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Functionality of *Varroa*-Resistant Honey Bees (Hymenoptera: Apidae) When Used in Migratory Beekeeping for Crop Pollination

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ABSTRACT Two types of honey bees, *Apis mellifera* L. (Hymenoptera: Apidae), bred for resistance to *Varroa destructor* Anderson & Trueman were evaluated for performance when used in migratory crop pollination. Colonies of Russian honey bees (RHB) and outcrossed bees with *Varroa*-sensitive hygiene (VSH) were managed without miticide treatments and compared with colonies of Italian honey bees that served as controls. Control colonies were managed as groups which either were treated twice each year against *V. destructor* (CT) or kept untreated (CU). Totals of 240 and 247 colonies were established initially for trials in 2008 and 2009, respectively. RHB and VSH colonies generally had adult and brood populations similar to those of the standard CT group regarding pollination requirements. For pollination of almonds [*Prunus dulcis* (Mill.) D.A.Webb] in February, percentages of colonies meeting the required six or more frames of adult bees were 57% (VSH), 56% (CT), 39% (RHB), and 34% (CU). RHB are known to have small colonies in early spring, but this can be overcome with appropriate feeding. For later pollination requirements in May to July, 94–100% of colonies in the four groups met pollination size requirements for apples (*Malus domestica* Borkh.), cranberries (*Vaccinium macrocarpon* Aiton), and lowbush blueberries (*Vaccinium angustifolium* Aiton). Infestations with *V. destructor* usually were lowest in CT colonies and tended to be lower in VSH colonies than in RHB and CU colonies. This study demonstrates that bees with the VSH trait and pure RHB offer alternatives for beekeepers to use for commercial crop pollination while reducing reliance on miticides. The high frequency of queen loss (only approximately one fourth of original queens survived each year) suggests that frequent requeening is necessary to maintain desired genetics.

KEY WORDS *Varroa destructor*, genetic resistance, migratory beekeeping, crop pollination, *Apis mellifera*

Honey bees, *Apis mellifera* L. (Hymenoptera: Apidae), serve as the chief pollinators of crops in the United States. This vital agricultural service is jeopardized as colony numbers decline from a myriad of health threats (National Research Council 2007). Parasitism by the mite *Varroa destructor* Anderson & Trueman ranks among the most consistently damaging of these threats. 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 resistant bees (Minnesota Hygienic, Russian honey bees [RHB], and bees with the trait of *Varroa*-sensitive hygiene [VSH]) are documented from field tests to suppress mite populations in colonies used for honey production (Rinderer et al. 2001a, Ibrahim et al. 2007, Ward et al. 2008). These bees have not been evaluated, however, for their performance when used during commercial-scale crop pollination. Colonies serving in crop pollination are managed differently and often more intensively than those used for honey production (Free 1993, Delaplane and Mayer 2000), and so bees may be stressed more when used for pollination. For example, colonies may be trucked frequently and over long distances to pollinate crops, whereas colonies usually remain stationary during honey production. Some crops, especially lowbush blueberry (principally *Vaccinium angustifolium* Aiton) and cranberry (*Vaccinium macrocarpon* Aiton), are notorious among beekeepers for being poor sources of nectar and pollen. In addition, exposure to pesticides is likely to be greater when bees are placed near intensively managed agricultural crops. Exposure to mites and other biological threats also may be greater because of the congregation of bees from many locations at important crops during bloom. These assorted stresses may interact to debilitate or kill colonies.

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Fig. 1. Map showing the routes and timing of shipments of honey bee colonies. The destination activities included winter management in St. Landry Parish, LA; almond pollination in Kern Co., CA; spring management in St. Landry Parish, LA; apple pollination in Columbia Co., NY; lowbush blueberry pollination in Washington Co., ME; cranberry pollination in Plymouth Co., MA; and recovery and honey production in Cattaraugus Co., NY. The migratory route was the same for each of the 2 yr of the study.

As noted above, previous research indicated the usefulness of RHB and bees with the VSH trait when used for honey production. In this study, we sought to determine how these two types of *Varroa*-resistant honey bees fared in an intensive crop pollination system in which honey production is incidental. We integrated these two stocks into a commercial beekeeping operation that primarily engages in pollination of major crops across the country. Here, we report good beekeeping functionality of the bees for migratory crop pollination. This information may be useful in helping beekeepers decide to move away from miticides and shift toward genetic solutions to manage *V. destructor*.

