Varroa destructor Infestation in Untreated Honey Bee (Hymenoptera: Apidae) Colonies Selected for Hygienic Behavior

MARLA SPIVAK AND GARY S. REUTER

Department of Entomology, 219 Hodson Hall, University of Minnesota, 1980 Folwell Avenue, St. Paul, MN 55108

J. Econ. Entomol. 94(2): 326–331 (2001)

ABSTRACT Honey bee (*Apis mellifera* L.) colonies bred for hygienic behavior were tested in a large field trial to determine if they were able to resist the parasitic mite *Varroa destructor* better than unselected colonies of "Starline" stock. Colonies bred for hygienic behavior are able to detect, uncap, and remove experimentally infested brood from the nest, although the extent to which the behavior actually reduces the overall mite-load in untreated, naturally infested colonies needed further verification. The results indicate that hygienic colonies with queens mated naturally to unselected drones had significantly fewer mites on adult bees and within worker brood cells than Starline colonies for up to 1 yr without treatment in a commercial, migratory beekeeping operation. Hygienic colonies actively defended themselves against the mites when mite levels were relatively low. At high mite infestations (>15% of worker brood and of adult bees), the majority of hygienic colonies required treatment to prevent collapse. Overall, the hygienic colonies had similar adult populations and brood areas, produced as much honey, and had less brood disease than the Starline colonies. Thus, honey bees bred for hygienic behavior performed as well if not better than other commercial lines of bees and maintained lower mite loads for up to one year without treatment.

KEY WORDS *Apis mellifera*, hygienic behavior, *Varroa destructor* mite resistance

BREEDING HONEY BEES *(Apis mellifera* L.) for resistance to the injurious parasitic mite *Varroa destructor* Anderson and Trueman (2000) (formerly called *Varroa jacobsoni* Oudemans) is a current priority in apiculture. A reasonable goal for a breeding program is to select honey bees that have heritable mechanisms of defense against the mites that allow them to tolerate infestation longer than unselected colonies before chemical treatments are required. Current chemical control practices for the mite within the United States most often involve the use of highly effective pesticides within the hive: either the synthetic pyrethroid fluvalinate (Apistan), or more recently, the organophosphate coumaphos (CheckMite). With the recent discovery of fluvalinate-resistant mites, many beekeepers are realizing that prolonged use of these high-efficacy pesticides is not a sustainable practice (Milani 1999) and that it is critical to implement more integrated control practices. Such integrated practices will vary depending on the size and associated labor costs of the hobby or commercial beekeeping operation. However, the foundation of any integrated program is the availability of selected lines of bees that demonstrate resistance mechanisms against the mites. These lines should retain genetic variability, and should have no fitness costs, such as reduced honey production or susceptibility to diseases, that may be associated with the traits that confer resistance (Bailey 1999).

The range of heritable defense mechanisms that honey bees display against *Varroa* has been reviewed recently in Harbo and Harris (1999) and Boecking and Spivak (1999). We are investigating one behavioral mechanism of defense, hygienic behavior, because it is known to be the primary mode of resistance against two diseases of honey bee brood, American foulbrood (Rothenbuhler 1964), and chalkbrood (reviewed in Spivak and Gilliam 1998a, 1998b), and thus is of broad economic interest in apiculture. Hygienic bees detect and remove diseased larvae and pupae from the wax cells. They also detect and remove a portion of worker pupae infested with *Varroa* (Peng et al. 1987; Boecking and Drescher 1991, 1992; Spivak 1996). The bees uncap and remove the majority of mite-infested cells 4–7 d after the cell is capped (Spivak 1996, Thakur et al. 1997), when offspring of the invading foundress mite are developing on the capped pupa. The removal of infested pupae thus limits the number of offspring of the mites by interrupting their reproductive cycle (Rath and Drescher 1990, Fries et al. 1994). An important goal of the present research was to determine the extent to which the behavior actually reduces the overall mite-load in infested colonies.

