Hygienic behaviour of honey bees and its application for control of brood diseases and varroa

Part I. Hygienic behaviour and resistance to American foulbrood

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There have been very few studies on hygienic behaviour as a mechanism of resistance to American foulbrood since Park, Woodrow, Rothenbuhler, and Rothenbuhler's students published their seminal work. The studies outlined in this part of the review form the core of information from which all later studies on hygienic behaviour have been based.

Introduction

The first detailed observations of hygienic behaviour of honey bees, *Apis mellifera*, were recorded in the 1930s during efforts to determine whether resistance to the bacterial disease American foulbrood (AFB) existed in bee colonies. This search was initiated because AFB is the most serious of the brood diseases of honey bees. Considerable research was conducted in the 1960s and 1970s on the role of hygienic behaviour as a mechanism of resistance to AFB. However, with the advent of sulfa drugs and antibiotics that are effective against AFB, research efforts in this area were not sustained.

Today, there is evidence in some areas of the USA that the bacterium that causes AFB (*Paenibacillus larvae* subsp. *larvae*; formerly *Bacillus larvae*) may be developing tolerance to the antibiotic oxytetracycline (Terramycin®), the only registered control in the country. Resistance of AFB to oxytetracycline has been a problem in Argentina for some years[2]. The introduction of the fungal disease chalkbrood into the USA in the late 1960s and the 1970s and the increased incidence of this disease in Europe and Asia have had serious negative effects on bee colonies throughout the world. There is no registered treatment for the disease in the USA or most other countries. The parasitic mite, *Varroa jacobsoni*, has also spread throughout most of the world, and the mites have already developed resistance to fluvalinate, one of the chemical controls and the only acaricide registered for use in the USA[6,11].

As a consequence of these problems, there has been a recent resurgence of interest in hygienic behaviour of honey bees. This behaviour has been demonstrated to be the primary mechanism of resistance to AFB and chalkbrood and is one defence against

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varroa. This review, divided into two parts, intends to bring readers up to date on the wealth of research already conducted on this interesting and important behaviour of honey bees, to point out the results that are available for immediate practical use in beekeeping, and to note areas where further research is needed. Part I of this review will cover the early research on hygienic behaviour as a mechanism of resistance to AFB. Part II (in the next issue) will contain sections on hygienic behaviour as a defence mechanism against chalkbrood, European foulbrood and varroa; hygienic behaviour in *Apis cerana*, subspecies of *A. mellifera,* and Africanized honey bees; assays to screen colonies for hygienic behaviour; and breeding programmes.

We wish to emphasize that selection and breeding for behaviour(s) of honey bees to control the most serious brood diseases and the most devastating mite pest are preferable to the introduction of foreign chemicals (antibiotics and pesticides) into bee colonies. Contamination of bee products such as honey, pollen, and wax with these chemicals is an increasing problem. Too often, beekeepers use improper formulations, dosages, and methods of dispensing chemical controls. This misuse can result in the lack of control of the disease or mite; the selection of pathogens and mites that are resistant to the chemicals; and, ultimately, devastation for the entire apicultural industry within an area, country, or even a continent.

Early research

In the 1930s, a search was conducted in the USA for honey bees resistant to AFB. The effort was initiated by O W Park (Entomologist at the Iowa Agricultural Experiment Station), F B Paddock (State Apiarist from the Agricultural Extension Service of Iowa State College), and F C Pellet (beekeeper and Field Editor of the *American Bee Journal*). To determine whether variation in resistance to AFB existed, various colonies of honey bees were presented with comb sections containing the remains of larvae killed by AFB (scales). The bees responded by either accepting the comb with no alteration, cleaning out the cells, or destroying and removing the cells. Although all colonies but one showed some symptoms of AFB early in the season, seven of 31 (23%) test colonies showed no symptoms by the end of the season[14,15]. The colonies that displayed the most resistance were those that removed the entire inoculation comb and then rebuilt it with new wax. Colonies that never showed symptoms of AFB as well as those that did but later recovered were considered resistant.

To determine whether the resistance was heritable, queens reared from one of the resistant colonies were allowed to mate with drones from other resistant colonies in an isolated mating apiary. Later in the season, the daughter colonies were presented with comb sections containing AFB scales. Nine of 27 (33%) of the colonies had no disease symptoms by the end of the season. Because the percentage of resistant colonies increased after one generation of selection, the authors concluded that the resistance was heritable[17]; however, this increase in resistant colonies was not statistically significant.

