0.5] > Russian honey bees [2.1 ± 0.3], and VSH [1.9 ± 0.3]). Although the numbers of *V. destructor* per 100 bees rose by January, similar significant differences ($F = 11.82$; df = 2, 398; $P < 0.001$) were found among stocks (CN [7.9 ± 0.7] > Russian honey bees [5.0 ± 0.5]), and VSH [5.0 ± 0.6]).

A very different pattern of *V. destructor* infestation was found in 2011–2012, although once again the main effects of bee type and sampling period interacted ($F = 36.34$; df = 4, 351; $P < 0.001$). In May, the stocks differed in infestations (Russian honey bees [4.9 ± 0.4] > CN [0.9 ± 0.2], and VSH [0.9 ± 0.1]). By September, *V. destructor* infestations in Russian honey bees had not increased from May levels (4.7 ± 0.4) and were less than that in CN (7.3 ± 0.9) or VSH (7.3 ± 0.9; $F = 6.42$, df = 2, 351, $P = 0.003$). Although the same pattern of significant differences was observed in January, mite infestations had reached damaging levels in some colonies (Russian honey bees [8.4 ± 0.7] < CN [17.2 ± 1.8] and VSH [18.0 ± 2.0]; $F = 31.48$, df = 2, 351, $P < 0.001$; Fig. 3).

Infestation With *A. woodi*. In 2010–2011, *A. woodi* was detected in 33% (139 of 427) of colony samples. The percentages of colonies infested by *A. woodi* were

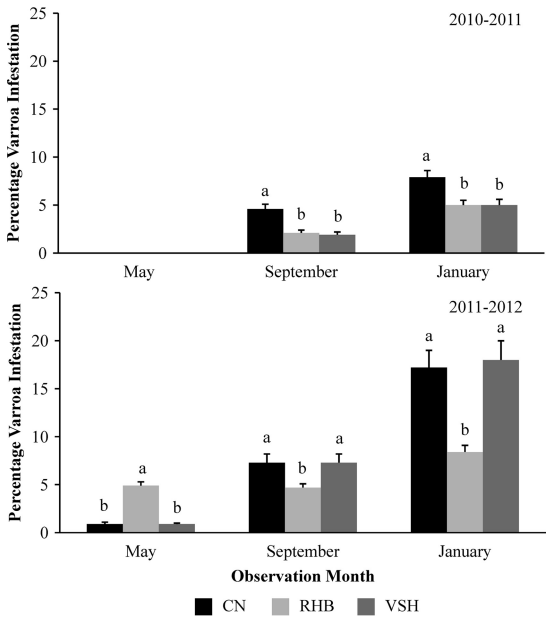

Fig. 3. Adult bee infestations by *V. destructor* (mean ± SD) in CN, Russian honey bee, and colonies with VSH trait for selected months for separate trials in 2010–2011 and 2011–2012. Means that do not share a common letter differ at $P \leq 0.05$ according to ANOVA for each month.

similar among honey bee stocks ($\chi^2 = 1.90$; df = 2; $P = 0.386$). In total, 37% of CN colonies, 29% of Russian honey bees, and 31% VSH outcross colonies had detectable mites. Overall, the percentage of bees infested within individual colonies averaged 7% (range 0–37%). Only six colony samples showed infestations at levels considered to be damaging (i.e., based on an economic threshold of ≥20% bees infested per colony; Nasr 2001). These included one VSH and one CN colony in May, two CN colonies in December, and two CN colonies in January.

In 2011–2012, *A. woodi* was detected in 35% (67 of 194) of colony samples. The proportion of colonies infested by *A. woodi* varied among bee types ($\chi^2 = 26.0$; df = 2; $P < 0.001$); ≈17% of Russian honey bee colonies were infested versus 45 and 56% of CN and VSH colonies, respectively. Overall, the percentage of bees infested within individual colonies averaged 8% (0–50%). Only five colony samples showed infestations at levels of 20% or more bees infested per colony. These included one VSH and three CN colonies in May and one CN colony in January.