In a previous study, we evaluated the performance of hygienic honey bee colonies in a commercial apiary (Spivak and Reuter 1998a). After 1 yr without treatment, the hygienic colonies had less disease, fewer mites on adult bees, and produced significantly more honey than the control colonies from an unselected line of honey bees. Although the results appeared promising, we repeated the study for several reasons. First, after 1 yr without treatment, all colonies had a very low level of mites, <2%, on the adult bees, which

0022-0493/01/0326–0331$02.00/0

was well under an estimation of the economic threshold for this pest (Delaplane and Hood 1999), and we wanted to test the hygienic colonies under higher parasite pressure. Second, in the previous study, we counted the number of mites only in their phoretic stage on adult bees, and did not count the number of mites in the colony that were reproducing within brood cells. Thus, in the current study we obtained a better estimation of mite load within the hygienic colonies by inspecting the infestation levels both on adults and within brood cells. Finally, we compared the hygienic bees with a commercial line of bees that is more renown for honey production to ensure that our previous finding that the hygienic colonies produced more honey was not misleading. As before, we compared colonies from a hygienic and a control line, each containing queens that were allowed to mate naturally with unselected drones in the same location to ensure that any observed differences in mite load or honey production were due solely to the genetic source of queens.

Materials and Methods

Hygienic Breeding Stock. The hygienic queens used in the experiment were bred from colonies of Italian-derived *Apis mellifera* and were maintained at the University of Minnesota. The degree of hygienic behavior was determined by a freeze-killed brood assay in which the time was recorded for colonies to detect, uncap, and remove brood from a comb section (5 by 6 cm, containing ≈100 capped larvae and pupae per side of the comb) that had been cut from a frame within the brood nest of the same or different colony, frozen at −20°C for 24 h, and placed in the nest of the test colony (Spivak and Downey 1998). Colonies that removed the freeze-killed brood from the comb section within 48 h on two trials were considered hygienic. To establish and maintain a hygienic line of bees, beginning in 1993, queen bees were raised from colonies that consistently removed at least 95% of the freeze-killed brood within 48 h. Each daughter queen was instrumentally inseminated (II) with 6–8 μl semen from drones collected from other unrelated colonies with similar removal rates. The colonies containing the II queens were wintered and tested again using the freeze-killed brood assay in the following spring. Only the colonies that removed 100% of the freeze-killed brood within 48 h and also had good wintering ability, strong populations in spring, and no visible signs of chalkbrood or other brood diseases were considered breeder colonies. Daughter queens were propagated in the next generation from these breeder colonies. To ensure adequate genetic variability, one or two new hygienic queens were selected each year from different queen producers throughout the United States and were included in the breeding program.

Field Methods. Hygienic breeder colonies containing fourth-generation II queens from the University of Minnesota were wintered in Amite County, MS, in 1996 in an apiary owned by a commercial beekeeper. The wintered colonies were treated for *Varroa* mites using Apistan strips (according to the label) in October 1996. All strips were removed from the colonies in December. In February 1997, daughter queens were reared from two of the hygienic queens. Additional queens were reared from two colonies with inseminated queens of commercial "Starline" stock (Italian descent) also wintered in Mississippi. The Starline colonies were chosen on the basis of the size of both the population of adult bees and the brood area, but were not selected for hygienic behavior.

Daughter queens from both hygienic and Starline colonies mated naturally with the drones from the surrounding area in Mississippi. Each queen was marked with enamel paint on the thorax to distinguish her by line. In May 1997, the hygienic and Starline colonies were transported to Minnesota and distributed among four apiaries. The colonies were situated on pallets, and each pallet contained two hygienic and two Starline colonies. After October 1996, the colonies were not treated to control *Varroa* until the termination of the experiment in March 1998. The colonies were not given any treatments for the tracheal mite, *Acarapis woodi*, but all colonies were given treatments of oxytetracycline (TM-25 in powdered sugar) to suppress American foulbrood, *Paenibacillus larvae*, in the fall of 1997, and again in spring of 1998, as part of the commercial beekeeper's routine practice.