Materials and Methods

Experimental Setup. This demonstration of performance by two resistant types of honey bees was conducted in collaboration with a beekeeping company (Evergreen Honey Company, Bunkie, LA) in 2008 and 2009. Colonies were established in August to early September before each of the two test years by introducing queens of four types into colony divisions. This timing ensured that bee populations were of the experimental types when colonies went into winter. Pure RHB colonies were established with pure-mated RHB queens produced by commercial sources (Brachmann 2009). Outcross VSH colonies were established with queen cells grafted from VSH breeder colonies maintained by us or a commercial source (Glenn Apiaries, Fallbrook, CA). The virgin VSH queens were in colonies that were located in apiaries with the colonies managed by the collaborator and therefore mated with locally available drones (presumably mostly from the collaborator's colonies) to produce VSH outcross colonies. The collaborator established control colonies (CT and CU as described below) with queen cells grafted from a commercial source of "Italian" honey bees (Latshaw Apiaries, New Albany, OH). Virgin queens from these cells also mated in the same area with local drones that presumably were of the same stock. Queens of all four types were paint-marked for identification. Colonies were kept in 10-frame Langstroth hives configured as one deep (23.7-cm) box and one medium (16.8-cm) box (in autumn, winter, and spring) or one deep and two medium boxes (in summer). Observations began on 240 colonies (CT, 61; CU, 39; RHB, 54; and VSH, 86) in 2008 and 247 colonies (CT, 62; CU, 52; RHB, 73; and VSH, 60) in 2009.

After the colonies were established, they were managed as usual by the collaborator except regarding treatments against *V. destructor*. Each colony was fed ≈1.8 kg of pollen substitute (BeePro patties, Mann Lake, Ltd., Hackensack, MN) and 6 liters of high-fructose corn syrup in October and January. All colonies were medicated in the spring and autumn against *Paenibacillus larvae* (causative agent of American foulbrood disease) with tylosin tartrate (Tylan, Elanco, Indianapolis, IN), against *Nosema* spp. with fumagillin (Fumagilin B, Medivet, High River, AB, Canada) and against *Aethina tumida* Murray (small hive beetles) with coumaphos (Check Mite+, Mann Lake, Ltd.) in bottom board traps. Half of the control colonies (group CT) were treated against *V. destructor*, once in March with τ-fluvalinate (Mavrik Aquaflow [diluted 1:4 with water], Wellmark International, Schaumburg, IL) and once in September with amitraz (undiluted Taktic, Hoechst Roussel Vet, Somerville, NJ). The other half of the control colonies (group CU) and the RHB and VSH colonies were not treated against *V. destructor*.

Evaluation of Colony Performance. Each year, colonies were transported four times to different sites to pollinate crops and three times to sites for recovery and possible honey production (Fig. 1). Soon after arrival at each site, each colony was inspected to verify the presence of the marked queen and to estimate population size. The data collected at each new site

reflected colony performance at the previous site. Colonies that lost their original queens were eliminated from later evaluations. Populations of adult bees were estimated as "frames of bees," a commonly used metric when assessing the size of colonies rented for pollination (Traynor 1993). In each hive box, we counted the number of spaces between the top bars of frames that were at least two thirds filled with bees (similar to Nasr et al. 1990). Populations of brood were measured as "frames with brood," i.e., the number of combs that had brood covering an area of $\geq$160 cm^2 ($\geq$25 in^2). This technique of estimating brood size is used by lowbush blueberries growers to assess the size of rental colonies. A medium-depth comb was converted to 0.67 of a deep comb for analyses of bee and brood populations. Crop growers and beekeepers traditionally establish pollination contracts for renting colonies based on minimum bee or brood populations. Typical requirements are six to eight frames of bees for almonds [*Prunus dulcis* (Mill.) D.A.Webb], six frames of bees for apples (*Malus domestica* Borkh.) and cranberries, and six to eight frames with brood for lowbush blueberries. Growers may pay bonuses for larger colonies.

