34 The Impact Of Temperature Gradients In The Brood Nest Of Honey Bees On The Reproduction Of *Varroa jacobsoni* Oud.: Field Experiments

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ABSTRACT

Since our laboratory experiments demonstrated a considerable impact of small temperature differences in worker brood cells on mite reproduction rates, we started temperature measurements inside these cells in both normal-sized and small colonies of honey bees. We used colonies of *A. mellifera mellifera*, *A. m. carnica*, and the race hybrid Buckfast.

When approximately similar ratios of adult worker bees to brood cells were maintained, we found no significant differences in brood nest temperatures between the three types of colonies. Their thermoregulatory capacities were quite similar. Regional differences in temperature within the brood nest tended to be dome-shaped, with a temperature close to 35°C in the center and near 33°C at the periphery. Even when small colonies (2 frames of brood covered by 1,000-1,500 bees) were kept at ambient temperatures of 4-8°C, no race-specific differences were found in the brood nest temperatures.

The two pure races and the Buckfast hybrid did, however, differ in their brood-rearing responses to seasonal changes. During the second half of March, the *A. m. carnica* and Buckfast colonies had adult populations comparable in size to, but brood nests on average much larger than the *A. m. mellifera* colonies. This had an impact on their thermoregulation. Brood cells of *A. m. carnica* colonies were characterized by a lower temperature when compared to cells of *A. m. mellifera*, on average, the difference was 0.3°C in the center and 1.3°C at the very periphery of the brood nest. When comparing Buckfast and *A. m. mellifera*, these differences were 0.5°C and 0.7°C, respectively. As a consequence, during spring *A. m. carnica* and Buckfast colonies had brood nest temperatures close to the optimum for the reproduction of Varroa mites.

In order to test the impact of brood nest temperature on mite propagation, two groups of 12 colonies each were placed in an area with no bee colonies, thus preventing mite immigration. The colonies were treated in early autumn with Apistan to remove any mites present. Then, each colony was re-infested with 200 mites. In one group, the experimental colonies, steps were taken to lower the average brood nest temperature: thin hive lid, open bottom board, stimulative feeding at the end of winter in order to increase brood nest size, and insertion of comb foundation in the brood nest in early spring. The other group, the control colonies, was kept in regular insulated hives and received neither stimulative

feeding nor comb foundation in the brood nest. Until February, no difference was found in the average number of dead mites that were collected twice a month from the bottom boards of the hives. From March onwards, however, the number of dead mites was higher in the experimental colonies. At the end of May, mite populations in all colonies were estimated using fluvalinate treatment. The control colonies contained on average less than half the number of mites as the experimental group, 476 ± 212 and 1,032 ± 563, respectively.

The average intrinsic rate of population growth in the experimental colonies was calculated to be 1.11 per week. The infestation rate at the end of May was high enough in 40% of the experimental colonies to reach catastrophic infestation levels before the next fall. None of the control colonies would have reached that level.

Considering these pronounced effects of small differences in brood cell temperature, we decided to study mite reproduction in relation to brood cell temperature in *A. cerana*. These measurements were made in cooperation with Dr. S. Tingek at the Agricultural Research Station at Tenom, Sabah, Malaysia. When external temperatures ranged from 18°C to 33°C, the temperature inside the worker brood cells at the center of the brood nest varied from 34.9°C to 36.5°C. At the periphery of the brood nest, the temperatures in worker brood cells were considerably affected by ambient conditions: from an average of 32.7°C with an external temperature of 18°C to 35.3°C with an external temperature of 33°C. Drone brood cells, usually located at the periphery of the brood nest, were on average 0.4°C lower in temperature than neighboring worker brood cells. As a consequence, the temperature in the drone brood cells of *A. cerana* colonies was on average 33°C. Our incubator studies have shown that this is the temperature at which Varroa reaches optimal reproductive success. Because the capped period of worker cells is too short for mite reproduction, Varroa specializes on the drone cells of its host. This explains why *V. jacobsoni* has its reproductive optimum at 33°C. Beekeepers, using *A. mellifera* colonies, could profit from this specialization of the mite by applying techniques that help their colonies maintain the preferred brood nest temperature of 35°C.