On 8 May 1997, each colony with a marked queen was evaluated for colony strength, degree of hygienic behavior, presence of brood diseases, and mite infestation on adult bees. Colony strength was estimated from the number of frames covered by bees (following Nasr et al. 1990), and the number of frames containing brood (cells contained egg, larvae, or pupae). The degree of hygienic behavior was evaluated using a modified assay, in which ≈160 cells containing sealed brood were freeze-killed with liquid nitrogen (Spivak and Reuter 1998b). The amount of freeze-killed brood completely removed after 24 h was recorded. The presence or absence of chalkbrood and American foulbrood infection was determined by examining three frames in the center of the brood nest containing brood of all stages in development. The number of *Varroa* mites on adult bees was calculated by collecting samples of ≈800–1000 bees from each colony into enough 70% ethanol to cover the bees, and handshaking each sample to dislodge the mites. The number of mites per sample was counted and from the weight of bees in each sample and a known weight of 100 wet bees, the number of mites per 100 bees was calculated.

After the May evaluations, the colonies were provided with honey supers ad libitum. From 18 to 20 August 1997 the honey was harvested. The amount of honey produced was measured by weighing each super (box) of honey as it was removed from each colony. The tare weight of the supers and frames was calculated by averaging the weight of 20 supers and frames after the honey was extracted.

On 28 September 1997, samples of adult bees were collected to determine *Varroa* infestation from all

colonies with marked queens. The number of cells containing sealed brood was determined for a subset of 12 hygienic and 12 Starline colonies (three colonies of each type were randomly selected from each apiary) by counting square centimeters of brood using a wire grid sectioned into 5 by 5-cm squares. At that time of year, there was relatively little brood in the colonies because most queens had ceased egg laying for the winter. The percentage of pupae infested with *Varroa* was determined in 11 colonies of each type (one of each of the 12 had no brood). One or two frames containing pupae within 1–3 d of ecolsion were removed from the colonies and taken to the laboratory where they were inspected under a dissection microscope for mite infestation. Depending on the brood area of the colony, 50–200 worker pupae were examined per colony. The number of foundress mites that were in the brood cells was counted. Foundresses were easily distinguished from daughter mites (deutonymphs) because the latter were lighter in cuticular coloration.

In November, a subset of the original colonies (39 hygienic and 37 Starline colonies) were transported to Mississippi for the winter. As is typical of migratory beekeeping operations, the colonies were first moved to a common "holding yard" or apiary in Minnesota before they were transported together to Mississippi on a netted truck. In Mississippi, the colonies were randomly assigned to one of two apiaries. In February 1998 all colonies were inspected for marked queens. *Varroa* infestation on adults was determined for all colonies, and brood infestation was determined as above for 12 hygienic and 12 nonhygienic colonies. The experiment was terminated at that time. In all, the colonies were left untreated for *Varroa* for 15 mo.

Measurements of honey production, number of *Varroa* on adult bees, and percent mite infestation in sealed worker brood were analyzed using two-way analysis of variance (ANOVA) to separate the effects of bee type (hygienic versus Starline) and apiary site (PROC GLM, SAS Institute 1995).

Results

Colony Evaluations and Honey Production. In May 1997, 64 hygienic colonies and 57 Starline colonies contained marked queens. The evaluations of colony strength indicated the mean numbers of frames of bees and brood in the two colony types were not significantly different: the hygienic and Starline colonies had (mean ± SD) 15.1 ± 2.56 and 13.6 ± 2.67 frames of bees and 9.3 ± 1.57 and 8.7 ± 2.14 frames of brood, respectively.

The May 1997 evaluations of presence or absence of chalkbrood mummies in the colonies demonstrated that 19 of the 64 (29.7%) hygienic colonies and 36 of the 57 (63.2%) Starline colonies had chalkbrood infections. No American foulbrood infection was noted in either the hygienic or Starline colonies.

The hygienic colonies removed significantly more freeze-killed brood within 24 h than the Starline colonies (61.6% ± 20.08 versus 49.9% ± 20.06, respectively; $F = 6.058$; df = 1,100; $P = 0.016$). There was a significant apiary effect ($F = 3.133$; df = 3, 100; $P = 0.029$); both hygienic and Starline colonies removed more brood at apiary 3 than at apiary 1, but there was no interaction between the two bee lines and apiary site.

