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Abstract – Colonies with naturally mated queens from a hygienic line of Italian honey bees (*Apis mellifera ligustica)* were compared to colonies from a commercial line of Italian bees not selected for hygienic behavior. The following characteristics were compared: rate of removal of freeze-killed brood; amount of chalkbrood; incidence of American foulbrood; honey production; and the number of mites, *Varroa jacobsoni*, on adult bees. The hygienic colonies removed significantly more freeze-killed brood than the commercial colonies, had significantly less chalkbrood, had no American foulbrood, and produced significantly more honey than the commercial colonies. Estimates of the number of *Varroa* mites on adult bees indicated that the hygienic colonies had fewer mites than the commercial colonies in three of four apiaries. In previous studies on the relation between hygienic behavior and resistance to diseases and mites, the test colonies contained instrumentally inseminated queens. This is the first study to evaluate hygienic stock in large field colonies with naturally mated queens. © Inra/DIB/AGIB/Elsevier, Paris

***Apis mellifera* / hygienic behavior / *Varroa* / chalkbrood / American foulbrood**

1. INTRODUCTION

For over 50 years, honey bee hygienic behavior has been recognized as an important mode of resistance to American foulbrood disease (AFB) [12]. Woodrow and Holst [24] stated, "... resistance to American foulbrood consists of the colony's ability to detect and remove diseased brood before the causative organism, *Bacillus larvae* White, reaches the infectious spore stage". Over 14 years ago, it was determined that hygienic behavior also is a mechanism of resistance to chalkbrood, caused by the fungus *Ascosphaera apis* (Maassen ex Claussen) Olive and Spiltoir [5]. Recently it has been demonstrated that hygienic bees detect and remove

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pupae infested with the parasitic mite, *Varroa jacobsoni* Oudemans [1, 2, 13, 18]. The removal of infested pupae interrupts the reproductive cycle of the mite [4, 14]. However, the extent to which the behavior actually reduces the mite-load in infested colonies remains to be determined.

Hygienic behavior occurs in approximately 10 % of most commercial honey bee populations thus far surveyed in the US (Spivak, unpublished observations). Despite the potential advantages of maintaining hygienic honey bee lines, few queen producers select for hygienic behavior.

Rothenbuhler [16] postulated that the behavior is controlled by two independently assorting, recessive genes: one for uncapping the diseased brood (*u*) and one for removing diseased brood from the nest (*r*) (but see Moritz [10]). When colonies were composed of mixtures of hygienic and non-hygienic bees (progeny of instrumentally inseminated queens from inbred lines), Trump et al. [23] concluded that "for removal of all dead brood from a small colony, the proportion of the bees that must be of the hygienic type is higher than 13 % but less than 50 %". These early experiments suggested that a small percentage of bees, possibly within particular patrilines, may actually perform the behavior.

The genetics of the trait may be controlled by raising queens from hygienic colonies and instrumentally inseminating them with semen from drones of other hygienic colonies [5]. However, instrumentally inseminated queens are used only as breeder stock in the beekeeping industry; commercial beekeepers use naturally mated queens in their production colonies. Genetic control may be obtained by saturating the mating area with hygienic drone mother colonies [8]. If lines of bees selected for hygienic behavior are to be utilized by the beekeeping industry, it is first necessary to examine colonies with queens reared from hygienic stock and mated naturally with unselected drones. Do colonies with naturally mated queens reared from a hygienic line remove freeze-killed brood more rapidly than commercial colonies? Do they have lower incidences of chalkbrood and AFB? Do they have fewer *Varroa* mites? And importantly, do they produce as much honey? The present study is the first to address these questions in a commercial apiary.

2. MATERIALS AND METHODS

2.1. Hygienic breeding stock

The hygienic queens used in the experiment were bred from 'Starline' stock, derived from Italian *Apis mellifera ligustica* Spinola, 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 5 cm × 6 cm comb section (containing approximately 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 [19, 21]. Colonies that removed the freeze-killed brood from the comb section within 48 h on two consecutive trials were considered hygienic [20, 21]. 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 4–6 μL semen from drones collected from other 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.