In subsequent years, Park, Pellet and Paddock began a selective breeding programme for colonies resistant to AFB[17,18]. They noted considerable variation among colonies in the propensity to develop AFB after inoculation with either comb sections containing AFB scales or sugar syrup containing *P. larvae* spores. The authors noted, 'We have observed that the bees sometimes remove and dispose of larvae very soon after they die, thus eliminating the evidence'[18]. Park[16] wondered whether the apparent resistance

was due to the housekeeping activities of the bees, physiological factors, or both.

In 1941, A W Woodrow (USDA Bureau of Entomology and Plant Quarantine, Laramie, Wyoming, USA) began experiments to determine whether the brood from the resistant and from the susceptible colonies truly varied in resistance to AFB. His goal was to understand 'what constitutes so-called resistance'[29]. He found that larvae derived either from resistant or from susceptible colonies were equally susceptible to AFB and concluded, '… colony resistance depends on behavioural factors rather than on physiological resistance in the larvae'[29].

Woodrow[30] observed individual larvae as they became diseased after feeding the colony sugar syrup inoculated with spores of *P. larvae*. Nine strains of bees were tested, and all colonies removed the diseased brood before it hardened to a scale. However, there was considerable variation in the development of the disease among the colonies which was not related to colony strength. The variation in the time required to clean out the cells was proportional to the amount of diseased brood present. In lightly infected colonies, most diseased brood was removed within seven days. In highly infected colonies, removal required a longer time, and some cells were neglected. The cells that were cleaned out were reused, and cultures obtained from them showed that they still contained spores. New brood reared in the cells of lightly diseased colonies did not become infected, but a third of the new brood in highly diseased colonies succumbed to AFB. Reinfection was attributed not to poor cleaning of the cell but rather to nurse and housecleaning bees. The cells that were cleaned out were also used for pollen and honey storage; however, honey was not stored in cells with visible remains of larvae with AFB.

The only evidence found for physiological resistance to AFB concerned the age of the larvae susceptible to infection: one-day larvae were most susceptible, while larvae inoculated more than two days five hours after hatching did not become infected[31]. In colonies of various strains and degrees of resistance, larvae during the susceptible stage (0–1 day) were inoculated by suspending *P. larvae* spores in larval food. When the brood cells were sealed, the comb was caged so that the bees could not remove the infected brood. Inspection of pupae before emergence revealed that 33–41% of the brood was removed before sealing and that 44–75% of those cells that were sealed contained diseased brood[31,32]. Spore inoculation of larvae when bees were 1–2 days or 2–4 days of age did not result in any removal of the larvae, and none of the inoculated larvae became diseased.

Woodrow and Holst[32] inoculated 0–1-day-old larvae with only the rod (vegetative) stage of the bacterium that precedes the spore stage in the infection. Only 0.5% of the larvae became diseased. These results demonstrated that the rod stage of the bacterium was not very infectious (also see Tarr[25]) and it was hypothesized that if infected larvae were removed before spores are produced, then further dissemination of the disease in the colony would be prevented[32].

Using one resistant and one susceptible colony, Woodrow and Holst[32] determined the stage of the disease at which the infected larvae were removed. Bees in the resistant colony removed larvae beginning on the sixth day after inoculation and, within 11 days, 138 of 200 (69%) of the inoculated larvae were removed. None of the removed larvae contained spores, but 23 of 25 larvae inspected contained rods of *P. larvae*. No additional larvae were removed after the 11th day, and the remaining pupae were not infected. Bees in the susceptible colony removed larvae beginning on the ninth day

after inoculation and continued to remove brood throughout development of larvae and pupae; *P. larvae* spores were found in all of the removed larvae. At the end of the experiment, 39 of 202 (19%) of the diseased pupae remained in the susceptible colony. Prior to removal, many of the cells containing inoculated larvae were uncapped by the bees in both colonies. In the resistant colony, the uncapped larvae were removed within 24 hours. In the susceptible colony, however, some of the uncapped larvae were resealed and later found to be infected. Woodrow and Holst[32] remarked, 'The bees are able to detect early stages of infection in the brood more readily than can the human observer… None of the larvae being removed from the resistant colony were discoloured or abnormal in appearance. Most of those examined, however, contained rods of *B. larvae*.'

