chamber is somewhat misleading, as the surface area counted in the brood chamber is larger than that of the inner cover.

Colonies showed a normal build-up of brood throughout the summer and reduction of brood in fall and winter. As colony strength increased, beetle numbers also increased as observed from this study and a previous investigation (Nolan and Hood 2008). While SHB population increased over the summer months, only two test colonies died during the study, however, the minimum colony losses could not be conclusively linked to SHB pressure. Trapping in both the bottom and top of the colonies may have contributed to the high survival rate (92%).

Apiary location was considered as a possible variable in beetle numbers and colony strength. Care was taken to select locations with similar sun and shade; however, other factors not realized might have played a role in both beetle and honey bee colony survivorship. Regardless of the unrealized differences in apiary location, the results show that beetle numbers and colony strength were similar in all five apiaries. This result is based on similar sun and shade exposure, similar colony strength measured in $25cm^2$ brood units, and similar existing beetle populations. Individual colonies did have different beetle numbers; however, the mechanism by which beetles "choose" one colony over another still needs to be investigated.

Conclusion and Recommendations

Our results support the practice of trapping small hive beetles in honey supers. Traps placed in the top honey super preformed equally to traps placed in the brood chamber. By trapping in the top honey super beekeepers can avoid damaging the queen and the lifting of heavy honey supers. Several traps are available for purchase that can be utilized in the top honey supers, as well as the brood chamber, including the Hood beetle trap used in this study. While trapping small hive beetles is one method of beetle control it is best used as part of an IPM program. Tapping will not eliminate small hive beetles from a colony but can decrease the population to below a critical level. The best method for small hive beetle control is to maintain strong healthy colonies by using good beekeeping practices.

Acknowledgements

We thank William Bridges, Department of Applied Economics and Statistics, Clemson University, Clemson, South Carolina, for his assistance with statistical analysis. Funding for this research was provided partially by USDA/ARS – CSREES Special Research Grants Program: Pest Management Alternatives Program. This material is based upon work supported by the CSREES/USDA, under project number SC-1700235. This is technical contribution No. 5545 of the Clemson University Experiment Station.

References

Ellis, J.D., K.S. Delaplane, R. Hepburn, and P.J. Elzen. 2002. *Controlling small hive beetles (*Aethina tumida *Murray) in honey bee (*Apis mellifera*) colonies using a modified hive entrance.* Am. Bee J. 142: 288-290.

Ellis, J.D., K.S. Delaplane, R. Hepburn, and P.J. Elzen. 2003. *Efficacy of modified hive entrances and a bottom screen device for controlling* Aethina tumida *(Coleoptera: Nitidulidae) infestations in* Apis mellifera *(Hymenoptera: Apidae) colonies.* J. Econ. Entomol. 96: 1647-1652.

Elzen, P. J., Baxter, J.R., Westervelt, D., Randall, C., Delaplane, K.S., Cutts, L., W. T. Wilson. 1999. *Field control and biology studies of a new pest species,* Aethina tumida *Murray (Coleoptera, Nitidulidae), attacking European honey bees in the Western Hemisphere.* Apidologie. 30: 361-366.

Hood, W.M. 2004. *The small hive beetle,* Aethina tumida: *a review.* Bee World 85(3): 51-59.

Hood, W.M. and G.A. Miller. 2003. *Trapping Small Hive Beetles (Coleoptera: Nitidulidae) Inside Colonies of Honey Bees (Hymenoptera: Apidae).* Am. Bee J. 143(5): 405-409.

Hood, W.M. and G.A. Miller 2005. *Evaluation of an Upper Hive Entrance for Control of* Aethina tumida *(Coleoptera: Nitidulidae) in Colonies of Honey Bees (Hymenoptera: Apidae).* J. Econ. Entomol. 98(6): 1791-1795.

Levot, G.W. 2008. *An Insecticidal Refuge Trap to Control Adult Small Hive Beetle,* Aethina tumida *Murray (Coleoptera: Nitidulidae) in Honey Bee Colonies.* J. Apic. Res. 47(3): 222-228.