INTRODUCTION

Small differences in temperature greatly affect reproduction of today's most important honey bee parasite, *Varroa jacobsoni* (Le Conte *et al.* 1990, Velthuis & Kraus, this Volume). The mite can reproduce exclusively within capped brood cells. We, therefore, conducted studies in order to learn about the factors which influence brood nest temperature and to understand why the mite's reproductive optimum is at the comparatively low temperature of 33°C. In cold and temperate climates *A. mellifera* colonies do not survive Varroa mite infestation without treatment (Ritter 1988, 1989), and acaricide resistance of *Varroa jacobsoni* (Lodesani et al. 1995). In addition, acaricide residues in bee products (Wallner 1995) are causing rapidly increasing problems to the bee keeping industry. Therefore, it is crucial to understand the factors which have an impact upon population growth of the parasite.

Honey bees have the most a well developed system of insect nest temperature control (Seeley & Heinrich 1981) and thermoregulation in honey bees is a well studied field of research (Heinrich 1981, 1993). Numerous studies of tem-

perature within the brood nest of *Apis mellifera* colonies have shown that the average temperature is kept within a small range (Gates 1914, Hess 1926, Vanssell 1930, Himmer 1932, Dunham 1933, Büdel 1960, Wohlgemuth 1957, Fahrenholz *et al.* 1989, Levin & Collinson 1990, Rosenkranz & Engels 1994). However, most studies conducted so far were aimed at describing the behavioral and physiological mechanisms involved in the process of thermoregulation. Furthermore, studies dealing with brood nest temperatures were often conducted only during summer, under experimental conditions in colonies of small size, and by measuring temperature in the space between brood combs, but not inside brood cells. We, therefore, conducted detailed studies on the effect of colony structure and ambient temperature upon brood nest temperature as well as on temperature distribution within the brood nest. The next step was to apply the results of these studies by testing the effect of beekeeping techniques upon brood nest temperature and population growth of *Varroa jacobsoni*.

A group of infested colonies was kept in poorly insulated hives while a second group of colonies was kept in well insulated hives. In the first group, additional brood rearing was stimulated while in the second group brood rearing was not stimulated. When considering the impact of temperature upon the parasite's population growth it is essential to know bee race dependent differences in brood nest temperature. Differences in thermoregulation of different *Apis mellifera* races have been found (Michel 1995) in laboratory tests conducted with groups of adult workers. We examined brood nest temperature within brood cells of colonies of the races *Apis mellifera mellifera*, the original race located in the North-West of Europe (Ruttner 1988), *Apis mellifera carnica*, a race originating from South-Eastern Europe (Ruttner 1988) which has been imported into numerous countries within and outside Europe due to its low defensiveness, and the race-Hybrid Buckfast which is kept by a growing number of beekeepers in Europe and North America.

In colonies of its original host Apis cerana the mite reproduces nearly exclusively in drone brood cells (Koeniger *et al.* 1981) while in colonies of *A. mellifera* reproduction of the mite is common in worker brood cells as well. In order to better understand the impact of temperature upon the parasite's reproduction we measured temperatures in different areas of the brood nest in worker and drone brood cells of *Apis cerana*, within the natural ambient temperature range of tropical *Apis cerana indica* in Malaysia, and within the same temperature range, in worker brood cells of *Apis mellifera* in the Netherlands (Kraus *et. al.*1998).

The central questions motivating our studies were:

- How is temperature in brood cells of honey bee colonies affected by bee race, season, position within the brood nest, number of worker bees and brood cells?

- How do bee keeping management techniques influence brood nest temperature and, therefore, population growth of *Varroa jacobsoni*?

- How does temperature dependency of the mite's reproduction fit together with conditions within the brood nest of colonies of its original host *Apis cerana*?