The experiments were repeated by Woodrow and States[33] with similar findings. Thus, the conclusions were as follows:

- 'The data show that resistance to American foulbrood in the honey bee colony consists in its ability to detect and remove diseased brood before the causative organism, *B. larvae*, reaches the infectious spore stage in the diseased larvae'[32].
- 'The early removal of diseased larvae while they contain only the non-infectious rods of *B. larvae* prevents dissemination of disease in the colony, whereas removal of infected brood containing the highly infectious spores results in spread of disease to other larvae'[32].
- 'Light infections sometimes are overcome whereas heavy ones are not, but the extent of infection is not determined entirely by the inoculating dose. The spread of disease within colonies depends rather on the extent to which general contamination of the colony is produced during diseased brood removal'[33].
- '… it is likely that [the removal of diseased brood] is a common behaviour of honey bees. Furthermore, since diseased brood was removed regardless of whether the colonies recovered from the disease, it is evident that colony resistance to American foulbrood does not depend entirely on this behaviour characteristic'[33].

The Rothenbuhler studies — larval resistance to AFB

In 1951, Walter C Rothenbuhler (Iowa State College, USA) began his long and productive research career on the mechanisms of resistance to AFB and on hygienic behaviour. He reviewed the work of Park and Woodrow and concluded that the ability of the bees to detect and remove diseased larvae (coining the term 'hygienic behaviour' to describe the process) was not a complete explanation of resistance[23]. He referred to work by Sturtevant and Revell[24] who discovered one possible physiological mode of resistance. These authors found that bees from colonies apparently resistant to AFB removed a higher percentage of spores from infected syrup than bees from susceptible colonies. The spores were removed from the syrup by the action of the proventricular valve, located between the crop (honey sac) and the ventriculus (midgut) in the digestive tract of the bees. The valve captures pollen grains and other small particles, including bacterial spores, suspended in nectar from the crop and passes them through the digestive system. In this way, spores are eliminated from the body rather than regurgitated with the nectar and possibly fed to larvae. A second mode of resistance was discovered by Victor Thompson,

a student of Rothenbuhler. Thompson (unpublished Master's thesis, cited in Rothenbuhler & Thompson[23]) found that nurse bees from a Hawaiian strain of bees were capable of preventing more of the larvae they fed from succumbing to AFB than were nurse bees from the highly susceptible line. The exact mechanism was not determined at the time, but it was speculated that the royal jelly secreted by adult bees from some lines may have a greater bactericidal effect[8] or that adult bees may disinfect the cell surface before the queen lays an egg in the cell[19].

Based on these previous studies, Rothenbuhler began his experiments by assuming that stocks of different origins have different potentials for resistance and that different types of inoculations conveying the pathogen to the host (e.g. spores in sugar syrup, spores in brood food, brood comb with spores) might result in the display of different mechanisms of resistance in bee colonies.

In all experiments, Rothenbuhler and his students compared a resistant line of bees to a highly susceptible (control) line. The most renowned resistant line originated in 1954 from E G Brown, a beekeeper from Sioux City, Iowa. Brown allowed his colonies to rob honey from infected combs brought to him for wax rendering. Four of his colonies were found to be highly resistant to AFB and provided queen and drone progeny for the Brown line used in all of Rothenbuhler's subsequent studies. The susceptible line began with a single queen from H Van Scoy of Candor, New York. Queens were reared from the Van Scoy colony and were inbred by mating them with drones from the same colony using instrumental insemination. The progeny colonies were selected for increased susceptibility.

In the first experiment, Rothenbuhler tested the hypothesis that larvae from different genetic lines of bees have different degrees of resistance to AFB. Two colonies of the Brown line, two from the Van Scoy line, and three from the Chartreuse line (bees with an eye-colour mutation) were tested. The larval food in cells containing one-day-old larvae was inoculated either with 50 000 *P. larvae* spores in tap water (spore treatment) or with tap water containing no spores ('check' treatment). From three rows of cells containing 30 larvae each, one row was chosen at random to be given either the spore or check treatment or to be untreated (control). Because many spore and check treatment larvae died within two days of inoculation, base counts of the number of treated larvae were made on the second day after inoculation and compared to the number of healthy, dead, or missing individuals on day 12. By day 12, 95% of the larvae receiving water survived; 67% of the larvae from the Brown line survived the spore treatment compared to 47% from the Chartreuse line and 25% from the Van Scoy line. Rothenbuhler and Thompson[23] concluded, 'These differences are interpreted to be due to different levels of innate resistance to American foulbrood in the larvae of the three lines.'

