Resistance to American foulbrood disease by honey bee colonies Apis mellifera bred for hygienic behavior

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Resistance to American foulbrood disease by honey bee colonies *Apis mellifera* bred for hygienic behavior

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Abstract – Honey bee colonies, selected for hygienic behavior on the basis of a freeze-killed brood assay, demonstrated resistance to American foulbrood disease. Over two summers in 1998 and 1999, 18 hygienic and 18 non-hygienic colonies containing instrumentally inseminated queens were challenged with comb sections containing spores of the bacterium *Paenibacillus larvae* subsp. *larvae* that causes the disease. The strain of bacterium was demonstrated to be resistant to oxytetracycline antibiotic. Seven (39%) hygienic colonies developed clinical symptoms of the disease but five of these recovered (had no visible symptoms) leaving two colonies (11%) with clinical symptoms. In contrast, 100% of the non-hygienic colonies that were challenged developed clinical symptoms, and only one recovered. All non-hygienic colonies had symptoms of naturally occurring chalkbrood disease (*Ascosphaera apis*) throughout both summers. In contrast 33% of the hygienic colonies developed clinical symptoms of chalkbrood after they were challenged with American foulbrood, but all recovered. The diseased non-hygienic colonies produced significantly less honey than the hygienic colonies.

***Apis mellifera* / hygienic behavior / American foulbrood / disease resistance**

1. INTRODUCTION

American foulbrood (AFB) disease, caused by the bacterium *Paenibacillus larvae* subsp. *larvae* (formerly *Bacillus larvae*) is the most serious of the diseases affecting honey bees *Apis mellifera* L. On a colony level, the most important mechanism of resistance to AFB is hygienic behavior of adult bees toward infected larvae (Rothenbuhler, 1964 a,b, reviewed in Spivak and Gilliam 1998 a,b). Worker bees that demonstrate this behavior rapidly detect, uncap, and remove infected brood from the

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nest. The spore, or infectious stage of the bacterium appears at approximately 10–11 days after egg-hatching, when the prepupae is developing within the 5th instar cuticle under a wax capped cell. Sporulation is accompanied by death of the prepupae (reviewed in Hansen and Brødsgaard, 1999). Hygienic bees uncap and remove larvae under capped cells when the bacterium is in the vegetative, non-infectious rod stage; i.e., before the bacteria sporulate in the hemocoel, and before the prepupae dies (Woodrow and Holst, 1942). In this way, the infection may be present in the colony, but the hygienic bees remove the infected brood before the disease is visible to the human eye.

Individual bees have inherent modes of resistance to the disease: young larvae (under 36–48 hours old) are susceptible to infection if they consume bacterial spores in their food secreted to them by nurse bees, but older larvae are increasingly resistant (Woodrow and Holst, 1942; Bambrick and Rothenbuhler, 1961; Brødsgaard et al., 1998; Crailsheim and Riessberger-Galle, 2001). In addition, a peptide fraction with an inhibitory effect against *P. l. larvae* was isolated from royal jelly (Bíliková et al., 2001), which may be a factor that contributes to the resistance of young larvae (Rose and Briggs, 1969). Adult bees transfer spores but never become infected themselves (Woodrow, 1942; Woodrow and Holst, 1942). The resistance of adult bees may be due to the action of the proventricular valve which filters the spores from the digestive tract (Sturtevant and Revell, 1953) and to substances with inhibitory activity found in their midgut, particularly in bees 8 days old (Crailsheim and Riessberger-Galle, 2001).

In North America, AFB has been controlled by the antibiotic oxytetracycline (trade name Terramycin®) for 50 years (Gochnauer, 1951). This antibiotic is the most commonly used treatment worldwide, although in some countries (e.g., New Zealand, Australia, Denmark) its use is prohibited, or regulated (e.g., Oldroyd et al., 1989; Van Eaton, 2000). The incidence of AFB that is resistant to the antibiotic oxytetracycline is rising dramatically in the US (Miyagi et al., 2000), Canada (Colter, 2000), and Argentina (Alippi, 1994). New antibiotics are being screened for use to treat AFB (Peng et al., 1996; Alippi et al., 1999; Kochansky et al., 1999, 2001), but as with oxytetracycline, they may leave residues in honey and *P. larvae* may develop resistance to them. A more sustainable approach is to focus efforts on breeding bees resistant to the disease to reduce or eliminate the need for antibiotics.