MATERIALS AND METHODS

Animals

Temperature measurements were conducted from February until October, 1995 and 1996 in a total of 103 colonies of the "land race" kept by most beekeepers within the Netherlands, predominately consisting of the common European black bee *Apis mellifera mellifera*, *Apis mellifera carnica* and the race hybrid, "Buckfast". Data were pooled because no differences in brood nest temperatures of colonies of different races were found. Bee colonies were kept in wooden hives holding 10 frames per box (frame size 37.0 x 22.3 cm). The number of bees and the size of the brood nest was estimated using the method developed by Gerig (1983): the percentages of comb areas covered by bees or brood cells were estimated by defining the total area of one comb side as 100%. The experiment on population growth of Varroa mites in differentially managed colonies was conducted from August 1996 until June 1997 with colonies of the "land race".

Temperature measurements

Measurements were conducted using the thermocouple reader Stanford Research Systems Model SR 630 with 15 copper-constantan thermocouples (Æ wire 0.2 mm, accuracy 0.1°C) in combination with a computer for data storage. Temperature was automatically monitored in 1-minute-intervals. During measurements conducted in the Netherlands and Germany, temperature was monitored from 2000 to 800 h. Because no foragers are outside, the ratio of number of adult bees and number of brood cells is better standardized during the night. Thermocouple tips were inserted under the cell cap and their wires were attached to the comb with liquid wax. Temperature was measured in worker brood cells, located at different places on the comb. These places were on two combs, one comb in the middle of the brood nest (middle comb), and the other comb located at the periphery of the brood nest (peripheral comb). On each comb thermocouples were inserted under the capping of brood cells, which were located at the edge of the comb as well as in its center. Four cells at the edge of the brood nest were selected having only two out of six neighboring cells containing brood, being respectively at the utmost upper, lower, right and left edge of the brood area. Three thermocouples were inserted in brood cells located in the center of each of the two combs, these cells being surrounded by six cells containing brood. The hole in the capping caused by inserting the thermocouple was closed with a small droplet of melted wax. The first temperature readings were made one hour after insertion of the thermocouples. Furthermore, the 15th thermocouple was used to record ambient temperatures during measurements. After measurements were finished, the position of the thermocouples and the condition of the monitored cell was checked. Worker bees exhibit hygienic behavior which results in opening brood cells and removing brood (Rothenbuhler 1964). Data from cells that were opened by the bees were excluded from analysis (within 12-18 h 16% of brood cells opened; n=210).

Ratio of worker bees and brood cells

In 10 colonies temperature was measured at average ambient temperatures ranging from 6-14°C during the test series. The following day, 50% of the brood cells were removed by replacing half of the number of brood combs with brood-

less combs. Temperature was measured again. After measurements, the brood cells were placed back in the colonies. The following day 40-50% of the workers (estimate according to Gerig, 1983) were removed and measurements were repeated.

Impact of beekeeping management methods upon population growth of Varroa jacobsoni

The colonies were located at the Flevoland Polder close to the Markerwaard lake, a former part of the North Sea shore, in an isolated wooded area belonging to a flood gate and closed to the public. In the whole area within flight range of our colonies, no bee colonies are known with the exception of 5 colonies belonging to a beekeeper. These colonies were treated by us with the Acaricide Apistan® before start of the experiment in order to avoid uncontrolled inoculation of the experimental colonies with Varroa mites. The colonies were positioned in two groups of 12 colonies each (low brood nest temperature group/high brood nest temperature group), with all hive entrances facing South East. Each group formed a row and the distance between both groups was about 0.5 km. Two colonies were queenless in spring and one colony swarmed before we finished the experiments. These colonies were thus excluded from the study.

Colony management - From August until end of April all colonies were housed in two stories. On April 24 the colonies received a honey supper (height 15 cm). The colonies were fed only during September and October 1996. The following management methods were differential in the high brood nest temperature group (HT group) and the low brood nest temperature group (LT group).