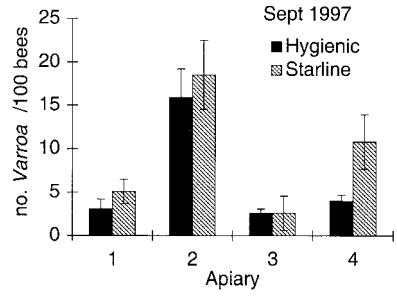

Fig. 1. *V. destructor* infestation on adult bees (mean ± SE), standardized as the number of mites/100 bees, 28 September 1997, within 63 hygienic and 54 Starline colonies distributed among four apiaries in Minnesota.

An average of 51.3 ± 20.98 kg (112.8 ± 46.16 lb) of honey was harvested from 63 hygienic colonies in late August 1996 (one queen had superseded since May). In comparison, an average of 46.1 ± 19.23 kg (101.5 ± 42.30 lb) honey was harvested from 54 Starline colonies (three queens had superseded since May). The difference in honey production was not significant ($F = 2.10$; df = 1, 110; $P = 0.150$). There was a significant apiary effect ($F = 5.63$; df = 3, 110; $P = 0.001$) because the colonies in apiary 4 produced significantly more honey than the colonies in apiary 3, but there was no significant interaction between the lines of bees and apiary site.

***Varroa* Infestations.** Of the samples of mites collected from adult bees in May 1997, six of the 64 (9.4%) hygienic colonies and 22 of 57 (38.6%) of the Starlines had detectable infestations. However, all colonies had fewer than one mite per 100 bees, indicating a very low infestation among all colonies. By late September, all colonies had detectable infestations of mites on adult bees (Fig. 1). The hygienic colonies had significantly fewer mites than the Starline colonies (log transformed data: $F = 4.563$; df = 1, 94; $P = 0.035$). There was a significant apiary effect; apiary 2 had the highest infestation of mites (>15 mites/100 bees on average). The colonies in the other three apiaries on average had ≤5 mites/100 bees, except for the Starline colonies in apiary 4, which had an average of 10.8 mites/100 bees.

An average of 12% of the sealed brood was inspected for mites from both the hygienic and Starline colonies (Table 1). The mean percent infestation of worker pupae in the hygienic colonies was significantly lower than in the Starline colonies ($F = 4.34$; df = 1, 14; $P = 0.0561$). There was a significant apiary effect ($F = 5.03$; df = 3, 14; $P = 0.0143$); the colonies in apiary 2 had significantly more infested brood, but there was no significant interaction term (Fig. 2).

There was no difference between the sets of colonies in the percentage of worker pupae infested by

Table 1. Percent of worker brood (mean ± SD) that was infested in a subset of the hygienic and Starline colonies in late September, 1997 and February, 1998

Date	Colony type	No. of colonies	% infested	% 1 foundress	% >1 foundress	No. cells inspected (% total brood)
Sept. 1997	Hygienic	11	15.7 ± 15.25a	9.1 ± 7.05a	6.6 ± 9.02a	164.0 ± 55.2 (11.8)
	Starline	11	32.2 ± 22.97b	12.0 ± 6.75a	20.2 ± 19.05b	86.0 ± 51.9 (12.0)
Feb. 1998	Hygienic	12	31.6 ± 8.36a	14.4 ± 4.26a	18.0 ± 6.40a	127.3 ± 44.5 (3.8)
	Starline	12	30.6 ± 12.01a	10.2 ± 3.75b	20.0 ± 9.54a	133.3 ± 49.2 (3.3)

Within a column and sample date, means followed by the same letter indicate no significant difference between hygienic and Starline colonies ($P < 0.05$).

one foundress ($F = 0.76$; df = 1, 14; $P = 0.3981$), but the Starline colonies had significantly more pupae infested by more than one foundress mite ($F = 4.35$; df = 1, 14; $P = 0.0552$) (Table 1). For both measures, there was a significant difference between colonies at apiaries 2 and 3, but no significant interaction terms. We did not estimate the foundress' fertility by counting the number of deutonymphs (female offspring) in the cells. However, of the total number of infested cells inspected, only seven cells (0.45%) among the hygienic colonies and two cells (0.30%) among the Starline colonies contained a live foundress with no offspring.