Populations of parasitic mites and *Nosema* infection in each colony were assessed several times each season. Infestations of *V. destructor* were measured near the beginning of each replication (October 2007 and January 2009) and in late spring, late summer, and autumn each year. Each colony was sampled by taking $\approx$300 adult worker bees from the broodnest. Samples were returned to the laboratory and kept frozen until analysis. We agitated the bees in a detergent solution for 30 min to dislodge mites from bees, and counted mites and bees to determine mites per 100 bees (Rinderer et al. 2004b). Infestations by *Acarapis woodi* Rennie (tracheal mites) and infections with *Nosema* spp. were measured from randomly selected colonies four times in 2008 (January, March, June, and October) and three times in 2009 (January, June, and November). Adult worker bees sampled for these evaluations were taken from inside the cover or from the upper box of each hive and stored frozen. The prothoracic tracheal trunks of 30 bees per colony were examined at 30$\times$ magnification with a stereomicroscope to determine prevalence of *A. woodi;* we report both the percentage of infested colonies for each of the four bee types, and the percentage of infested bees within a colony. Fifty bees were ground whole and submitted to a multiplex, real-time polymerase chain reaction (PCR) assay to quantify DNA sequences of both *N. apis* and *N. ceranae* (Bourgeois et al. 2010). *N. apis* was found to represent just 0.002% of total *Nosema* DNA in the first two sample periods (January and March 2008), so subsequent analyses only quantified DNA of *N. ceranae.*

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 using analysis of variance (ANOVA) (Proc MIXED; all statistical tests performed with SAS 9.2, SAS Institute 2009) applied to a split-plot design. Main units were the four bee types (CT, CU, RHB, and VSH) arranged completely randomly with colonies as replicates within each type; subunits were repeated measures over the eight sampling dates (four sampling dates for *V. destructor*) each year. Data within each year were analyzed separately. Several repeated measure covariance parameters were considered for the subunit, and the parameter with the best fit was used for analysis of each variable. In cases where there was a significant interaction of bee type and date for each 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 chi-square tests (Proc FREQ). Effects were considered to be significant at $P \leq 0.05$.

We evaluated survivorship of colonies and original queens for the four bee types in two ways. We compared the frequencies of colonies of three classes (those that retained original queens through the entire season, those that superseded queens at any time, and those that died) with chi-square tests on data summed within each year (Proc FREQ). We also estimated longevity of colonies with original queens and compared this among bee types within each year (logrank test in Proc LIFETEST, followed by verification and mean separation with ANOVA). We used the last date that an original queen was seen to calculate the survival time for that colony. Observations were censored if the later fate was unknown; this included colonies that survived with original queens until the end of each year. Because we do not know how long these queens and colonies continued to live, our estimated longevities underestimate actual longevities.

Results

Population of Adult Bees. In 2008, the analysis of population size showed no significant interaction between honey bee stock and sampling date (with date representing the location and crop being pollinated) ($F = 1.14$; df = 21, 1,374; $P = 0.298$). Adult bee populations averaged over the entire season differed among the four honey bee types ($F = 3.78$; df = 3, 1,374; $P = 0.010$). CT and VSH outcross colonies were larger than CU colonies; RHB colonies were intermediate in size and not different from the larger or smaller groups (Fig. 2a). Sizes of adult bee populations varied through time ($F = 494.76$; df = 7, 1,374; $P < 0.001$). Populations showed a slight increase in March after pollinating almonds and then doubled in size during spring management in Louisiana (March–April) and peaked in late May after pollinating apples. Populations then declined steadily during pollination of blueberries and cranberries, during postpollination management into autumn and upon relocation to Louisiana in October for overwintering. Colonies averaged 5.2 frames of bees while pollinating almonds and averaged 13–17 frames of bees while pollinating apples, blueberries, and cranberries.