Nolan IV, M.P. and W.M. Hood. 2008. *Comparison of Two Attractants to Small Hive Beetles,* Aethina tumida, *in Honey Bee Colonies.* J. Apic. Res. 47(3): 229-233.

Torto, B., R.T. Arbogast, H. Alborn, A. Suazo, D.V. Engelsdorp, D. Boucias, J.H. Tumlinson, P.E.A. Teal. 2007a. *Composition of volatiles form fermenting pollen dough and attractiveness to the small hive beetle* Aethina tumida, *a parasite of the honey bee* Apis mellifera. Apidologie 38: 380-389.

Torto, B., R.T. Arbogast, D.V. Engelsdorp, S. Willms, D. Purcell, D. Boucias, J.H. Tumlinson, P.E.A. Teal. 2007b. *Trapping of* Aethina tumida *Murray (Coleoptera: Nitidulidae) from* Apis mellifera *L. (Hymenoptera: Apidae) Colonies with an In-Hive Baited Trap.* Envir. Entomol. 36(5): 1018-1024.

SAS Institute. 1992. *SAS/STAT user's guide, version 6, 4th ed.* SAS Institute, Cary, NC.

Hygienic responses to Varroa destructor by commercial and feral honey bees from the Big Island of Hawaii before exposure to mites

Robert G. Danka, Jeffrey W. Harris and José D. Villa

USDA-ARS Honey Bee Breeding, Genetics and Physiology Laboratory, 1157 Ben Hur Road, Baton Rouge, Louisiana 70820

Summary

The important honey bee queen production industry on the Big Island of Hawaii is threatened by the recent discovery of *Varroa destructor* on the island. We tested the pre-exposure level of resistance to mites of three sources of commercial Hawaiian bees and feral Hawaiian bees based on their expression of varroa sensitive hygiene (VSH), i.e., the removal of mite infested brood. Experimental colonies were started in Baton Rouge, LA, from local mite infested bees and test queens from Hawaii. We included reference groups of bees with high VSH, and mite susceptible bees. After worker populations represented the test queens, we added a comb of mite infested brood to each colony for one week and measured the subsequent change in infestation resulting from hygiene. Colonies started from commercial and feral Hawaiian queens hygienically removed similar amounts (33-45% on average per source) of mite infested brood in one week. These responses were numerically intermediate between those of the resistant VSH bees (91% removal) and the susceptible bees (9% removal). There was large colony-to-colony variation within each commercial and feral source. We also measured the mite population growth in

colonies during nine weeks. Mite population growth did not differ among the sources although it ranged from -51% for VSH bees to -11 to +53% for the other types. The results indicate that existing commercial and feral Hawaiian bees have some resistance to *V. destructor* based on hygienic response to mite infested brood. This response in commercial stock probably is derived from selection for general hygiene and from importations of germplasm from the U.S. mainland. The variable response of individual colonies suggests that resistance could be improved by testing and selection within the existing Hawaiian bee population.

Keywords: *Apis mellifera*, mite resistance, varroa sensitive hygiene, VSH, bee breeding

Introduction

Hawaii supports an important honey bee (*Apis mellifera*) queen rearing industry that produces more than 250,000 queens annually. The parasitic mite *Varroa destructor* only recently was discovered on the Big Island of Hawaii (August 2008) but is expected to soon threaten the bees used for queen production. Mites were found on the eastern side of the island, away from the three queen production operations on the western side. We sought to gauge the potential impact of *V. destructor* on managed colonies used for queen production when the mite population expands to the west. Commercially produced queens in Hawaii are propagated from a variety of breeder sources, most of which have been selected for hygienic response to freeze-killed brood. Such selection for general hygiene improves resistance to *V. destructor* (Spivak and Reuter 2001). Information about the relative mite resistance of the island's bees, even before the bees experience direct selection pressure from the mite, could help guide beekeeper decisions about strategies for managing mites and what breeding sources to use for queen production. Simple variation in response to *V. destructor* among existing colonies would suggest that there is an opportunity to select for improved mite resistance.