Infection With *N. ceranae*. In 2010–2011, there was an interaction between the main factors of bee type and sampling period for numbers of *N. ceranae* per bee ($F = 4.76$; df = 6, 516; $P < 0.001$). In May, Russian honey bees (2.00 ± 0.64 × 106) had fewer *N. ceranae* per bee than CN (8.53 ± 5.84 × 106) and VSH (7.83 ± 1.81 × 106; $F = 10.67$, df = 2, 516, $P < 0.001$). In both September (≈0.196–0.30 × 106) and December (≈0.27–0.95 × 106), numbers of *N. ceranae* per bee did not differ between stocks ($F = 0.60$; df = 2, 516; $P = 0.551$ and $F = 0.45$; df = 2, 516; $P = 0.635$, respectively). In January, CN (1.18 ± 0.34 × 106) had fewer *N. ceranae* than Russian honey bees (2.20 ± 0.49 × 106). The number of *N. ceranae* per bee for VSH (1.51 ± 0.45 × 106) did not differ significantly from those for either CN or Russian honey bees.

In 2011–2012, numbers of *N. ceranae* per bee followed a similar pattern to that seen in 2010–2011. Counts were less in September than they were in May and then became greater in January. There was an interaction between bee type and sampling period for *N. ceranae* counts ($F = 8.39$; df = 4, 211; $P < 0.001$). In May, Russian honey bees (5.76 ± 0.68 × 106) had more *N. ceranae* per bee than either CN (1.40 ± 0.99 × 106) or VSH (0.52 ± 0.16 × 106). In September VSH (0.50 ± 0.16 × 106) had more *N. ceranae* then either the CN (0.02 ± 0.00 × 106) or Russian honey bees (0.02 ± 0.01 × 106). By January, all stocks had similar numbers of *N. ceranae* per bee (≈0.39–0.98 × 106).

Honey Production. In 2010, all stocks produced similar amounts of honey ($F = 0.05$; df = 2, 173; $P = 0.947$; ≈28–132 pounds of honey). In 2011, there were significant differences in honey production ($F = 3.38$; df = = 2, 125; $P = 0.037$) among stocks (CN [161.8 ± 10.5] > Russian honey bees [127.2 ± 6.8] with VSH (138.2 ± 9.6) not differing from either CN or Russian honey bees.

Effects of Winter Feeding. Overall, the effects of winter feeding were minimal. In 2010–2011, feeding did not increase the frames of bees (fed = 5.20 ± 0.27 frames of bees, nonfed = 4.78 ± 0.27; $F = 0.96$; df = 1, 134; $P = 0.330$). However, feeding did produce somewhat more brood (fed = 0.58 ± 0.04 frames of brood, nonfed = 0.14 ± 0.02, $F = 87.98$; df = 1, 134; $P < 0.001$). For both frames of bees and frames of brood, the three stocks responded similarly to feeding ($F = 0.75$; df = 2, 134; $P = 0.474$ and $F = 2.01$; df = 2, 134; $P = 0.138$, respectively).

In 2011–2012, feeding did not increase the number of frames of bees (fed = 7.73 ± 0.49, nonfed = 7.87 ± 0.44, $F = 0.03$; df = 1, 97; $P = 0.864$) or the number of frames of brood (fed = 1.82 ± 0.11, nonfed = 1.79 ± 0.15, $F = 0.54$; df = 1, 97; $P = 0.465$).

Effect of Parasites on Colony Performance. Survival and changes in colony populations were affected to differing degrees by parasite load, whereas supersedure of original queens was not. Undetectable levels of *V. destructor* in May of Trial 1 precluded an estimation of the relationship between colony mortality and initial infestation for the May–September period. For all of the other three subsequent time periods (September–January of Trial 1 and May–September and September–January of Trial 2), colony mortality increased significantly with initial *V. destructor* infestation at the beginning of the time period (respective odds ratios of 1.331, 1.155, and 1.148 for an increase in 1% infestation; with corresponding $P > \beta$ of <0.001, 0.02, and 0.004). In contrast, the initial infection with *N. ceranae* at the beginning of a time period did not have clear effects on survival except for the suggestion of an effect during September–January of Trial 2 (odds ratio of 2.464 for an increase in 1 million *N. ceranae*

individuals per bee; $P > \beta$ of 0.06). Supersedure of original queens was not affected by either of the two parasites (for all estimable time period–parasite combinations $P > \beta$ ranged from 0.13 to 0.57). For odds ratios that were significant overall, no differences between stocks were detected.