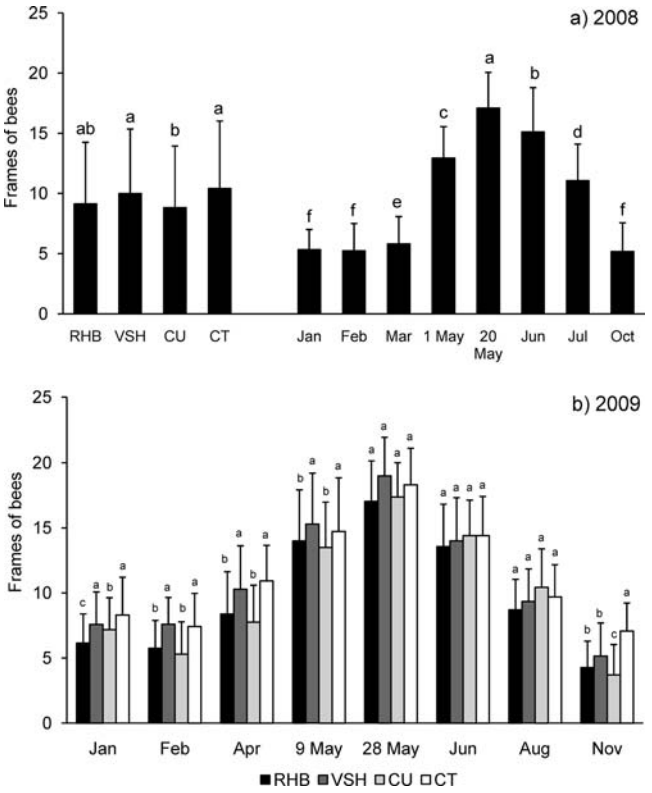

Fig. 2. Populations of adult bees (mean ± SD) in test colonies in 2008 (a) and 2009 (b). The main effects of bee type and sampling date were significant in 2008; these effects interacted in 2009. For comparisons between bee types and between sampling dates (2008) or within each date (2009), means that do not share a common letter differ at $P \leq 0.05$ according to ANOVA.

The general pattern of seasonal population dynamics observed in 2008 was repeated in 2009. Bee populations grew from January until colonies were placed in blueberries in mid-May, then populations declined through autumn (Fig. 2b). However, there was a significant interaction between honey bee type and sampling date ($F = 2.41$; df = 21, 1,064; $P < 0.001$). CT and VSH colonies were larger then CU and RHB colonies until mid-May. All bee types then had similar bee populations until November, when CT were largest, CU were smallest, and RHB and VSH were intermediate. Colonies had ≈5.5 (CU and RHB) to 7.5 (CT and VSH) frames of bees during almond pollination and ≈13–19 frames of bees during pollination of apples, blueberries, and cranberries.

Population of Brood. In 2008, the number of frames with brood varied with sampling date ($F = 630.79$; df = 6, 1,159; $P < 0.001$) but not with bee type ($F = 1.50$; df = 3, 1159; $P = 0.212$), and there was no interaction between these main effects ($F = 1.53$; df = 18, 1,159; $P = 0.071$). Brood populations averaged for all bee types increased from 1.3 frames with brood in February to 12.2 frames with brood in late May when bees were located on blueberries and then declined to 1.8 frames with brood in late autumn (Fig. 3a).

In 2009, brood populations were affected by bee type ($F = 10.61$; df = 3, 1,064; $P < 0.001$) and sampling date ($F = 312.12$; df = 7, 1064; $P < 0.001$) and there was no interaction between the main effects ($F = 1.36$; df = 21, 1,064; $P = 0.130$) (Fig. 3b). Overall, CT and VSH outcross colonies produced more brood (6.4 frames with brood) than CU and RHB colonies (5.4 frames with brood). The seasonal production of brood followed the trend observed in 2008. Colonies averaged 11.0 frames of brood when they were rented for blueberry pollination.

Infestation With *V. destructor*. Analysis of mite infestations on adult bees showed a significant interaction between honey bee type and sampling date ($F = 8.86$; df = 3, 595; $P < 0.001$) in 2008. In October 2007, soon after colonies were established and before they were composed fully of worker bees from the test queens, infestations were relatively low (0.5–2.2 *V. destructor* per 100 bees) but varied among the initial colony divisions that were randomly assigned to the different bee types (VSH ≥ RHB ≥ CU > CT) (Fig. 4a). Infestations increased in all untreated groups through the rest of the season but at varying rates among bee types. Infestations rose most in CU and RHB colonies and reached 14.2 *V. destructor* per 100 bees in October. Infestation in the VSH outcross colonies rose until midsummer and then rose only slightly more (to 8.1 *V. destructor* per 100 bees) by October. In the CT group, infestation increased to 6.8 *V. de-*