Our goal in this study was to challenge colonies selected for hygienic behavior with AFB spores to determine if the colonies were resistant to the disease. We first selectively bred colonies for the behavior using freeze-killed brood assay and subsequently challenged them with *P. larvae*. This approach differs from that used by Rothenbuhler (1964 a,b) and others (Park, 1937; Woodrow, 1942) who first located colonies resistant to AFB and later determined the mode of resistance to be hygienic behavior. We modeled our approach after Gilliam et al. (1983, 1988) who found that colonies selected for rapid removal of freeze-killed brood were also resistant to chalkbrood (caused by the fungus, *Ascosphaera apis)* when subsequently challenged with the pathogen. A similar but more correlative approach was taken by Palacio et al. (2000) in Argentina who found that the degree of hygienic behavior increased in colonies after four years of selection solely on the queens, and that the hygienic colonies had a lower frequency of naturally occurring brood disease than non-hygienic colonies.

Because of the rising incidence of AFB resistant to oxytetracycline, we challenged the hygienic colonies in this study with this resistant strain of bacteria. As controls, we also inoculated colonies bred for non-hygienic behavior.

2. METHODS

2.1. Colonies

The hygienic and non-hygienic bees used in the study were derived from the breeding program at the University of Minnesota. The breeding program was initiated in 1993 by selecting colonies of Italian-derived *A. mellifera* bees using a freeze-killed brood assay described in Spivak and Downey (1998) and Spivak and Reuter (1998a). Colonies that uncapped and removed 95–100% of the freeze-killed brood within 48 hours were considered hygienic, those that took over six days to perform the same task were considered non-hygienic. To establish and maintain the lines, queen bees were raised from colonies that displayed the most rapid and least rapid removal rates. For each generation, the daughter hygienic queens were instrumentally inseminated with a mixture of 6–8 μl semen from drones collected from different hygienic colonies. Similarly, daughters from the most non-hygienic queens were inseminated with 6–8 μl sperm of drones from the most non-hygienic colonies.

Queens were inseminated in the summers of 1997 and 1998. In August each year, the queens were sent to California where they were wintered in colonies owned by a commercial beekeeper. We assayed the wintered colonies for hygienic behavior in California in March of 1998 and 1999. In both years, the hygienic colonies removed 95–100% of the freeze-killed brood within 48 hours. The non-hygienic colonies removed 32–73% of the brood in the same time.

In April of each year, 1.4 kg of bees and the inseminated queens from each colony were shipped back to Minnesota in standard shipping packages for bees. The bees and queen were each hived together in one standard Langstroth deep hive body containing nine frames of drawn comb. During the summer, honey supers were added over a queen excluder to the colonies as needed.

2.2. Inoculation with AFB

In 1998, frames of comb containing AFB spores (in scale form, from the dried remains of infected brood) were obtained from a commercial beekeeper who was unable to successfully suppress AFB in his colonies after repeated treatments with oxytetracycline. Sections of comb with AFB spores were analyzed for resistance to in the lab of C. Peng at the University of California – Davis. Confirmation that the AFB was resistant was published by Miyagi et al. (2000).

Comb sections (15 cm × 15 cm) were cut from the remaining frames containing AFB spores. In 1998, 80% of the cells in the cut sections contained scales, determined visually. After the trial ended in 1998, combs from the most infected colonies were frozen at –20 °C over the winter and new 15 cm × 15 cm sections were cut out for use in the next year. In 1999, approximately 50% of the cells within the cut sections contained AFB scale.