In an elaborate second study, Thompson and Rothenbuhler[27] tested the hypothesis that adult bees have mechanisms to protect the larvae from infection. Larvae from resistant and susceptible lines were transferred into colonies containing resistant or susceptible adults. One resistant colony and one susceptible colony were fed daily for 20 days with sugar syrup containing *P. larvae* spores. Another pair of resistant and susceptible colonies were not inoculated. Fewer larvae succumbed to AFB within the resistant colony (8.7%) than in the susceptible colony (11.6%), and only six of 20 combs containing eggs introduced into the resistant colony showed larvae with symptoms of AFB compared to 17 of 20 combs

introduced into the susceptible colony. Thompson and Rothenbuhler[27] concluded that the effect of the adult bees on conferring resistance to the larvae was small relative to the effect of larval resistance itself.

Between 1961 and 1967, Rothenbuhler and his students continued their studies on physiological mechanisms of larval resistance to AFB[3,4,5,10]. At the same time, however, Rothenbuhler turned his attention back to the early studies on hygienic behaviour as a mechanism of resistance to AFB.

The Rothenbuhler studies — genetics of hygienic behaviour

Beginning in 1964, Rothenbuhler and his students published the now famous six-part series of articles on the behavioural genetics of hygienic behaviour in honey bees[9,12,21,22,26,28]. This work, based on elegant experimental designs, is recognized as a classic example of the effects of Mendelian inherited genes on behaviour[1,7]. It demonstrated both the genetic and environmental effects on this economically important trait.

Rothenbuhler began by testing the ability of the resistant Brown and susceptible Van Scoy lines of bees as well as resistant and susceptible colonies from a new line (Squire) to remove larvae with AFB[21]. To assess resistance or susceptibility of the colonies, they were inoculated with *P. larvae* by inserting a section of comb containing 75 AFB-killed larvae in the centre of the brood nest (following Park[16]). Daughter queens were propagated from the most resistant and from the most susceptible colonies, and controlled matings were ensured by instrumentally inseminating the daughter queens with drones from selected colonies. Selection pressure was applied to successive generations by increasing the numbers of AFB-killed larvae presented during the test.

For the experiment, either water alone or water containing about 7000 *P. larvae* spores was suspended in the food for rows of larvae in comb cells of both the resistant and the susceptible lines. The base count for number of live larvae was made on the following day, and the number of larvae removed from the cells was counted each successive day. Two or three days before the end of the experiment, the remaining brood was uncapped to determine viability.

Over two trials, colonies from the Brown line removed the majority of the spore-inoculated larvae, whereas the Van Scoy line removed relatively few (see figs 1 and 2 in Rothenbuhler[21]). In addition, the Brown colony had no AFB-killed pupae on the 16th day after inoculation in any of the trials, whereas a high percentage (35–57%) of the inoculated larvae in the Van Scoy colonies were killed by AFB and then left in the cells under a wax capping. The results from the resistant and susceptible Squire lines were similar to those of the Brown and Van Scoy lines, respectively, although the resistant Squire colonies removed fewer infected larvae than did the Brown line.

Rothenbuhler then set out to determine the mode of inheritance of the trait and the number of loci (positions of the genes in the chromosome) responsible for its expression[22]. First, he instrumentally inseminated queens from the inbred Brown line with single drones from the inbred Van Scoy line to produce F_1 hybrid worker progeny. He then inoculated five F_1 colonies by introducing either AFB spores or water into the larval food. The F_1 bees did not remove the diseased larvae from the comb. From this, Rothenbuhler deduced that hygienic behaviour was a recessive trait.