The extensive population of feral honey bees on Hawaii could impact the dynamics of infestation there. These bees may be descended largely from bees that survived the extensive colony mortality caused by American foulbrood disease that occurred in the islands beginning about 1930 (Roddy and Arita-Tsutsumi 1997, Eckert 1950). The existing feral population may have hygiene that was enhanced by this selection event. Hygiene against brood diseases also affords some resistance to *V. destructor* (Spivak 1996).

We tested commercial and feral bees of Hawaii for their hygienic response to *V. destructor* when colonies are challenged with mite infested brood. We routinely make such measurements as part of a breeding program that is focused on enhancing varroa sensitive hygiene (VSH), a well-characterized mechanism that confers significant mite resistance (Harbo and Harris 2005, Villa et al. 2009). In colonies with bees expressing high VSH, mite infested pupae are removed and there is poor fecundity of remaining mites; mite populations therefore tend to decline or grow only slowly. Our measures of VSH should serve to show the status of, and potential for, one means of resistance to *V. destructor* mites in Hawaiian bees.

Materials and Methods

Thirty-two colonies were made by dividing colonies infested with *V. destructor* at Baton Rouge, LA in late April 2009. Each colony was started with three to four combs having adhering bees, unsealed brood, honey, pollen and empty cells. Colonies were in single story, 10-frame standard hives. Small hive beetles, *Aethina tumida*, were managed by placing a treatment station (half of a coumaphos [CheckMite®; Mann Lake, Hackensack, MN] strip under a piece of corrugated plastic) on the bottom board for the first week after colonies were established. The density of *V. destructor* was measured on samples of adult bees (324 ± 89 [std. dev.] bees per colony), and then colonies were assigned to six treatment groups (queen sources) that had similar average mite density (8.7 ± 0.3 mites per 100 bees). Each of the three commercial sources (Hawaiian Queen Co., Kona Queen Co. and Olivarez Honey Bees) and the feral Hawaiian population were represented by six queens. Two reference types, mite resistant VSH and a mite susceptible control, were represented by four queens each. Feral queens were captured by Hawaii Department of Agriculture (HDOA) personnel from colonies in swarm traps around Hilo, HI, within the three weeks prior to the test. These queens produced colonies of dark bees that often were nervous on the combs. VSH queens were pure VSH from our breeding program. The susceptible queens were purchased from a commercial, U.S. mainland source whose bees in previous testing showed relatively little resistance to *V. destructor* (unpub. obs.). For nine weeks, empty combs were added to the colonies as needed. Queens that failed were replaced with queens of the same type when possible. Data from commercial sources are reported anonymously.

VSH activity was evaluated in each colony 10 weeks after the test was set up (when all bees were from the resident queen). Mite infestation was measured in a brood comb from a donor colony (not in the test), and then the comb was inserted into the broodnest of a test colony. The comb was retrieved after one week and mite infestation was measured again in bees of the same age cohort. We measured initial infestation in 150 cells containing larvae, prepupae or white-eyed pupae; beginning infestation was 14.4 ± 4.4%. We measured final infestation in 200 cells containing bees that ranged from purple-eyed, tan-bodied pupae to pre-emergent adults. The percentage removal of infested brood was calculated as ([initial infestation – final infestation] / initial infestation)*100.

We also estimated the mite population growth for colonies that still had original queens nine weeks after the test was started (n = 5-6 for commercial and feral bees, and n = 2 for susceptible and VSH bees). The initial mite population of each colony in April was calculated from the total weight of adult bees (measuring hives with bees and then after removing bees) and the mite density in a sample of bees (66.7 ± 10.1 g). After nine weeks, we repeated the calculation of mites on adult bees, and also calculated mites in brood based on a measurement of the total area of sealed brood per colony and infestation in a sample of 200 cells of sealed brood. The percentage change in the mite population was calculated as ([final population – initial population] / initial population)*100.

Effects of queen types on response variables were made with analysis of variance (PROC MIXED; SAS Institute 2000) after verifying normality and homogeneity of variances between queen types. Means separation was based on t-tests of least square means. Pearson's correlation (PROC CORR) was used to assess linear relationships between variables.