The comb sections with AFB scale were introduced into the middle frame (frame 5) in each colony in June each year. Only colonies that still contained the tagged and clipped inseminated queens were inoculated. In 1998, eight hygienic colonies, derived from five sublines (unrelated queen lines), and nine non-hygienic colonies (derived from four sublines) were inoculated. In 1999, 10 hygienic colonies (from six sublines) and nine non-hygienic colonies (five sublines) were tested.

After inoculation, the colonies were inspected every 7–14 days. Every frame was inspected on both sides for AFB by shaking off the bees and counting the number of cells that showed clinical symptoms (sunken wax cappings and uncapped cells containing discolored, ropy brood). A severity score from 0–3 was given for each frame with brood: 0 = 0 cells containing AFB per frame; 1 = 1–5 cells per frame; 2 = 6–25 cells per frame; and 3 ≥ 25 cells per frame. An overall severity score for each colony on each

inspection date was later obtained by calculating the mean (± s.d) of the individual frame scores. An overall score of 1 corresponded to a colony with only slight clinical symptoms, possibly not noted by cursory inspection. An overall score of 2 would indicate noticeable symptoms, and a score of 3 corresponded to a highly symptomatic colony. The scores were compared between the diseased hygienic and diseased non-hygienic colonies using Wilcoxon 2-sample tests on dates when the number of symptomatic colonies of each type ≥ 3.

The colonies also were inspected for chalkbrood mummies on three frames (frames 3, 5, and 7) each visit. The colonies were not inoculated with this pathogen so the observed incidence of disease occurred naturally. The three frames were scored for chalkbrood severity using the same criteria as for AFB, and were analyzed using the same statistics. In addition, the incidence of queen supersedure or queen loss was noted, and in 1999, honey production was measured.

3. RESULTS

3.1. AFB

In 1998, three of the eight hygienic colonies, and nine of the nine non-hygienic colonies developed clinical symptoms of AFB (Tab. I; $P = 0.009$ based on Fisher Exact Test). One of the infected hygienic colonies recovered on its own (had no visible symptoms) by 21 August when the

Table I. Number of colonies on each inspection date with clinical symptoms of American foulbrood after inoculation with comb sections containing AFB scale in 1998 (top) and 1999 (bottom), and number of colonies on each inspection date with naturally occurring chalkbrood symptoms.

Date inspected	Colonies with AFB		Colonies with chalkbrood	
Inoculated 19 June 1998	Hygienic $n = 8$	Non-hyg $n = 9$	Hygienic $n = 8$	Non-hyg $n = 9$
26 Jun.	0	0	0	8
3 Jul.	1	1	1	8
10 Jul.	3	5	2	8
17 Jul.	3	9	4	7
27 Jul.	3	9	2	7
6 Aug.	3	9	3	5
14 Aug.	3	9	0	8
21 Aug.	2	9	0	9
Inoculated 8 June 1999	$n = 10$	$n = 9$	$n = 10$	$n = 9$
22 Jun.	0	5	0	9
2 Jul.	4	9	1	7
9 Jul.	2	8	0	6
18 Jul.	0	8	0	7
9 Jul.	0	7[1]	1	5
13 Aug.	0	7[1]	0	7

[1] One of the nine non-hygienic colonies became queenless by 29 July, and due to lack of brood, had no symptoms of AFB.

experiment was terminated, while the other two colonies remained symptomatic, and all nine non-hygienic colonies remained symptomatic ($P = 0.250$). In 1999, four of the 10 hygienic colonies, and nine of nine non-hygienic colonies had AFB symptoms by 2 July ($P = 0.011$). The four infected hygienic colonies recovered by the end of experiment on 13 August. In contrast, seven of the non-hygienic colonies were still infected on 13 August ($P = 0.021$) One of the nine infected non-hygienic colonies apparently recovered on its own, but the other became queenless, and because there was no brood, AFB symptoms disappeared.