To estimate the number of loci governing the expression of the trait, Rothenbuhler next backcrossed the F_1 progeny colonies to the original Brown parent. He raised

daughter queens from two of the F_1 colonies and then allowed the F_1 queens to produce drones. He collected semen from 29 drones and used it to inseminate 29 daughter queens raised from the Brown line parent colony by single-pair matings. He assumed *a priori* that if half of the colonies backcrossed to the Brown line (expected to be homozygous recessive) displayed hygienic behaviour, then one locus would be involved; if 25% were hygienic, then the behaviour would be controlled by two loci. He also performed the reciprocal backcross; eight drones from the F_1 queens were mated to daughter queens of the original (also inbred) Van Scoy parent colony. None of the colonies from this backcross to the susceptible (homozygous dominant) line should have expressed the behaviour. He then inoculated the backcrossed colonies and compared their hygienic response to pure Brown and pure Van Scoy colonies.

Of 29 colonies backcrossed to the Brown line, six (21% of the colonies) rapidly removed all the diseased larvae, and no diseased pupae remained in the combs. Thus, he concluded that hygienic behaviour was controlled by two loci. Contrary to expectation, one of the colonies from the backcross to the Van Scoy line removed all of the diseased larvae within two days. These results were problematic to Rothenbuhler because he assumed that if the trait were recessive, none of the progeny from the cross to the dominant parent would express the behaviour. With candor, he stated, 'We cannot disregard this result, regardless of how much we would like to, but we are basing the genetic hypothesis on the other data.'

None of the other 23 colonies from the backcross to the Brown line removed the diseased larvae; nine of these uncapped dead larvae, but 14 did not. At this point, Rothenbuhler speculated that one of the loci might be responsible for uncapping the cells and the other for removing the diseased larvae from the cells. He proceeded to uncap the cells that remained sealed in the 14 colonies and noted that six of the colonies removed most of the diseased brood within two days. Of the remaining eight colonies, three did not remove any diseased larvae, and the rest removed from 3–47%. Rothenbuhler[22] stated, 'Although this group of 14 colonies is not as discretely separated into removers and non-removers as the whole group of 29 was separated into uncappers and non-uncappers, for the time being the 14 are interpreted as falling into two groups. This seems to be the simplest possible explanation and justifiable in the light of inadequate knowledge about the environmental factors that affect the removal rate.'

The stinging behaviour of the backcrossed colonies was also recorded because of the prevalent notion that disease-resistant bees were highly defensive and that defending the nest against diseases and intruders (including beekeepers) were manifestations of the same trait. Rothenbuhler's results clearly showed that stinging behaviour and hygienic behaviour assorted independently and, therefore, were not controlled by the same sets of genes or linked on the same chromosome.

To summarize the results of his landmark experiment, Rothenbuhler[22] developed a two-locus model of inheritance for hygienic behaviour. The process of uncapping a cell containing dead brood and removing the contents was thought to be dependent upon homozygosity for two recessive genes. Workers heterozygous at one or both loci should not be hygienic. Homozygosity at one of the two loci should result in workers that either remove or uncap.

Although Rothenbuhler's work is most renown for determining the genetic basis of a complex behaviour, he and his

students were far-sighted in their approach to understanding both the genetic and environmental effects on the behaviour. In later years, Rothenbuhler was not certain that his Mendelian two-locus model was sufficient to explain the variability in the expression of the trait (A Collins, personal communication). In a subsequent re-evaluation of Rothenbuhler's model, Moritz[13] suggested that a three-locus model may better fit the original data set and that even more complex patterns of inheritance might be involved. Although Rothenbuhler's work demonstrated major gene effects on hygienic behaviour, he did not continue with the genetic line of research after 1964. Instead, he and his students concentrated on environmental effects on the behaviour — how colony demography, previous experience of the bees, and resource conditions influenced the expression of the trait.

Age of hygienic bees

Thompson[26] published the first article on the age at which bees tend to perform hygienic duties. The colonies in the experiment were comprised of a single-age (within four days) cohort of bees from the Brown line. Since no brood was allowed to emerge in the colonies, the age of all adult bees was known. Combs containing brood inoculated with spores of *P. larvae* via the larval food were introduced into the test colonies at various intervals when the bees were 4–68 days of age. When the Brown bees in the colony were all less than 27 days of age, they removed all of the diseased larvae. However, as the age cohort of bees exceeded four weeks, the bees removed all of the diseased larvae only when nectar was being brought into the nest. One colony composed of old bees was an exception and removed all of the diseased brood even when no nectar was available. It was concluded that young resistant bees will remove all diseased brood regardless of nectar availability, but bees older than about four weeks remove the larvae only during a nectar flow.