For colonies that survived with either supersedure or original queens, only during one of the four time periods (September–January of Trial 2) increasing levels of *V. destructor* and *N. ceranae* were significantly associated with decreasing colony populations (respective slopes of linear regressions: −0.35 frames per 1 mite per 100 bees with *V. destructor* and −1.92 frames per 1 million *N. ceranae* per bee; corresponding $P > \beta$ of <0.0001 and 0.03). There was also a significant positive effect of *N. ceranae* on colony growth in the May–September period of the first year (an increase of 0.07 frames per 1 million *N. ceranae* per bee; $P > \beta$ of 0.04). This effect was relatively small to observe negative effects and was probably biologically inconsequential.

Discussion

Overall, the two Varroa-resistant stocks functioned adequately in this model beekeeping system that relies on both honey production in Montana and almond pollination in California. Indeed, Russian honey bees appeared to be particularly well-suited for this U.S. beekeeping operation.

Survival of colonies, either with original queens or with supersedure queens, was similar among honey bee stocks for both years. In 2010–2011, 38% of 185 colonies had supersedure queens at the end of the experiment and 19% of the colonies died (40% of dead colonies previously had superseded). The 19% mortality compares favorably to the 30% losses estimated from a survey of U.S. beekeepers during the same period (vanEngelsdorp et al. 2012, Spleen et al. 2013). In 2011–2012, 23% of 175 colonies had supersedure queens at the end of the experiment and 41% of the colonies died (18% of dead colonies previously had superseded). More colonies were lost in this study than the overall nationwide report of 23%, but the 41% mortality is within the range reported by beekeepers (vanEngelsdorp et al. 2012). We did note high numbers of *V. destructor* in many of the colonies in the January 2012 inspection of the colonies, and *V. destructor* infestation associated significantly with mortality of colonies in 2011–2012. The frequencies of colony mortality and queen supersedures we observed each season are similar to those found in other contemporary studies of commercially managed honey bees (mortality, 25–68%; suprsedure 24–32%; Danka et al. 2012, Rinderer et al. 2013). The collective mortality and supersedure events in commercial colonies demonstrate both the general challenge of managing honey bees successfully and the specific challenge of maintaining the genetic integrity of the desirable stock.

Colony size is central to both honey production and almond pollination. Larger colonies tend to produce more honey and are considered more suitable for almond pollination. Colonies were markedly larger in May 2010 than in May 2011. In September, shortly after the honey production season, colonies of all stocks were reasonably large, although in 2010 Russian honey bee colonies had significantly fewer bees than CN colonies and significantly less brood than either CN or VSH colonies. Nonetheless, in 2010, Russian honey bees colonies produced as much honey as CN and VSH colonies. These results are consistent with other studies comparing Russian honey bees and Italian honey bees that found that Russian honey bees both produced as much or more honey as Italian honey bees and quickly reduced colony size when resources became scarce (Rinderer et al. 2001, 2004; Tubbs et al. 2003; Ward et al. 2008). All colonies were of similar size in January 2011, with ≈35% of CN and VSH, and 26% of Russian honey bee colonies having six frames or more of bees. In 2011–2012, no differences among stocks were noted for frames of bees or frames of brood during May or September, except Russian honey bees had more frames of brood in May. Despite having a similar size, Russian honey bee colonies produced less honey than CN colonies; VSH colonies were intermediate between the other stocks. This result stands in contrast to other studies where Russian honey bees produced as much or more honey than other stocks (Rinderer et al. 2001, 2004; Tubbs et al. 2003; Ward et al. 2008). In January 2012, Russian honey bees had more frames of bees than either CN or VSH colonies and more brood than CN colonies. In all, 48% of CN colonies, 40% of VSH colonies, and 87% of Russian honey bee colonies had six frames of bees, and 74% of Russian honey bee colonies had eight frames of bees. These differences likely arose from the differential parasitism of *V. destructor* or *N. ceranae* among the stocks given the significant negative associations we found between parasite level and colony growth for the September–January period.

Infestations of *V. destructor* in 2010–2011 were lower in Russian honey bee and VSH outcross colonies than in CN colonies in both September and January. By January, infestations in CN colonies were approaching a treatment threshold of 10% infestation even though in May infestations were below detectable levels. This comparatively lower level of *V. destructor* infestation is consistent with other studies of these two types of Varroa-resistant honey bees (reviewed in Rinderer et al. 2010c).