Overall the results were consistent over both years: of 18 hygienic and 18 non-hygienic colonies challenged with AFB spores, seven hygienic (39%) and 18 non-hygienic (100%) developed symptoms of the disease. There was a highly significant association between hygienic behavior of the colonies and the low incidence of AFB symptoms ($\chi^2 = 14.9564$; $P = 0.0001$, based on a Mantel-Haenszel test to obtain a combined estimate over the two years, Sokal and Rolf, 1995). Of the hygienic colonies with symptoms of AFB, 5 of 7 (71%) recovered, leaving only two of 18 colonies infected (11%). In contrast, only one of 17 (6%) non-hygienic colonies recovered (excluding the colony that became queenless). Again, there was a highly significant association between hygienic behavior and the likelihood that a colony recovered from AFB (Mantel-Haenszel $\chi^2 = 9.0373$; $P = 0.0026$).

The AFB severity scores of the colonies that became infected are shown in Figure 1. There were no significant differences in the severity of infection in 1998 (10, 17, 27 July, and 6, 14 August), or 1999 (2 July) (Wilcoxon 2-sample tests: all $P \geq 0.05$). All colonies with clinical symptoms of AFB had symptoms on all brood frames.

The response of the bees to the comb sections containing AFB spores used as the inoculum in each colony varied. In 1998, one week after the colonies were inoculated, the majority of hygienic and non-hygienic colonies had cleaned and polished the cells within the comb section, and the queen had laid eggs in the cells and/or the cells were filled with nectar and pollen. Three of the hygienic colonies had destroyed either all or most of the comb section down to the midrib and rebuilt new wax cells (as indicated by their light yellow color) (Fig. 2). One non-hygienic colony also tore the entire comb section down, and three others

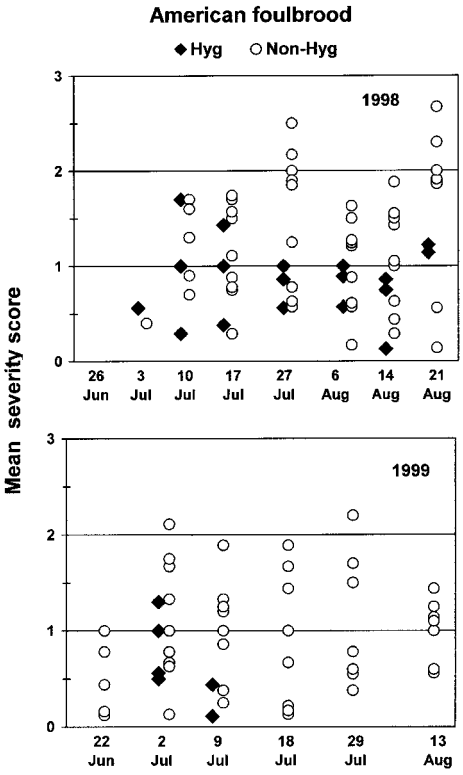

Figure 1. Overall severity scores of American foulbrood in hygienic and non-hygienic colonies with clinical symptoms on each date of inspection in 1998 (top) and 1999 (bottom). Scores of individual combs were averaged to give overall score for colony (see text). Overall score of 0 = no AFB; 1 = 1–5 cells of AFB (colony slightly symptomatic); 2 = 6–25 infected cells (moderately symptomatic); 3 ≥ 25 cells (highly symptomatic).

destroyed small portions of the comb. All colonies eventually raised brood in the comb section (Fig. 3), and if they became diseased, AFB symptoms were seen both in the comb section and on all other frames containing brood. The non-hygienic colony