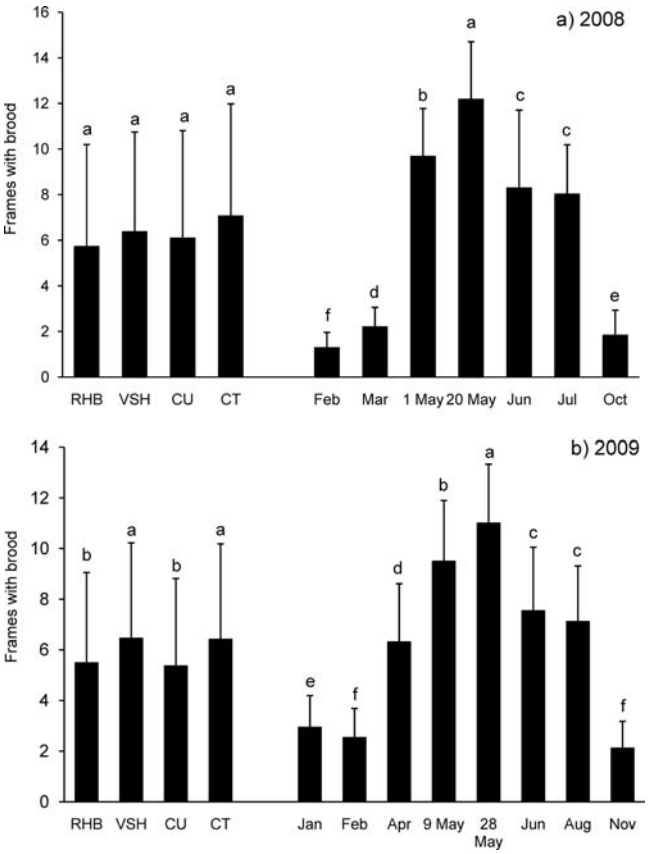

Fig. 3. Number of frames (mean ± SD) with significant patches of brood (i.e., brood covering ≥160 cm^2) in test colonies in 2008 (a) and 2009 (b). The main effects of bee type and sampling date were significant in each year. For comparisons between bee types and between sampling dates, means that do not share a common letter differ at $P \leq 0.05$ according to ANOVA.

structor per 100 bees in summer despite a miticide treatment in April, and then decreased to 1.1 *V. destructor* per 100 bees in the autumn after a miticide treatment in August.

A significant interaction ($F = 10.60$; df = 9, 490; $P < 0.001$) between honey bee type and sampling date also occurred in 2009. The initial measurement of infestation in January (when colonies had the genetics of test queens) showed relatively high infestations in the untreated groups and significant differences among the four bee types (Fig. 4b). The overall infestations decreased by April, when CU and RHB had greater infestations (6.2 *V. destructor* per 100 bees) than CT and VSH (3.8 *V. destructor* per 100 bees). Infestations increased slightly by June and were similar among all bee types. In November, infestations increased in CU, RHB, and VSH outcross colonies (6.7–10.5 *V. destructor* per 100 bees) but decreased in CT colonies (1.4 *V. destructor* per 100 bees) after a late summer miticide treatment.

Infestation With *A. woodi*. *A. woodi* were detected in 19% (105 of 548) of colony samples. The proportion of colonies infested by *A. woodi* varied among the bee types ($\chi^2 = 18.01$, df = 3, $P < 0.001$); 32% of CT colonies versus 13–17% of CU, RHB, and VSH outcross colonies had detectable mites. Overall, the percentage of bees infested within individual colonies averaged 1% (range, 0–40%). Only five colony samples showed infestations at levels considered to be damaging (i.e., 20% or more bees infested per colony; Nasr 2001). These included two CT colonies in January 2008, one CT colony in March 2008, one CU colony in June 2008, and one VSH colony in October 2008.

Infection With *Nosema*. In 2008, a significant interaction between honey bee type and sampling date ($F = 2.76$; df = 9, 144; $P = 0.005$) was detected for the number of *Nosema* per bee. *Nosema* infections were greater in CT colonies than in the other three groups in January, July, and October (Fig. 5a). CU colonies had greater infections than the other groups in March. Season-long averages of the number of *Nosema* per bee were 1.6×10^6 for CT, 0.6×10^6 for CU, 0.3×10^6 for VSH, and 0.2×10^6 for RHB.