2.2. Experiment 1

Hygienic queens were reared from one breeder colony containing a second generation II queen at the University of Minnesota in late May 1995. This colony consistently removed 100 % of freeze-killed brood within 48 h. The sister queens emerged in an incubator (34 °C and 70 % RH) and were marked with a dot of enamel paint on the thorax. They were then introduced into mating nuclei distributed in three apiaries owned by a commercial beekeeper near Hammond, Wisconsin. The apiaries were located over 80 km from the University of Minnesota. The queens mated naturally, and when they had begun laying eggs, were introduced into colonies in the same three apiaries in Wisconsin. Each apiary contained at least 24 colonies on pallets, with four colonies per pallet. Each colony occupied two standard Langstroth deep hive bodies. For comparison, half of the colonies in each apiary (two per pallet) contained laying queens reared from commercial Italian stock that had mated naturally in eastern Texas in March 1995. The commercial queens were reared from two different breeder queens, and therefore, were not sisters.

Beginning in late August 1995, when all the bees in the hygienic colonies were progeny of the introduced queens, three consecutive freeze-killed brood assays were conducted on all colonies in the three apiaries. The brood for the first assay was obtained by cutting sections of comb containing capped brood from colonies located at the University of Minnesota. These sections were frozen for 24 h and then introduced into the test colonies in Wisconsin. As an individual section of frozen brood was inserted into each test colony, a comb section of similar size that contained sealed brood was cut out from the colony. These new sections were frozen and used in the second assay. Freeze-killed brood for the third assay was obtained in the same way as that for the second assay. Previous experiments demonstrated that the source of the freeze-killed brood (from the same or different colony) had no effect on its removal rate by hygienic colonies [19]. In all trials, the number of intact capped cells was counted before the sections were inserted, and the number of emptied cells was counted after 48 h. Cells were not counted as empty if they were only uncapped or if any part of the dead brood remained. In this way, the most conservative measure of removal was recorded. The results of the three successive tests were analyzed using a repeated measures 2-way ANOVA [17].

The weather during the first assay (24–26 August 1995) was rainy and relatively cool (daytime highs: 20–25 °C). By the time of the second assay (29–31 August), the weather cleared and mean daytime temperatures remained above 26 °C. In addition, a goldenrod (*Solidago* spp.) bloom provided a strong source of incoming nectar during both this second assay and third assay (8–10 September).

2.3. Experiment 2

Hygienic breeder colonies containing third generation II queens from the University of Minnesota were wintered in eastern Texas in 1995 in an apiary owned by the same commercial beekeeper whose apiaries located in Wisconsin were used in experiment 1. In February 1996, the colonies in Texas were evaluated for population size and brood area, and were tested for hygienic behavior using the freeze-killed brood assay. Daughter queens were reared from the most populous colony that consistently removed freeze-killed brood within 48 h. The breeder colony also removed 65 % of pupae experimentally infested with *Varroa* mites in subsequent tests conducted in July 1996 at the University of Minnesota (after the breeder colony was transported back to Minnesota from Texas in May) using techniques described in Spivak [18].

Additional queens were reared from one colony of commercial stock of Italian descent also wintered in Texas. The commercial colony was chosen on the basis of the size of both the population of adult bees and the brood area. A freeze-killed brood assay indicated that the commercial colony removed less than 50 % of the frozen brood within 48 h. This colony was not tested for its ability to remove pupae experimentally infested with *Varroa*.

Daughter queens from both hygienic and commercial lines emerged within mating nuclei and mated naturally with the drones from the surrounding area in Texas. Two weeks after the queens emerged, they were marked with enamel paint on the thorax. In May 1996, all colonies with naturally mated queens were transported to Wisconsin. The hygienic and commercial colonies were equally distributed among four apiaries. The colonies were situated on pallets, and each pallet contained two hygienic and two commercial colonies.