There was no significant difference in the number of *Varroa* on adult bees in February 1998: the 30 surviving hygienic colonies had 36.9 ± 19.97 mites per 100 bees, and the 32 surviving Starline colonies had 37.4 ± 24.05 ($F = 0.091$; df = 1, 52; $P = 0.764$). The mite levels in the hygienic colonies were higher than in the Starline colonies in apiary 1, whereas the reverse was true in apiary 2 (interaction term between bee line and apiary: $F = 3.504$; df = 1, 52; $P = 0.067$). However, when the apiaries were analyzed separately, there were no significant differences in the number of mites on adult bees between the hygienic and Starline colonies (*t*-test: apiary 1, $P = 0.134$; apiary 2, $P = 0.279$). On average, 3.5% of the sealed worker brood was inspected in all colonies for *Varroa* infestation, and there was no difference in overall percent infestation of the brood ($F = 0.13$; df = 1, 20; $P = 0.727$ (Table 1). The hygienic colonies had significantly more cells infested with just one foundress ($F = 7.03$; df = 1, 20; $P = 0.015$) but there was no difference between bee lines in the percent cell infested by more than one foundress ($F = 0.24$; df = 1, 20; $P = 0.629$). There was no difference in infestation among the two apiaries.

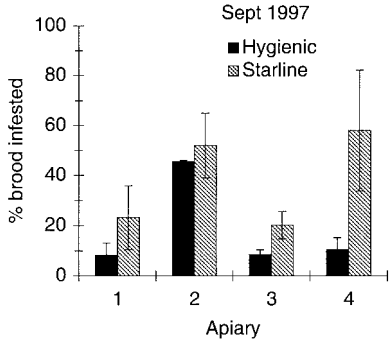

Fig. 2. Percentage of worker brood cells infested with *V. destructor* mites (mean ± SE) on 28 September 1997 within 11 hygienic and 11 Starline colonies distributed among four apiaries in Minnesota.

Discussion

The results show that colonies with naturally mated queens from a line bred for hygienic behavior had fewer mites on adult bees and within worker brood cells than colonies not bred for this behavior for up to 1 yr without treatment in a commercial beekeeping operation. The migratory beekeeping practice of temporarily situating colonies in a common apiary before transporting them together by truck to another state apparently had the effect of ameliorating differences in mite loads. Placing many colonies in close proximity increases the opportunity for infested bees to drift from one colony to another, and for robbing bees that steal honey from weak, mite-infested colonies to bring mites back into their own colony, leading to more uniform distribution of mites throughout all the colonies.

In late September 1997 the four apiaries had very different levels of mite infestation on adult bees even though the apiaries were situated within 5 km of each other. The percentage of worker brood that was infested with mites in the hygienic colonies, however, was half of that in the Starline colonies. The hygienic colonies also had a significantly smaller proportion of worker cells infested with more than one foundress, which supports studies showing that colonies remove more experimentally mite-infested pupae if the pupae are infested with more than one foundress (Boecking and Drescher 1991, 1992; Spivak 1996). The proportion of cells infested by more than one foundress, particularly in the Starline colonies was very high, most likely due to the paucity of fifth-instar worker brood that the mites could invade at the time of year when the colonies were sampled (28 September). The percentage of mites that were infertile (laid no progeny) is small relative to other studies (e.g., Rosenkranz

and Engels 1994, Boot et al. 1997, Medina and Martin 1999) but the reason is unknown.