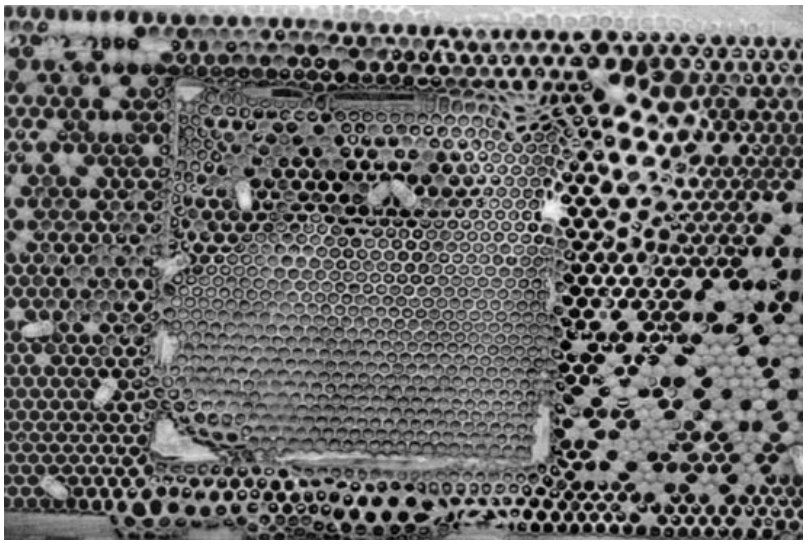

Figure 2. An example of a comb section (15 cm × 15 cm) that was used to inoculate the colonies with AFB spores. In this case, the wax cells were torn down to the midrib, and new wax cells were beginning to be built in the center of the comb section. The cells in the upper left corner of the section were not torn down, and were filled with nectar and pollen.

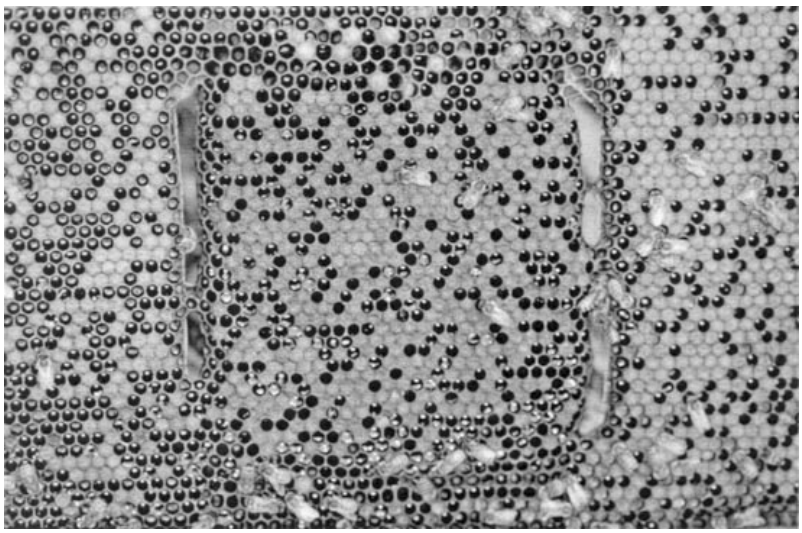

Figure 3. All colonies raised brood in the comb section with the AFB spore innoculum, whether the cells were torn down and rebuilt or not. Most colonies attached the comb section to the rest of the comb in the frame, as shown here shown on the top and bottom of the section.

that completely rebuilt the comb section, and one of the hygienic colonies that rebuilt most of the comb section with new wax later developed symptoms of AFB. Thus, the destruction of the inoculum comb did not prevent the bees from later becoming diseased.

3.2. Chalkbrood

In both years, no hygienic colonies had clinical symptoms of chalkbrood when the experiment began. After the colonies were inoculated with AFB, four of the hygienic colonies developed symptoms of chalkbrood in 1998 (Tab. I). In contrast to AFB, chalkbrood symptoms were not always persistent in the colonies. Two hygienic colonies with symptoms on 10 July remained symptomatic until 14 August. Two additional colonies had symptoms on 17 July; one of these recovered after that date, and the other was again observed with symptoms on 27 July. In 1999, two different colonies showed temporary symptoms of chalkbrood; one on 2 July, and the other on 29 July (Tab. I). Over the two years, four of the six hygienic colonies with chalkbrood also showed symptoms of AFB.