This experiment should be re-evaluated in light of more recent studies which demonstrated that the bees in single-age cohort colonies distribute their tasks irrespective of age; some young bees become precocious foragers, and some old bees revert to in-hive tasks such as brood feeding (reviewed in Robinson[20]). Therefore, in Thompson's study, some of the young bees must have performed tasks, such as foraging, which are characteristic of older bees, and thus they may have differed physiologically from same-age bees that continued performing in-hive tasks. More recent studies by Spivak (unpublished) in which two hygienic colonies were composed of bees of 11 different age cohorts (ranging from 3–33 days old), the mean ages of the bees that uncapped and removed freeze-killed brood were 15 and 17.5 days, respectively (n = 265 and 311 bees). The mean ages of the bees foraging in the same colonies were significantly greater, 22 and 20 days, respectively (n = 79 and 66 bees; t tests $P < 0.01$). This experiment indicated that hygienic bees are middle-aged; they have brood-rearing experience but have not necessarily begun foraging.

Bee experience

Trump *et al.*[28] determined whether hygienic behaviour could be modified by learning. The rate of removal of AFB-diseased brood was compared among single-age cohort colonies of the Brown line, the Van Scoy line, and a 50 : 50 mix of the two lines. Subsequently, the bees in the mixed colonies were sorted by colour (Brown bees were dark and Van Scoy bees yellow) and introduced into new colonies containing bees of the same kind. The results showed that the mixed colonies removed diseased brood as quickly as the pure Brown colonies. However, when the bees were later sorted

into their respective types, the Brown bees removed the diseased brood, and the Van Scoy bees did not. These results indicated that the Van Scoy bees did not learn the behaviour from the Brown bees and suggested that the bees that performed the behaviour in the mixed colonies were probably all from the Brown line.

They then determined the proportion of bees of the hygienic type necessary for a colony to express the behaviour. When one colony was composed of 1300 Van Scoy bees and 200 Brown bees (13% hygienic bees), 23% of the diseased brood was removed on the first test and 47% on the second. Another colony composed of 11% Brown bees removed 89% on the first test and 47% on the second. These results emphasized the variability in the expression of the behaviour and indicated that for colony level expression of hygienic behaviour, 13–50% of the bees in the colony must carry the genes for the behaviour.

Interaction of age, genotype and nectar flow

Momot and Rothenbuhler[12] examined the interaction effects of age of hygienic bees, genotype, and resource availability. Four mixed colonies were established; two had adult bees from the Brown line and emerging brood from the Van Scoy line. The other two contained adult bees of the Van Scoy line and brood from the Brown line. The amount of cyanide-killed brood (this procedure is discussed in Part II) that was removed by the colonies over three months was recorded. During this time, the older bees died, and the new adults emerged. Thus, the composition of the first two colonies changed from Brown to Van Scoy bees and that of the second two colonies from Van Scoy to Brown bees. As predicted, the colonies originally composed of Brown-line adults removed fewer dead larvae as the Van Scoy bees emerged and aged, and vice versa. When the composition of the colonies had completely reverted to the opposite type in one set of trials, the Brown bees removed all the dead brood within 21 hours, whereas the Van Scoy bees removed only 16% in the same time.

The effects of incoming nectar affected the expression of the trait within the colonies as noted previously by Thompson[26]. For example, one colony composed originally of all Brown bees removed all of the dead brood in one test when all of the Brown bees were over 30 days of age. There was a considerable nectar flow at the time of that test. However, one week later, during a nectar dearth, the same colony took four times as long to remove the dead brood.

Momot and Rothenbuhler[12] wondered whether Brown bees that had begun foraging would return to the hive duty of removing diseased brood. A colony was established that contained Brown bees of foraging age and emerging Van Scoy brood, and another colony was established with the opposite mix. The colonies were given a comb section of dead brood two weeks after they were established. The colony with the Brown foragers and Van Scoy hive bees removed the dead brood much more slowly than the colony with the opposite mix. Because resource conditions also influenced the removal rates in these experiments, it was tentatively concluded that young (in-hive) Brown bees are invariably hygienic, but old (foraging) Brown bees could be induced by a nectar flow to be hygienic. Neither young nor old Van Scoy bees were hygienic, and nectar flow did not influence their propensity to remove dead brood.

Part II of this review, 'Studies on hygienic behaviour since the Rothenbuhler era' will follow in the next issue of Bee World.

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