Each colony that was transported to Texas was treated for *Varroa* mites using one Apistan® strip per colony in the fall of 1995. No subsequent Apistan treatment was given to any colony (mating nucleus or full-size colony) until after the experiment was terminated in September 1996. All colonies transported from Texas to Wisconsin were treated with 50 g menthol crystals for the tracheal mite, *Acarapis woodi* (Rennie), and were given six preventative dustings of oxytetracycline (a total of 1 000 mg TM-25® in powdered sugar) for European foulbrood and AFB in May and June 1996.

In June, the hygienic and commercial queens in every colony in the four apiaries in Wisconsin were located to determine if they were marked. If they were, the colony was evaluated on colony strength and degree of chalkbrood infection and incidence of AFB. Initially, four colonies were evaluated together by all recorders to standardize the scoring procedure. After that, two people evaluated each remaining colony, and both scores were averaged for each criterion.

Colony strength was estimated from the number of frames covered by bees [11] and numbers of frames containing brood (cells contained egg, larvae, and/or pupae) were estimated.

The degree of chalkbrood infection was measured by counting the number of chalkbrood mummies found in cells on both sides of two frames containing capped brood per colony. Only mummies in uncapped cells were counted. The colony was given a score from 0 to 3 (uninfected to highly infected), where 0 = no mummies, 1 = 1–5 mummies, 2 = 5–20 mummies, and 3 = over 20 mummies. Colonies were scored 0 or 1 for incidence of AFB: 0 = not infected; 1 = at least one infected pupa in an uncapped cell was noticed. If AFB was found, the colony was treated with oxytetracycline dust, as before.

After the June evaluations, the colonies were provided with honey supers *ad libitum*. In early September, the honey was harvested, and the colonies (in two deep hive bodies) were evaluated again for degree of chalkbrood infection and incidence of AFB. In addition, each colony was evaluated for honey production, removal of freeze-killed brood, and levels of *Varroa*. The amount of honey produced was measured by marking each super of honey as it was removed to indicate its colony of origin and then weighing the super on a floor scale in a honey extraction facility. The tare weight of the supers and frames was calculated by weighing the extracted supers.

To assay for hygienic behavior, each colony was assayed once with freeze-killed brood, and the amount of brood removed from each comb section was recorded after 48 h. At the time of the assay, goldenrod was in bloom, and mean daytime temperatures were over 26 °C.

The number of *Varroa* mites on adult bees was calculated by collecting samples of approximately 300–400 bees from each colony into enough 70 % ethanol to cover the bees, and hand-shaking 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.

All measures, except incidences of AFB, were analyzed using a 2-way ANOVA to separate the effects of bee type (hygienic versus commercial) and apiary site [17].

3. RESULTS

3.1. Experiment 1

Forty-three marked, laying queens from the hygienic stock were successfully introduced into the full-size colonies in June. At the time of the freeze-killed brood assays in August, seven of the marked queens had been superseded. Colonies headed by these queens comprised the hygienic-supersedure group.

The results of the three successive freeze-killed brood assays are shown in *figure 1*. In all tests, the 36 hygienic colonies (with marked, sister queens) removed significantly more brood than the 56 commercial colonies ($F = 16.1$, d.f. = 2, 96; $P < 0.001$). The mean percent (± s.d.) brood removed over the three tests by the hygienic, hygienic-supersedure, and commercial colonies was 82.9 % ± 10.49, 58.9 % ± 28.79, and 58.9 % ± 21.52, respectively. In the second and third tests, the hygienic colonies removed signifi-