The non-hygienic colonies had chalkbrood symptoms before the colonies were inoculated with AFB in both years (eight colonies in 1998 and nine in 1999), and the majority of them continued to be symptomatic throughout the duration of the experiment. In 1998, five of the nine colonies had chalkbrood symptoms on each inspection date, but symptoms in the remaining four were observed sporadically (i.e., the same colonies did not always show chalkbrood symptoms week to week). In 1999, only one non-hygienic colony had persistent symptoms of chalkbrood on each inspection date. After 22 June, one of the nine colonies did not show symptoms the rest of the experiment, and symptoms in the remaining colonies were observed sporadically.

The chalkbrood severity scores for those colonies that had clinical symptoms of this disease are shown in Figure 4. Only two comparisons were made (17 July and 6 August, 1998) as these were the only dates when the number of diseased colonies of each type ≥ 3. There were no significant differences in the severity of the symptoms between hygienic and non-hygienic colonies on those dates (Wilcoxon 2-sample tests: all $P \geq 0.05$).

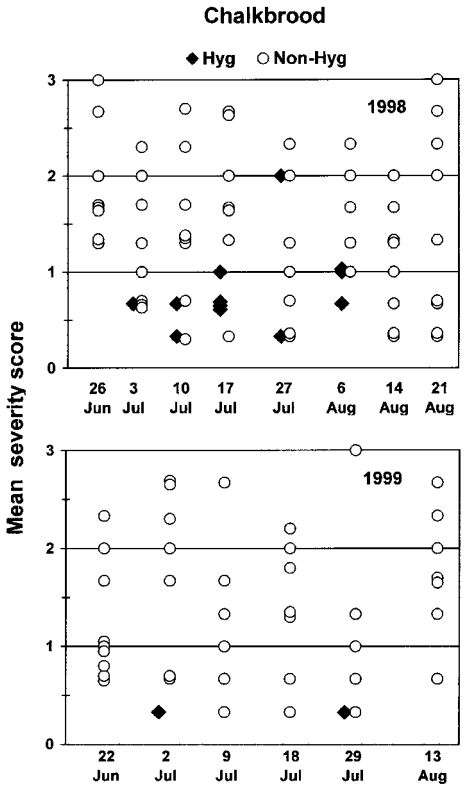

Figure 4. Overall severity scores of chalkbrood in the symptomatic hygienic and non-hygienic colonies on each date of inspection in 1998 (top) and 1999 (bottom). Scores of individual combs were averaged to give overall score for colony (see text). Overall score of 0 = no chalkbrood mummies; 1 = 1–5 cells containing chalkbrood mummies (slightly symptomatic); 2 = 6–25 infected cells (moderately symptomatic); 3 ≥ 25 cells (highly symptomatic).

3.3. Queen supersedure

In 1998, two hygienic and one non-hygienic queens were superseded by 21 August. In 1999, eight hygienic and four non-hygienic queens were superseded by 13 August. Successful queen replacement did not correspond with recovery from AFB except in one case in 1999 when a diseased non-hygienic colony superseded its queen during the first week of the experiment (22 June) and after 18 July had no further visible symptoms.

3.4. Honey production

In 1999, the hygienic colonies produced an average of 25.7 kg ± 13.4 (s.d.) of honey, and the non-hygienic colonies produced 14.0 kg ± 16.4 ($t = 2.110$, $P = 0.105$). One of the non-hygienic colonies (the one that superseded its queen the first week of the experiment, had no chalkbrood symptoms thereafter, and had no AFB symptoms by the end of the experiment) produced 51 kg of honey, more than any other hygienic or non-hygienic colony. If this colony was excluded from the analysis, the remaining non-hygienic colonies produced on average 9.3 kg ± 9.4 ($t = 2.120$, $P = 0.0098$).