V. destructor infestations in 2011–2012 followed a very different pattern. In May, Russian honey bee colonies had significantly more mites on adult bees than either CN or VSH colonies. However, this was reversed by September and January when Russian honey bee colonies had significantly fewer mites than CN and VSH colonies. In January, the numbers of mites per 100 bees in CN and VSH colonies (17.2 and 18.0, respectively) were at levels considered to be damaging. This is the first instance of VSH not displaying resistance to *V. destructor*. The result was not strongly related to colonies that had supersedures of original VSH queens (i.e., colonies being outcrosses of

outcrosses) rather than original queens; infestations were 15.6 ± 7.4 ($n = 9$) and 20.3 ± 10.0 ($n = 11$) mites per 100 bees in colonies with original and supersedure queens, respectively. The unexpectedly poor colonies observed in mid-January, which in some cases had bees that appeared to be lethargic and in disorganized clusters, prompted a preliminary analysis of viral infections to supplement our standard analyses for levels of mites and *N. ceranae.* Titers of deformed wing virus and Israeli acute paralysis virus were greater in CN and VSH colonies than in Russian honey bees colonies, while titers of black queen cell virus were more similar among the bee types (J. Chen, unpublished data). Titers of all viruses were greater in colonies that were smaller than average than in colonies that were larger than average. This raises the possibility that deformed wing virus or Israeli acute paralysis virus infections diminished hygiene in VSH colonies. However, whatever factor diminished the expression of resistance in VSH bees did not affect the Varroa resistance of Russian honey bee colonies.

Although our results confirm the predictable destructive and debilitating effects of *V. destructor* on colonies, they provide another example of how *N. ceranae* manifests inconsistent effects at the field level. Colony survival and colony populations were affected by *N. ceranae* only in the period from September to January of Trial 2. *Apis mellifera carnica* Pollmann colonies in Switzerland (Dainat et al. 2012) were not affected by infections with *N. ceranae*, while Villa et al. (2013) in the United States and Higes et al. (2009) Spain, colony depopulation effects were found. No bee type showed any apparent resistance to *N. ceranae.*

In both years of this study *A. woodi* was detected in ≈35% of the colonies. However, infestations were generally low with only 11 colonies having infestations exceeding a treatment threshold of >20% (Nasr 2001). Despite the generally low levels of infestation, the data reported here reflect the previously reported resistance of Russian honey bees to *A. woodi* (de Guzman et al. 2001a,b; Villa and Rinderer 2008).

Numerous experiments have reported that winter feeding helps maintain or improve the size of colonies before the almond pollination season (DeGrandi-Hoffman et al. 2008; Rinderer et al. 2010a,b). However, in this experiment, the effects of short-term, midwinter feeding were minor. January colony sizes were numerically greater in 2010–2011 and identical in 2011–2012 for fed colonies compared with unfed colonies. The amount of brood in January was significantly more in fed colonies in 2010–2011 and numerically more in fed colonies in 2011–2012. Hence, although feeding had small positive effects, the chief determinants of colony sizes appear to be other environmental factors.

Our findings generally suggest that *V. destructor*-resistant honey bees are suitable to a beekeeping enterprise that focuses on both western honey production and almond pollination. However, their use, as is the case with all beekeeping, requires management that attends to the consequences of unusual weather patterns and is vigilant for unusual epizootic events.

Acknowledgments

We thank Honey Land Apiaries (Jim and Craig Rodenberg) for providing the colonies used in these experiments and for valuable beekeeping collaboration. Jack Brumley kindly fed the colonies while they were wintering in California. Technical assistance was provided by U.S. Department of Agriculture–Agricultural Research Service (USDA–ARS) personnel, Lorraine Beaman, Garrett Dodds, David Dodge, Sandra Hineman, Victor Rainey, J. Anthony Stelzer, Joshua Wales, Jeremy Wagnitz, Rachel Watts, and Daniel Winfrey. Judy Chen (USDA–ARS, Beltsville, MD) measured virus titers. Funding was provided by the USDA–ARS Areawide Program "Improving Honey Bee Health, Survivorship and Pollination Availability."

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Received 24 September 2013; accepted 7 February 2014.