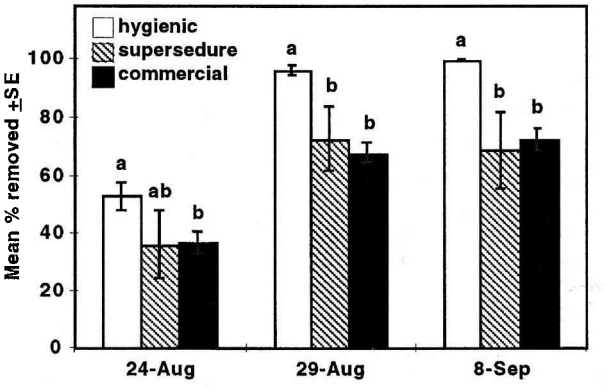

Figure 1. Removal of freeze-killed capped brood on three test dates in 1995 by colonies with naturally mated hygienic queens (n = 36), hygienic queens that were superseded (n = 7), and commercial queens (n = 56). Bars within one test date followed by different letters are significantly different at $P < 0.05$ (Tukey's HSD test).

cantly more brood than the seven colonies containing supersedure queens (Tukey's HSD test) [17]. In addition, there was a significant effect of test date ($F = 61.9$; d.f. = 2, 192; $P < 0.001$). All colonies removed less freeze-killed brood in the first test compared to the two successive tests.

Seven (19.4 %) hygienic and two (3.6 %) commercial colonies removed 95 % of the freeze-killed brood within 48 h on all three tests. Eleven (30.6 %) hygienic and three (5.4 %) commercial colonies removed 90 % or more of the brood in all tests.

3.2. Experiment 2

In June 1996, 49 marked queens were found in the hygienic colonies and 46 marked queens in the commercial colonies. The evaluations of colony strength indicated that the mean numbers of frames of bees and brood in the two colony types were not significantly different. The hygienic colonies had 17.4 ± 1.43 frames of bees and 10.1 ± 1.85 frames of brood. The commercial colonies had 17.3 ± 1.74 frames of bees and 10.0 ± 1.52 frames of brood.

The evaluations of chalkbrood mummies on two frames containing capped brood in June and September are shown in *figure 2*. In all apiaries, the hygienic colonies had significantly less chalkbrood than the commercial colonies in both June ($F = 32.24$; d.f. = 1, 87; $P < 0.001$) and September ($F = 17.74$; d.f. = 1, 87; $P < 0.001$). There was no significant apiary effect and no significant interaction between bee type and apiary.

At least one AFB-infected pupa was noted in six (13 %) of the commercial colonies in June and in five (10.9 %) of the colonies in September. No AFB was noted in any hygienic colonies during either inspection.

An average of 40.5 ± 16.45 kg (90.0 ± 36.56 lb; mean ± s.d.) of honey was harvested from the 49 hygienic colonies in late August 1996 (*figure 3*). In comparison, 30.1 ± 14.49 kg (66.8 ± 32.20 lb) honey was harvested from the 46 commercial colonies. The difference was highly significant ($F = 9.81$; d.f. = 1, 87; $P = 0.002$). There was no significant apiary effect and no significant interaction between bee type and apiary.