4. DISCUSSION

Hygienic colonies, selected on the basis of a freeze-killed brood assay, demonstrated resistance to AFB. Of 18 hygienic colonies challenged with AFB over two years, seven (39%) developed clinical symptoms of the disease and five of these recovered from the disease on their own, leaving 11% symptomatic. In contrast, all 18 non-hygienic colonies that were challenged with AFB developed clinical symptoms, and only one recovered on its own (another became queenless).

The combs were destroyed in the colonies when the experiments were terminated each year, so it is unknown whether the disease would have persisted in the remaining colonies. Destroying (burning, irradiating) infected combs is essential in control because AFB spores can successfully germinate in 35 year old combs (Haseman, 1961). Due to the presence of spores in old combs, many beekeepers routinely apply antibiotics as a prophylactic measure to prevent disease outbreak. Routine replacement of old combs in thriving colonies is a potentially important component of disease prevention (Stanley, 2000; Van Eaton, 2000), and together with the use of hygienic stocks of bees, could eliminate the routine use of antibiotics to prevent disease outbreak, and reduce the need to treat colonies that become diseased.

Colonies that display hygienic behavior demonstrated resistance to chalkbrood as well as AFB (Gilliam et al., 1983). The hygienic colonies in this study did not have chalkbrood at the beginning of the experiment, but after inoculation with AFB spores, six colonies developed symptoms of chalkbrood. Most likely, chalkbrood spores are present in most colonies, but if larvae become infected in hygienic colonies, the bees remove the diseased larvae from the nest before the typical symptoms of the disease appear in the larvae. We presume that the hygienic colonies were not able to remove all larvae infected with either chalkbrood or AFB, and so disease symptoms appeared temporarily. As the AFB infection recovered, the chalkbrood infection also recovered in both years. The non-hygienic colonies had chalkbrood symptoms prior to inoculation with AFB spores in both years, and after inoculation had symptoms of both diseases. In a laboratory study, Feldlaufer et al. (1993) isolated an antimicrobial compound, linoleic acid, in chalkbrood that was active against *P. l. larvae*. However, the results from our field study indicated that the presence of chalkbrood in colonies did not inhibit the development of AFB symptoms.

It is unclear why so many colonies superseded the queens in 1999. It is not known

whether the supersedures were related to being challenged with AFB, or if they were due to the queens themselves. As mentioned, successful queen replacement did not correspond with recovery from AFB.

Diseased, non-hygienic colonies produce less honey than healthy, hygienic colonies, as demonstrated in this experiment. An interesting exception was the non-hygienic colony that recovered from AFB and collected more honey than any other colony in the apiary in 1999. It is possible that the amount of nectar being processed by this colony helped in diluting the number of spores transferred to larvae by adult bees if the adults eliminated spores through the proventricular valve and midgut (Bailey and Ball, 1991).

It is important to note that the hygienic colonies in this study were part of the 4th and 5th generation of selection, contained queens instrumentally inseminated with semen of drones from hygienic colonies, and consistently removed 95%–100% freeze-killed brood within 48 hours. It is not clear if resistance to AFB would be maintained in colonies with queens mated to unselected drones and with slower removal rates. Diligent selection and breeding efforts are required to obtain and maintain hygienic colonies with consistently rapid removal rates. However, the benefits of having colonies resistant to AFB, chalkbrood disease, and partially resistant to *Varroa destructor* (Spivak and Reuter, 1998b, 2001) are evident. The maintenance of resistant bee colonies is the foundation for effective integrated disease and pest management, and in the long run is the most sustainable alternative to the risks and problems associated with the prolonged use of antibiotics and pesticides.