Results

Colonies of the various queen sources differed in their expression of VSH ($P < 0.001$). Hawaiian commercial and feral colonies hygienically removed similar amounts of mite infested brood within one week (P = 0.433 – 0.932 for pairwise

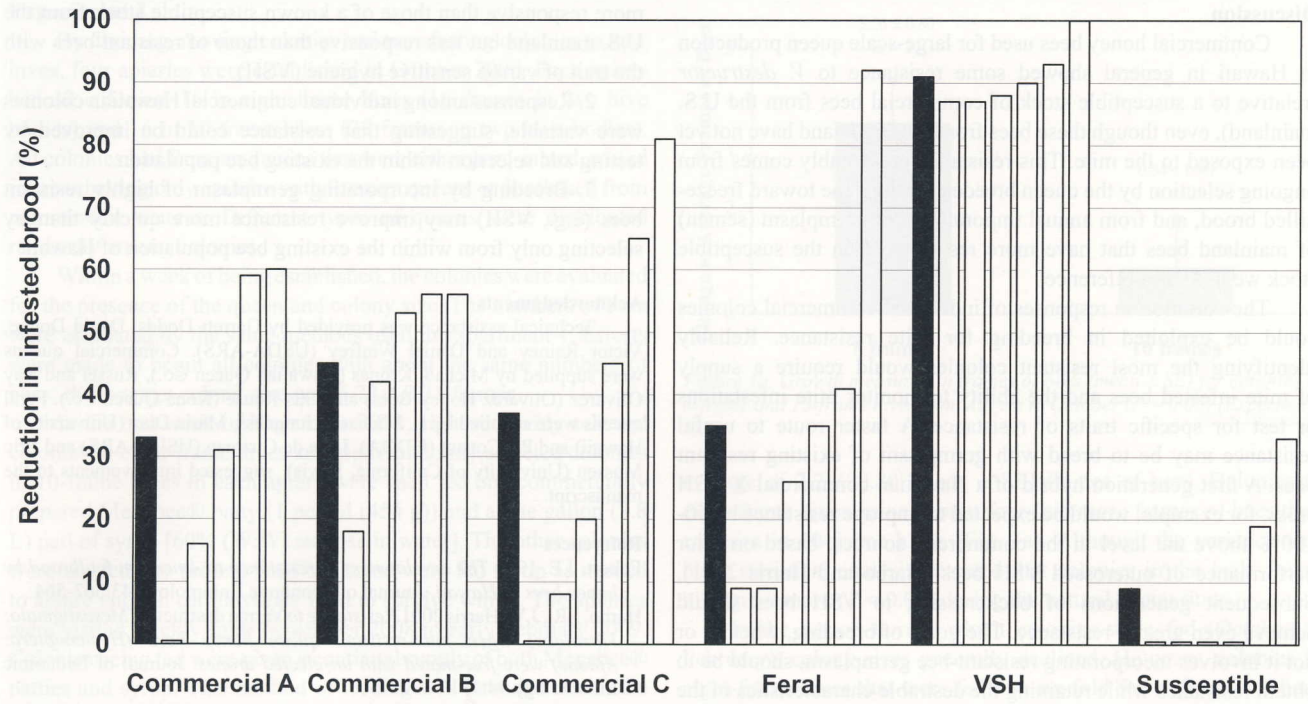

Figure 1*. Decrease in infestation of V. destructor in brood after a 1-week exposure in test colonies. Black bars are the average response of each queen source. White bars are data for individual colonies, arranged in ascending order within each queen source.*

comparisons), with averages of 33-45% removal per group (Figure 1). These responses were numerically intermediate between those of the resistant VSH (91% removal) and the susceptible control (9% removal). Commercial source B removed significantly more mites than the susceptible control ($P = 0.020$). Responses of the two other commercial sources and the feral bees trended toward greater removal than the susceptible control but did not differ significantly ($P = 0.059$ - 0.112) from it. There was large colony-to-colony variation within each of the commercial and feral sources. Pure VSH colonies removed significantly more mite infested pupae than all other bee types did ($P < 0.001$ - 0.005).