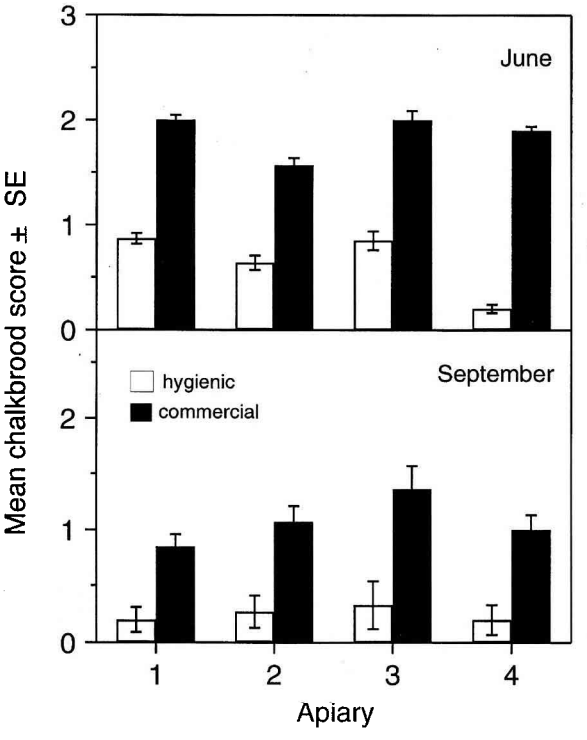

Figure 2. Abundance of chalkbrood mummies on two frames of capped brood within 49 hygienic and 46 commercial colonies distributed among four apiaries in June and September 1996. Colonies were scored from 0 to 3 (uninfected to highly infected), where 0 = no mummies, 1 = 1–5 mummies, 2 = 5–20 mummies, and 3 = over 20 mummies. The hygienic colonies had significantly less chalkbrood than the commercial colonies in both months ($P < 0.001$).

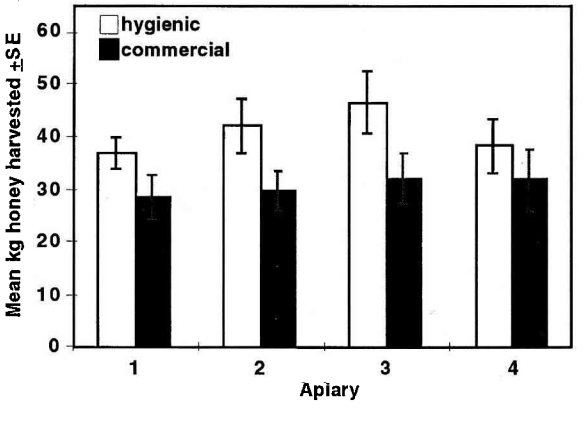

Figure 3. Honey production by 49 hygienic and 46 commercial colonies, distributed among four apiaries, in early September 1996. The hygienic colonies produced significantly more honey than the commercial colonies ($P = 0.002$).

The results of the single hygienic assay in September 1996 indicated that the hygienic colonies removed significantly more freeze-killed brood (94.2 % ± 12.16; mean ± s.d.) than the commercial colonies (82.31 % ± 22.91) ($F = 10.71$; d.f. = 1, 87; $P = 0.002$). Thirty-eight (77.6 %) of the hygienic colonies and 20 (43.5 %) of the commercial colonies removed 95 % or more of the freeze-killed brood within 48 h.

The hygienic colonies had 0.6 ± 0.86 (mean ± s.d.) *Varroa* mites per 100 adult bees, and the commercial colonies had 1.0 ± 1.0 (*figure 4*). Overall, the hygienic colonies had significantly fewer mites than the commercial colonies ($F = 5.78$; d.f. = 1, 87; $P = 0.013$). However, there was a significant interaction between bee type and apiary ($F = 4.06$; d.f. = 3, 87; $P = 0.009$) because on average, more mites were found in the second apiary within the hygienic colonies than in the commercial colonies.

4. DISCUSSION

The results of these experiments demonstrated that colonies with naturally mated queens from stock bred for hygienic behavior were significantly more hygienic (based on rate of removal of freeze-killed brood) and had lower incidences of chalkbrood and AFB than commercial colonies not selected for hygienic behavior. The hygienic colonies in this study also produced more honey than the commercial colonies. These results indicate that it is possible to select for hygienic behavior without compromising honey production; it does not imply that hygienic behavior and honey production are genetically linked traits. It is very important for queen breeders to simultaneously select for hygienic behavior and other commercially desirable traits (honey production, wintering ability). Finally, estimates of the number of *Varroa* mites on adult bees indicated that most of the hygienic colonies had fewer mites than unselected commercial colonies.