ACKNOWLEDGEMENTS

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Résumé – Résistance à la loque américaine de colonies d'abeilles *Apis mellifera* sélectionnées pour leur comportement hygiénique. Les colonies d'abeilles, sélectionnées pour leur comportement hygiénique sur la base d'un test de couvain tué par congélation (« colonies hygiéniques ») ont présenté une résistance à la loque américaine. Au cours des étés 1998 et 1999, 18 colonies hygiéniques et 18 colonies non hygiéniques possédant des reines inséminées artificiellement, ont été testées avec des sections de rayons renfermant des spores de la bactérie *Paenibacillus larvae* subsp. *larvae*, agent de la loque américaine. On a montré que la souche de bactérie était résistante à l'antibiotique oxytétracycline. Sept colonies hygiéniques (39 %) ont contracté la maladie mais cinq d'entre elles ont guéri (pas de symptômes visibles), donc seules deux colonies (11 %) sont restées malades. Par contre 100 % des colonies témoins ont été infectées et une seule a guéri. Toutes les colonies non hygiéniques ont eu du couvain plâtré (*Ascosphaera apis*) spontané durant les deux étés. En revanche six (33 %) colonies hygiéniques ont développé du couvain plâtré après avoir été infectées avec des spores de loque américaine, mais elles ont guéri. Les colonies malades non hygiéniques ont produit significativement moins de miel que les hygiéniques. Les colonies hygiéniques de cette étude étaient issues de la 4^{e} et 5^{e} génération de sélection, possédaient des reines inséminées artificiellement avec du sperme de mâles issus de colonies hygiéniques et ont régulièrement éliminé en 48 h,

95 à 100 % du couvain tué par le froid. Il n'est pas certain que la résistance à la loque américaine se maintienne si les reines étaient fécondées par des mâles non sélectionnés et avec un taux d'élimination plus faible.

***Apis mellifera* / comportement hygiénique / loque américaine / résistance aux maladies**

Zusammenfassung – Resistenz gegen Amerikanische Faulbrut von auf Hygieneverhalten gezüchteten Bienenvölkern (*Apis mellifera*). Völker der Honigbienen, die durch Tests mit durch Gefrieren getöteter Brut auf Hygieneverhalten gezüchtet wurden, zeigten eine Resistenz gegen die Amerikanische Faulbrut (AFB). Während der Sommer 1998 und 1999 wurden 18 auf Hygiene und 18 nicht auf Hygiene gezüchtete Völker mit instrumentell besamten Königinnen getestet, in die Wabenstücke mit Sporen des Bakteriums *Paenibacillus larvae* subsp. *larvae* gegeben wurden, durch die die Krankheit hervorgerufen wird. Der Bakteriumstamm war nachweislich resistent gegen das Antibiotikum Oxytetracyclin. Bei 7 Hygiene – Völkern (39 %) entwickelten sich klinische Symptome der Krankheit, von denen sich 5 Völker wieder erholten (es waren keine Symptome zu erkennen). Es blieben nur 2 Völker (11 %) mit Symptomen übrig. Im Gegensatz dazu erkrankten alle Kontrollvölker (100 %), von denen sich nur eines wieder erholte. Bei allen nicht auf Hygiene selektierten Völkern (100 %) trat Kalkbrut (*Ascosphaera apis*) während beider Sommer durch natürliche Infektion auf. Im Gegensatz dazu entwickelten sich nur bei 6 Hygiene-Völkern (33 %) nach der Infektion mit Faulbrutsporen Symptome der Kalkbrut, aber alle wurden wieder gesund. Die erkrankten nicht-hygienischen Völker erzeugten signifikant weniger Honig als die hygienischen Völker. Die hygienischen Völker in dieser Untersuchung stammten von der 4. und 5. Generation einer Selektion, die durch instrumentelle Besamung von Königinnen mit Drohnen aus Hygiene – Völkern erhalten wurden, und die durchgehend immer innerhalb von 48 Stunden 95 bis 100 % der durch Gefrieren abgetöteten Brut entfernten. Es ist noch nicht geklärt, ob die Resistenz gegen AFB sich in Völkern erhält, wenn sich die Königinnen mit nicht selektierten Drohnen paaren oder mit solchen, deren Völker eine langsamere Rate bei der Entfernung der Brut aufweisen.

***Apis mellifera* / Hygieneverhalten / Amerikanische Faulbrut / Krankheitsresistenz**

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