In late February 1998, after the colonies were moved to Mississippi, the remaining hygienic and Starline colonies had very high and equivalent mite loads, both on adult bees and within worker brood. Most colonies were near collapse at that time due to the high degree of parasitism. The average percent infestation of mites in worker brood in February was equivalent to that of the Starline colonies in September 1997; approximately one-third of the brood was infested. There was more worker brood in the hygienic colonies that was infested with one foundress, but a similar amount of brood was infested with more than one foundress. This finding suggests that the hygienic colonies were not able to actively reduce the mite load as effectively under high parasite pressure. Our interpretation is that hygienic behavior is mediated by olfactory cues emanating from abnormal (dead, diseased, or parasitized) brood (Masterman et al. 2000, Spivak and Gilliam 1998b); and at very high mite levels, the bees may habituate to the odor cues that elicit the behavior and are not able to detect individually infested cells. In this case, the bees may cease to detect and remove abnormal brood. Other data reflect this possibility (Spivak 1996), but physiological experiments to confirm habituation or precise behavioral experiments to confirm the reduction of the behavior under extreme disease or parasite pressure have not been performed.

The hygienic colonies had similar populations and brood areas, produced as much honey, and had less chalkbrood than the Starline colonies. These results confirm our previous findings (Spivak and Reuter 1998a) that colonies bred for hygienic behavior suffer no apparent fitness costs, and perform as well, if not better, than commercial stocks not bred for hygienic behavior in two different commercial beekeeping operations. In both experiments, the differences between the hygienic and commercial colonies was due to the genetic effects of the queens, because all queens took their mating flights at the same time from common apiaries, and so encountered the same pool of unselected drones with which to mate.

An important goal of the present research was to determine the extent to which hygienic behavior reduces the mite-load in infested colonies. This experiment and our previous one (Spivak and Reuter 1998a) demonstrate that colonies with naturally mated queens bred for hygienic behavior actively defend themselves against the mites when mite levels are relatively low. At high mite infestations (e.g., >15% of worker brood and >15% of adult bees) hygienic colonies eventually will collapse unless treated. It remains to be determined if colonies with hygienic queens mated to a greater proportion of hygienic drones would survive mite infestations for longer periods, as would occur if more beekeepers selected for the trait from among their commercial stocks of bees. It is currently unreasonable to assume that honey bees bred for hygienic behavior will survive indefinitely without some sort of periodic treatment. However, it is encouraging that lines bred for hygienic behavior may require less frequent treatments than unselected lines. Any reduction in pesticide use within colonies translates into lower operating costs for the commercial beekeeper and decreased risk of contaminating honey and hive products.

Acknowledgments

We thank Pete Vos for providing us with the commercial bee colonies and apiary sites, raising and marking the queens, keeping track of the colonies as they were transported back and forth between Mississippi and Minnesota, and helping with the colony evaluations. We also thank David Tronrud and Rebecca Masterman for their assistance with different phases of this study. The research was funded by grants from the North Central Region Sustainable Agriculture Research and Education (SARE LWF 62-016-03716) with matching funds from beekeeping associations in Minnesota, Wisconsin, North Dakota, South Dakota, and California. Special thanks to the Minnesota Honey Producers Association and the Minnesota Hobby Beekeeping Association for their ongoing generous support of this research. This is publication no. 00117-0022 from the Agricultural Experiment Station of the University of Minnesota.

References Cited

Anderson, D., and J.W.H. Trueman. 2000. *Varroa jacobsoni* (Acari: Varroidae) is more than one species. Exp. Appl. Acarol. 24: 165–189.

Bailey, L. 1999. The quest for a super-bee. Bee World 80: 55–60.

Boecking, O., and W. Drescher. 1991. Response of *Apis mellifera* L. colonies infested with *Varroa jacobsoni* Oud. Apidologie 22: 237–241.

Boecking, O., and W. Drescher. 1992. The removal responses of *Apis mellifera* L. colonies to brood in wax and plastic cells after artificial and natural infestation with *Varroa jacobsoni* Oud. and to freeze-killed brood. Exp. Appl. Acarol. 16: 321–329.

Boecking, O., and M. Spivak. 1999. Behavioral defenses of honey bees against *Varroa jacobsoni* Oud. Apidologie 30: 141–158.