The hygienic colonies removed a higher percentage of freeze-killed brood than the commercial colonies in all three tests in 1995 and in the single test in September 1996. Rothenbuhler [16] found that F1 progeny of hygienic queens from the Brown line, each instrumentally inseminated with semen of a single drone from the non-hygienic Van Scoy line, removed

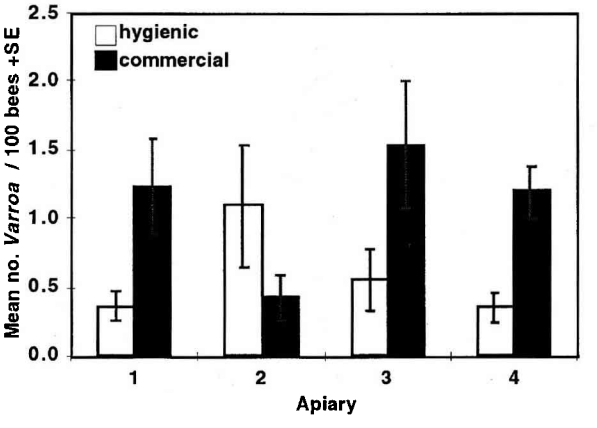

Figure 4. Abundance of *Varroa* mites per 100 adult bees from each of 49 hygienic and 46 commercial colonies in 1996. The hygienic colonies had fewer *Varroa* mites than the commercial colonies in all apiaries except the second one ($P = 0.013$)

diseased brood at the same rate as the non-hygienic line. The hygienic queens in the present study were mated with multiple drones of unknown genotype, but still removed significantly more freeze-killed brood within 48 h than the commercial colonies. These results suggest the inheritance of hygienic behavior may be controlled by more than two recessive loci (also see Moritz [10]).

The increased rate of removal by all colonies in the second and third assays in 1995 coincided with a goldenrod bloom. The expression of hygienic behavior is known to be strongly influenced by environmental factors, particularly the amount of nectar available to the colonies. A strong nectar flow increases the expression of hygienic behavior [9, 22]. In 1996, both the hygienic and commercial colonies had relatively rapid rates of removal, although the hygienic colonies had significantly higher removal rates. There was a strong nectar flow from goldenrod during the assay; however, it is unclear why so many of the commercial colonies removed high percentages of the freeze-killed brood. It is possible that a second assay, conducted before or after the goldenrod bloom, would have resulted in lower rates of removal by the commercial colonies.

The difference in the degree of chalkbrood infection between the hygienic and commercial colonies was very apparent during the field inspections. It also was notable that no hygienic colonies became infected with AFB, despite the fact that infected commercial colonies were located on the same pallets. In previous experiments on the relation between hygienic behavior and resistance to chalkbrood and AFB, the test colonies contained instrumentally inseminated queens [5, 6, 16, 20]. The present study is the first to compare levels of chalkbrood and AFB in large field colonies with naturally mated queens raised from hygienic stock.

Colonies infected with chalkbrood may experience a reduction in foraging force and a subsequent reduction in honey production [7]. In the present study, the commercial colonies produced, on average, 26 % less honey than the hygienic colonies; however there was no significant negative correlation between amount of chalkbrood in June and honey yield in September (Pearson correlation = –0.181, $P = 0.079$). The lower yields by the commercial colonies could have been due to the source of Italian stock from which the queens were reared and not to their degree of chalkbrood infection.

Hygienic behaviour may limit the population growth of *Varroa* mites in three ways: 1) the immature mites may be killed when the infested pupae are removed, which would decrease the average number of offspring per reproducing mite; 2) the phoretic period of adult female mites that survive removal of the pupae may be extended; and 3) the mortality of the adult mites may increase if they are damaged by grooming adult bees when the mites escape through the opened cell [4]. The hygienic queens in the present study were reared from colonies that removed significantly more pupae infested with *Varroa* mites than non-hygienic colonies in a previous study [18]; thus, it was hypothesized that the hygienic colonies would have lower levels of *Varroa* mites. In fact, most of the hygienic colonies in the 1996 experiment had fewer *Varroa* mites than the commercial colonies. However, the *Varroa* mite counts estimated the number of mites found on adult bees only; mites within brood cells were not assessed. Overall, the mean numbers of *Varroa* mites found on the adult bees in all the colonies were relatively low and may have been underestimated because the mites were shaken off the bees by hand rather than by a mechanical shaker [3]. Studies are in progress to compare the levels of *Varroa* mites in hygienic and commercial colonies left untreated for a longer time and to obtain more detailed estimates of the

number of mites by examination of both adult bees and cells containing worker and drone brood.