Mite population growth during nine weeks did not differ among the queen sources ($P = 0.683$) (Figure 2) and was quite variable among colonies. On average, mite populations declined by 51% in VSH colonies and either declined slightly (-11 to -16%) or increased (31 to 53%) in the other groups. Mite population growth was related inversely to removal of infested brood ($r = -0.411$, $n = 27$, $P = 0.033$).

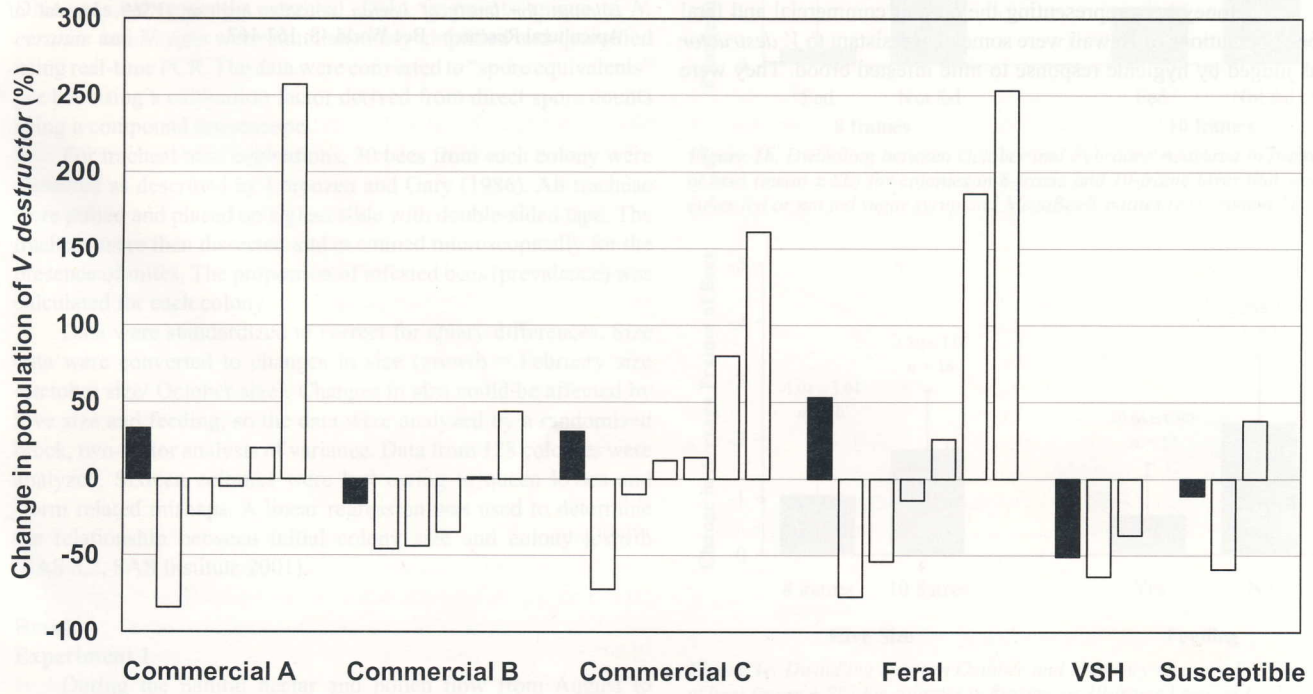

Figure 2. *Change in populations of V. destructor during the nine-week test. Data arranged as in Figure 1.*

Discussion

Commercial honey bees used for large-scale queen production in Hawaii in general showed some resistance to *V. destructor* (relative to a susceptible stock of commercial bees from the U.S. mainland), even though these bees from the Big Island have not yet been exposed to the mite. This resistance presumably comes from ongoing selection by the queen breeders for hygiene toward freeze-killed brood, and from annual importations of germplasm (semen) of mainland bees that have more resistance than the susceptible stock we used as a reference.