In 2009, no effects occurred due to bee type ($F = 2.20$; df = 3, 238; $P = 0.088$) or sampling time ($F = 2.65$; df = 2, 238; $P = 0.073$), and there was no two-way interaction ($F = 1.63$; df = 6, 238; $P = 0.138$) (Fig. 5b). Season-long averages of the number of *Nosema* per

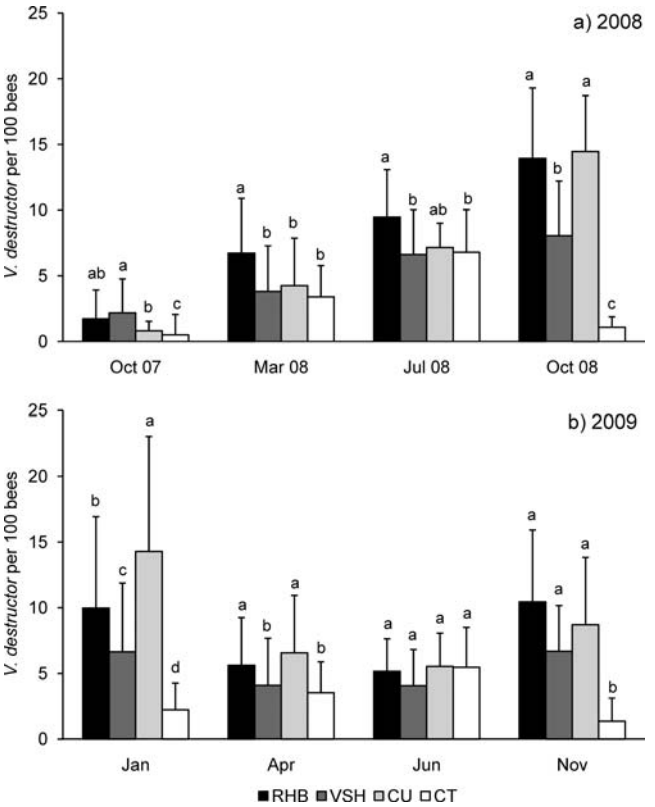

Fig. 4. Adult bee infestations by *V. destructor* (mean ± SD) in test colonies in 2008 (a) and 2009 (b). The main effects of bee type and sampling date interacted in each year. For comparisons within each date, means that do not share a common letter differ at $P \leq 0.05$ according to ANOVA.

bee were 0.9×10^6 for CU, 0.9×10^6 for VSH 0.6×10^6 for RHB and 0.5×10^6 for CT.

Longevity of Colonies. Similar proportions of the four bee types had colonies that were classified as live with original queen, live with superseded queen (or with queen cells), or dead (2008: $\chi^2 = 5.37$, df = 6, $P = 0.498$; 2009: $\chi^2 = 11.80$, df = 6, $P = 0.065$). Only approximately one fourth (29% in 2008; 23% in 2009) of colonies that were shipped to California for almond pollination in February were alive with original queens when they returned to Louisiana in October (Table 1). Overall, queens were superseded in 35% of colonies in 2008 and 53% in 2009, and 37% of colonies died in 2008 and 24% in 2009. The estimated longevities of original queens did not differ among the bee types in 2008 ($\chi^2 = 3.79$, df = 3, $P = 0.286$). In 2009, however, longevities of queens differed among the bee types ($\chi^2 = 10.35$, df = 3, $P = 0.016$). Estimated longevities were greatest for CT and VSH queens, significantly less for RHB queens and intermediate (and similar to the other groups) for CU queens (Table 1).

Discussion

This study demonstrated that two types of *Varroa*-resistant honey bees functioned well for commercial crop pollination in a migratory beekeeping operation. Colony performance in each of 2 yr indicated that both RHB and outcrossed VSH bees not treated against mites generally compared favorably with the miticide-treated honey bees that currently are being used by our collaborator.