Despite the obvious advantages of selecting bee colonies for hygienic behavior, few commercial queen producers select for the trait in their breeding programs. One reason may be due to the common misconception that hygienic colonies are highly defensive. This assumption stems from the reputation of the Brown line of hygienic bees, maintained by Rothenbuhler, which was notoriously defensive. However, in backcrossed colonies between the inbred Brown line and the inbred Van Scoy (non-hygienic) line, Rothenbuhler [16] demonstrated clearly that stinging and hygienic behaviors were inherited separately. The colonies used in the present study were gentle and the recorders, none of whom wore gloves, received few if any stings throughout the evaluations.

A second reason why apiculturists have not selected for the trait may be due to inconsistencies inherent in the assay for hygienic behavior. The rate of removal of the freeze-killed brood within a particular colony even under the same environmental conditions is not always consistent between assays [15]. In addition Rodrigues et al. [15] found that colonies more quickly removed freeze-killed brood that had been recently capped than brood that had been capped for 5 days. However, in similar experiments, Spivak and Downey [19] found that the developmental stage of the brood did not influence the removal rate by colonies that consistently removed freeze-killed brood within 48 h.

In conclusion, the results of this study demonstrate that hygienic behavior is a highly desirable trait. The hygienic colonies in the present study were derived from an Italian line of bees; however, the behavior can be found in other races of bees (e.g. Carniolan bees, pers. obs.). The amount of chalkbrood and AFB in colonies can be reduced by requeening colonies with naturally mated queens from a hygienic line of bees. Continuing studies will determine the extent to which the *Varroa* mite load is reduced in hygienic colonies.

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Résumé – Les performances dans un rucher commercial de colonies d'abeilles présentant un comportement hygiénique. Le comportement hygiénique des abeilles est un mécanisme de résistance à la loque européenne et aux maladies cryptogamiques et l'un des modes de défense contre *Varroa jacobsoni*. Malgré les avantages évidents que procurent des colonies d'abeilles sélectionnées pour leur comportement hygiénique, peu d'éleveurs de reines sélectionnent ce caractère. Le but de cette étude était de comparer des colonies, avec des reines fécondées naturellement, issues d'une lignée hygiénique d'abeilles italiennes (*Apis mellifera ligustica* Spinola) avec des colonies issues

d'une lignée commerciale d'abeilles italiennes non sélectionnées pour ce caractère. Dans les études précédentes sur la relation entre le comportement hygiénique et la résistance aux maladies et aux acariens, les colonies testées possédaient des reines inséminées artificiellement. Ceci est la première étude qui évalue les performances d'une lignée hygiénique dans de fortes colonies avec des reines fécondées naturellement. On a évalué les paramètres suivants : taux d'élimination du couvain tué par le froid, quantité de couvain plâtré, présence de loque américaine, production de miel et nombre de varroas sur les abeilles adultes. Au cours de deux études indépendantes (1995 et 1996) les colonies hygiéniques (respectivement 36 et 49 colonies) ont éliminé significativement plus de couvain tué par le froid que les colonies commerciales témoins (respectivement 56 et 46 colonies) (*figure 1*). Les comparaisons en 1996 montrent que les colonies hygiéniques avaient significativement moins de couvain plâtré que les colonies commerciales et n'avaient pas de loque américaine, malgré le fait que les colonies commerciales aient été placées sur les mêmes palettes (*figure 2*). Les colonies de la lignée hygiénique ont aussi produit plus de miel (40,5 ± 16,45 kg) que celles de la lignée commerciale (30,1 ± 14,49 kg) (*figure 3*), ce qui montre qu'il est possible de sélectionner le caractère hygiénique sans pour autant compromettre la production de miel. Les estimations du nombre de varroas sur les abeilles adultes indiquent que les colonies de la lignée hygiénique ont eu, dans trois cas sur quatre, des niveaux d'acariens inférieurs à celles de la lignée commerciale (*figure 4*). Dans l'ensemble les colonies de la lignée hygiénique avaient 0,6 ± 0,86 varroas pour 100 abeilles adultes tandis que celles de la lignée commerciale en avaient 1,0 ± 1,0. Pourtant, des estimations plus précises du parasitisme, qui devraient inclure les comptages d'acariens dans les cellules de couvain, sont nécessaires avant de tirer des conclusions définitives concernant le rôle du comportement hygiénique sur la prévalence de *Varroa* dans les colonies d'abeilles.