The variation in responses of individual commercial colonies could be exploited in breeding for mite resistance. Reliably identifying the most resistant colonies would require a supply of mite infested bees and the ability to monitor mite infestations or test for specific traits of resistance. A faster route to useful resistance may be to breed with germplasm of existing resistant bees. A first generation hybrid of a Hawaiian commercial X VSH cross, for example, would be expected to improve resistance by 50-100% above the level in the commercial sources, based on prior performance of outcrossed VSH bees (Harbo and Harris 2001). Subsequent generations of backcrossing to VSH bees should achieve even greater resistance. The goals of breeding, whether or not it involves incorporating resistant-bee germplasm, should be to obtain resistance while retaining the desirable characteristics of the bees currently produced in Hawaii.

Feral Hawaiian bees responded to *V. destructor* about the same as the commercial bees did, and therefore do not appear to offer an immediate source of mite resistant breeding material. The mechanism of resistance in the feral bees is unknown. If these bees have a resistance mechanism other than that found in the commercial bees, some feral bees might be beneficial breeding sources. We only monitored expression of VSH based resistance to *V. destructor*, but other mechanisms of resistance occur (Spivak and Boecking 2001).

Conclusions and Recommendations

1. Honey bees representing the current commercial and feral bee populations of Hawaii were somewhat resistant to *V. destructor* as judged by hygienic response to mite infested brood. They were more responsive than those of a known susceptible stock from the U.S. mainland but less responsive than those of resistant bees with the trait of varroa sensitive hygiene (VSH).

2. Responses among individual commercial Hawaiian colonies were variable, suggesting that resistance could be improved by testing and selection within the existing bee population.

3. Breeding by incorporating germplasm of highly resistant bees (e.g., VSH) may improve resistance more quickly than by selecting only from within the existing bee population of Hawaii.

Acknowledgments

Technical assistance was provided by Garrett Dodds, David Dodge, Victor Rainey and Daniel Winfrey (USDA-ARS). Commercial queens were supplied by Michael Krones (Hawaiian Queen Co.), Russel and Ray Olivarez (Olivarez Honey Bees) and Gus Rouse (Kona Queen Co.). Feral queens were supplied by L. Michael Klungness, Maria Diaz (University of Hawaii) and Pat Conant (HDOA). Lilia de Guzman (USDA-ARS) and Eric Mussen (University of California, Davis) suggested improvements to the manuscript.

References

Eckert, J.E. 1950 *The development of resistance to American foulbrood by honey bees in Hawaii.* Journal of Economic Entomology 43:562-564.

Harbo, J.R., J.W. Harris 2001 *Resistance to* Varroa destructor *(Mesostigmata: Varroidae) when mite-resistant queen honey bees (Hymenoptera: Apidae) were free-mated with unselected drones.* Journal of Economic Entomology 94:1319-1323.

Harbo, J.R., J.W. Harris 2005 *Suppressed mite reproduction explained by the behaviour of adult bees. Journal of Apicultural Research* 44: 21-23.

Roddy, K.M., L. Arita-Tsutsumi 1997 *A History of Honey Bees in the Hawaiian Islands.* Journal for Hawaiian and Pacific Agriculture 8:59-70.

SAS Institute 2000 *The SAS system, version 8.01.* SAS Institute, Cary, NC.

Spivak, M. 1996 *Honey bee hygienic behavior and defense against Varroa jacobsoni. Apidologie* 27: 245-260.

Spivak, M., O. Boecking 2001 *Honey bee resistance to varroa mites.* In Mites of the Honey Bee, Webster, T.C., K.S. Delaplane eds. Dadant & Sons, Hamilton, IL, 205-227.

Spivak, M., G.S. Reuter 2001 Varroa jacobsoni *infestation in untreated honey bee* (Apis mellifera) *colonies selected for hygienic behavior.* Journal of Economic Entomology 94:326-331.

Villa, J.D., R.G. Danka, J.W. Harris 2009 *Simplified methods of evaluating colonies for levels of varroa sensitive hygiene (VSH).* Journal of Apicultural Research / Bee World 48: 162-167.