The adult bee population of a colony normally is the key consideration when establishing rental contracts for pollination of three of the crops (almonds, apples, and cranberries) involved in this study. The minimal acceptable colony size is typically six combs that are two thirds covered by adult bees. All bee types had sufficient size to rent for pollination of apples in early May (94–99% of colonies of the four bee types) and cranberries in June (95–100% of colonies). There was a marked difference among bee types, however, for the percentage of colonies meeting the standard for almond pollination in February. VSH outcross (57%) and CT (56%) colonies performed better than RHB (39%) and CU (34%) colonies. This discrepancy in bee populations in early spring may be due to differences in characteristics of the stocks. In contrast to Italian honey bees, RHB tend to have small populations in early spring but build up rapidly when pollen becomes reliably available (Tubbs et al. 2003), as was seen here. Supplemental feeding of 50:50 pollen and pollen substitute over winter can boost colony sizes of RHB in

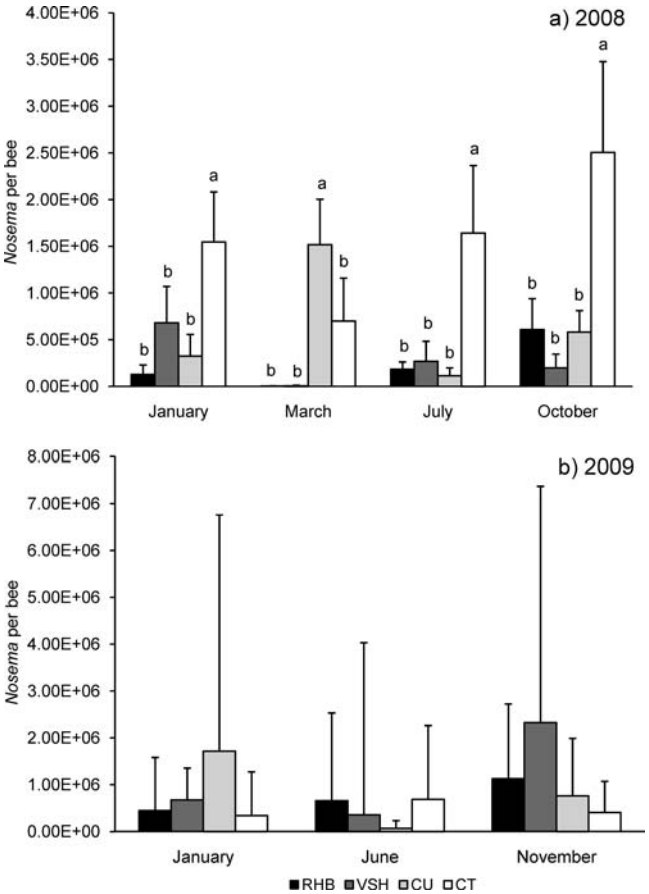

Fig. 5. Number of *Nosema* per bee (mean ± SD) in fumagillin-treated test colonies in 2008 (a) and 2009 (b). The main effects of bee type and sampling date interacted in 2008; no effects were seen in 2009. Within each date in 2008, means that do not share a common letter differ at $P \leq 0.05$ according to ANOVA.

early spring to an average of >10 frames of bees (Rinderer et al. 2012).

Brood populations (assessed as the number of frames with brood) were used to evaluate colonies for rental for pollination of lowbush blueberries in late May. All bee types met the rental requirement of six frames with brood, and 71–87% had 10 or more frames with brood and so earned a bonus payment.

Table 1. Percentages of colonies known to survive with original queens (OQ) and with supersedure queens (SSQ), and colonies known to die with original queens

Yr	Bee type	*n*	OQ[a]	SSQ[a]	Dead[a]	Longevity (d)[b]
2008	CT	61	25	29	46	188 ± 10a
	CU	39	23	31	46	168 ± 15a
	RHB	54	30	44	26	177 ± 13a
	VSH	86	37	35	28	201 ± 10a
2009	CT	62	29	52	19	175 ± 13a
	CU	52	21	60	19	163 ± 13ab
	RHB	73	12	51	37	130 ± 11b
	VSH	60	28	50	22	177 ± 12a

Also shown are estimated longevities (mean ± SE) of colonies with OQ. Bee types are given in the text.

[a] Within each year, the four bee types did not differ ($P \leq 0.05$; chi-square test) in the distribution of colonies among the three fates (OQ, SSQ, and dead).

[b] Within each year, means followed by the same letter are not significantly different ($P \leq 0.05$; ANOVA).