***Apis mellifera* / comportement hygiénique / *Varroa* / couvain plâtré / loque américaine**

Zusammenfassung – Eigenschaften hygienischer Bienenvölker in einer kommerziellen Bienenhaltung. Das hygienische Verhalten von Honigbienen (das Ausräumen erkrankter Brut) ist ein Resistenzmechanismus gegenüber der Amerikanischen Faulbrut sowie der Kalkbrut und ist einer der Abwehrmechanismen gegen *Varroa jacobsoni*. Trotz der offensichtlichen Vorteile einer Selektion auf hohes hygienisches Verhalten wird diese nur von sehr wenigen professionellen Königinnenzüchtern durchgeführt. In unserer Untersuchung sollten Völker mit natürlich gepaarten Königinnen einer hygienischen Zuchtlinie von Italienerbienen (*Apis mellifera ligustica*) mit einer kommerziellen Linie von Italienerbienen verglichen werden, welche nicht auf dieses Verhalten hin selektiert war. Die in früheren Untersuchungen über die Beziehung zwischen dem hygienischen Verhalten und der Resistenz gegenüber Krankheiten und Milbenbefall verwendeten Völker hatten künstlich besamte Königinnen. Dies ist daher die erste Studie, in der die Eigenschaften einer hygienischen Linie in großen Völkern mit natürlich gepaarten Königinnen bewertet werden. In den Völkern wurde das Entfernen von durch Kälteeinwirkung abgetöteter Brut, der Kalkbrutbefall, das Vorkommen Amerikanischer Faulbrut, die Honigproduktion und die Anzahl von Varroamilben auf den Bienenarbeiterinnen erfaßt. In zwei getrennten Untersuchungen (1995 und 1996) entfernten die Völker der hygienischen Linie

(36 bzw. 49 Völker) signifikant mehr der abgetöteten Brut als die kommerzielle Linie (56 bzw. 46 Völker, *Abb. 1*). Die Untersuchung 1996 zeigte, daß in der hygienischen Linie der Befall durch Kalkbrut signifikant geringer war, und in diesen keine Amerikanische Faulbrut angetroffen wurde, obwohl befallene Völker der kommerziellen Linie auf den gleichen Paletten standen (*Abb. 2*). Die Völker der hygienischen Linie produzierten mehr Honig (40.5 ± 16.45 kg) als die kommerziellen Völker (30.1 ± 14.49 kg, *Abb. 3*). Dies belegt, daß eine Selektion auf hygienisches Verhalten ohne negative Auswirkungen auf die Honigproduktion durchgeführt werden kann. Die Schätzungen der Anzahl von *Varroa* auf Arbeiterinnen deuten darauf hin, daß die Völker der hygienischen Linie auf vier der Bienenstände einen geringeren Milbenbefall aufwiesen als die kommerzielle Linie (*Abb. 4*). Insgesamt hatten die hygienischen Völker 0.6 ± 0.86 *Varroa*milben pro 100 Arbeiterinnen, während es in den kommerziellen Völkern 1.0 ± 1.0 waren. Bevor sichere Schlußfolgerungen bezüglich einer Auswirkung des hygienischen Verhaltens auf den Milbenbefall gezogen werden können, sind jedoch genauere Messungen des Milbenbefalls nötig, in die auch Zählungen der Milben in der Brut einbezogen werden.

***Apis mellifera* / hygienisches Verhalten / *Varroa* / Kalkbrut / Amerikanische Faulbrut**

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