Boot, W. J., N. Q. Tan, P. C. Dien, L. V. Huan, N. V. Dung, L. T. Long, and J. Beetsma. 1997. Reproductive success of *Varroa jacobsoni* in brood of its original host, *Apis cerana*, in comparison to that of its new host, *A. mellifera* (Hymenoptera: Apidae). Bull. Entomol. Res. 87: 119–126.

Delaplane, K. S., and W. M. Hood. 1999. Economic threshold for *Varroa jacobsoni* Oud. in the southeastern USA. Apidologie 30: 383–395.

Fries, I., S. Camazine, and J. Sneyd. 1994. Population dynamics of *Varroa jacobsoni:* a model and a review. Bee World 75: 5–28.

Harbo, J. R., and J. W. Harris. 1999. Selecting honey bees for resistance to *Varroa jacobsoni.* Apidologie 30: 183–196.

Masterman, R., B. H. Smith, and M. Spivak. 2000. Evaluation of brood odor discrimination abilities in honey bees (*Apis mellifera* L.) using proboscis extension reflex conditioning. J. Insect Behav. 13: 87–101.

Medina, L. M., and S. J. Martin. 1999. A comparative study of *Varroa jacobsoni* reproduction in worker cells of honey bees (*Apis mellifera*) in England and Africanized bees in Yucatan, Mexico. Exp. Appl. Acarol. 23: 659–667.

Milani, N. 1999. The resistance of *Varroa jacobsoni* Oud. to acaricides. Apidologie 30: 229–234.

Nasr, M. E., R. W. Thorp, T. L. Tyler, and D. L. Briggs. 1990. Estimating honey bee (Hymenoptera: Apidae) colony strength by a simple method: measuring cluster size. J. Econ. Entomol. 83: 748–754.

Peng, Y. S., Y. Fang, S. Xu, L. Ge, and M. E. Nasr. 1987. Response of foster Asian honey bee (*Apis cerana* Fabr.) colonies to the brood of European honey bee (*Apis mellifera* L.) infested with parasitic mite *Varroa jacobsoni* Oudemans. J. Invertebr. Pathol. 49: 25–264.

Rath, W., and W. Drescher. 1990. Response of *Apis cerana* Fabr. towards brood infested with *Varroa jacobsoni* Oud. and infestation rate of colonies in Thailand. Apidologie 21: 311–321.

Rosenkranz, P., and W. Engels. 1994. Infertility of *Varroa jacobsoni* females after invasion into *Apis mellifera* worker brood as a tolerance factor against varroatosis. Apidologie 25: 402–411.

Rothenbuhler, W. C. 1964. Behavior genetics of nest cleaning in honey bees. IV. Responses of F_1 and backcross generations to disease-killed brood. Am. Zool. 4: 111–123.

SAS Institute. 1995. SAS user's guide, version 6.10. SAS Institute, Cary, NC.

Spivak, M. 1996. Hygienic behavior and defense against *Varroa jacobsoni.* Apidologie 27: 245–260.

Spivak, M., and D. Downey. 1998. Field assays for hygienic behavior in honey bees (Hymenoptera: Apidae). J. Econ. Entomol. 91: 64–70.

Spivak, M., and M. Gilliam. 1998a. Hygienic behaviour of honey bees and its application for control of brood diseases and varroa mites. Part I: Hygienic behaviour and resistance to American foulbrood. Bee World 79: 124–134.

Spivak, M., and M. Gilliam. 1998b. Hygienic behaviour of honey bees and its application for control of brood diseases and *Varroa* mites. Part II: Studies on hygienic behaviour since the Rothenbuhler era. Bee World 79: 165–182.

Spivak, M., and G. S. Reuter. 1998a. Performance of hygienic honey bee colonies in a commercial apiary. Apidologie 29: 285–296.

Spivak, M., and G. S. Reuter. 1998b. Honey bee hygienic behavior. Am. Bee J. 138: 283–286.

Thakur, R. K., K. Bienefeld, and R. Keller. 1997. *Varroa* defense behavior in *A. mellifera carnica.* Am. Bee J. 137: 143–148.

Received for publication 8 February 2000; accepted 5 October 2000.