Colony health is an important factor in successfully using honey bees for pollination. Response to the primary health threat, *V. destructor*, varied among bee types. Among the untreated groups, VSH outcross bees tended to have comparatively good resistance to *V. destructor*. Importantly, an economically useful level of resistance was achieved in these VSH colonies despite half of the genetics of the worker bees presumably having come from drones of the mite-susceptible control stock. It is a common practice in large-scale commercial beekeeping to introduce queens via queen cells and then let the queens outcross with local drones. Using VSH to introgress mite resistance seems to be well suited for this application. Because the VSH trait is genetically additive (Harbo and Harris 2001), mite resistance in a beekeeping operation should improve over time as colonies with VSH queens begin producing drones with the VSH trait, and newly introduced queens mate with those drones.

Infestations of *V. destructor* on adult bees in RHB colonies during much of the beekeeping season were greater than those we have seen in tests of RHB used for honey production (Rinderer et al. 2001a, Ward et al. 2008) and often were similar to those of CU bees. This observation could reflect greater invasion rates from *V. destructor* produced in CU colonies present in the same apiaries. Indeed, Rinderer et al. (2004a) found that infestations of *V. destructor* in RHB colonies tend to increase when they were intermingled with susceptible bees in an apiary. This trend may be exacerbated during pollination if the colonies are weakened significantly. It is also common for RHB colonies to have a higher proportion of *V. destructor* on adult bees while maintaining lower infestations in capped brood (infestation of brood was not measured here), thereby protecting developing brood (Rinderer et al. 2001b; de Guzman et al. 2007). The mite loads during January to April tended to be greater in RHB and CU colonies than in VSH and CT colonies, but we cannot say whether this impacted colony sizes during almond pollination. Despite variation in mite loads, all groups performed well enough in the time frame of the experiment to meet rental requirements for pollination of apples, blueberries, and cranberries.

The two different miticide treatments used in CT bees produced different outcomes against *V. destructor*. The late summer treatment with amitraz was very effective despite known resistance to this miticide in some populations of *V. destructor* around the world (Elzen et al. 2000, Rodríguez-Dehaibes et al. 2005). Conversely, the spring treatment with fluvalinate had no apparent effect, i.e., infestations of *V. destructor* changed similarly in CT and CU colonies from spring to summer in both years. Poor control may have come from resistance of the mites to fluvalinate, which is known to occur in the United States (Elzen et al. 1999). Our observations suggest that the spring treatment in this beekeeping operation could be eliminated, and just the summer treatment relied upon to suppress *V. destructor*.

Infestations of *A. woodi* and infection with *Nosema* spp. generally were below thresholds recognized as causing damage to bees, and there was no apparent relationship between levels of these parasites and colony size or survival. The proportion of colonies infested by *A. woodi* increased during each year in all four bee types, but in CT bees the late summer treatment with amitraz against *V. destructor* also markedly reduced infestation with *A. woodi*. Semiannual treatment with fumagillin may have suppressed any potential differences between stocks for response to *Nosema*. RHB colonies consistently had comparatively low infestations of *A. woodi* and infections with *Nosema* spp.

There was a relatively high frequency of loss of queens of all types, with only approximately one fourth of the original queens surviving through a year. Queen failures came at various times during the season and probably came from many causes. We observed some swarming activities when colonies were most populous in May and June during pollination of apples and lowbush blueberries. During this time, supersedure rates or queenlessness were high both in 2008 (RHB, 39%; VSH, 28%; CU, 26%; and CT, 16%) and in 2009 (RHB, 33%; VSH, 30%; CU, 31%; and CT, 35%). Frequent queen losses are an obstacle to successfully applying genetic solutions such as mite resistance to beekeeping issues. It suggests that requeening colonies at least annually would be needed to maintain desirable genetics. Discovering the causes and remedies for queen failures would be useful to support implementation of resistance as a mite management strategy. Note that the pure RHB queens and their daughters and the VSH queens would produce drones having genetics for mite resistance that would be passed to colonies produced from new introduced virgin queens or supersedure queens.

Our findings suggest that pure RHB (with management considerations) and outcross VSH bees offer functional options for the expanding sector of beekeeping engaged in commercial crop pollination. The adoption of resistant bees would help beekeepers, who currently rely extensively on miticides for managing *V. destructor*, to maintain colony health with reduced in-hive pesticide use.

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