In the LT group January 30, 1997 the insulation of the hives was reduced. The thick pine covering the hive was elevated with sticks 13.5 cm above the hive. The hive was thus on top only insulated by a 0.5 plywood lid. The hive was put on 8 cm wooden feet and the removable board which is usually fit into the hive directly under the mesh wire bottom and provides insulation, was removed and placed on the ground16 cm below the mesh wire. The bottom of the hive was thus open. The HT group was kept in regular insulated hives.

The colonies of the LT group each received ½ kg of Nectapoll (sugar, pollen and water) on January 30 and one kg on February 27. This food stimulates brood production. The aim was to achieve a comparably low ratio of worker force and brood nest size. At the beginning of the experiment September 20 - October 10, 1997, the colonies of both groups contained similar numbers of bees (LT group: 6,060 ± 2,329, HT group: 6,429 ± 3,571). March 13, the colonies of the LT group had a smaller worker force but a larger brood nest as compared to colonies of the HT group (bees - LT group: 3726 ± 1245, HT group: 4,872 ± 2,972; brood cells - LT group: 9,714 ± 1,970, HT group: 8,193 ± 1,859). The ratio of workers per brood cell was thus on average clearly lower in colonies of the LT group (LT group: 0.38 ± 0.08, HT group: 0.58 ± 0.30).

The brood nest was enlarged in colonies of the LT group by inserting an empty comb per colony in the middle of the brood nest on March 3, and by inserting a comb foundation per colony (thin wax sheets where the bees have to build cells on) on April 2, 16 and 23.

Varroa mite population - The experimental colonies were treated with the highly efficient acaricide Apistan® for a 5 week period from end of August until

beginning of October 1996. The almost mite free colonies were inoculated with 200 Varroa mites each between 14th and 24th of October. 200 young workers were removed from a brood comb and kept in queen cages. Varroa mites sampled from bees of a highly infested colony were removed from their host bees and transferred to the workers. The bees, along with the 200 mites, were put back into their colony. The hives were provided with a removable bottom board which was placed under the mesh wire bottom of the hives. The boards were covered with paper and coated with a layer of Vaseline. The number of dead mites was counted weekly or in two weeks intervals. From 21st of May 1997 until 16th of June the test colonies were treated with Apistanâ and the number of dead Varroa mites was counted daily during the first week of the treatment and after the first week in 2 to 4 days intervals. We removed the queens of the colonies at the 22nd of May and provided young unmated queens at the 4th of June. The colonies did not contain any capped brood for a period of at least 5 days. At the end of this period no mites were found on the removable board.

RESULTS

Effect of position within the brood nest and ambient temperature

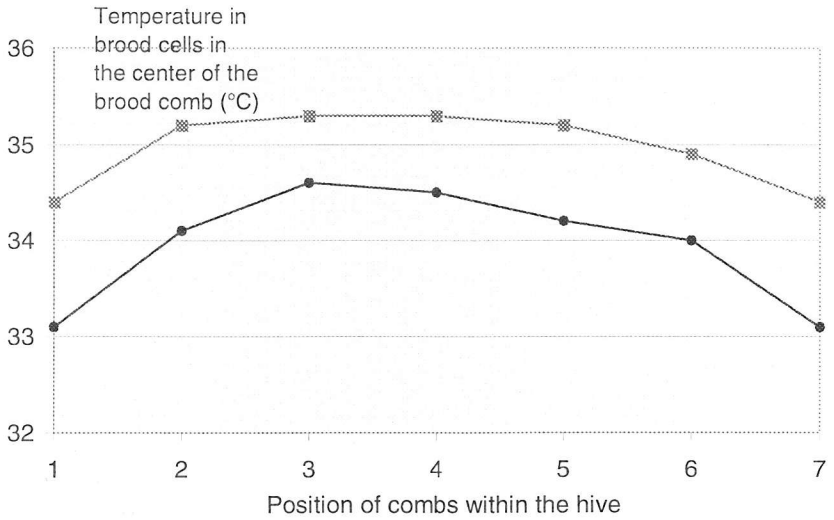
Average temperatures in brood cells varied from 30.7-35.6°C between brood cells. Temperature ranges during one night varied from 0.2-5.2°C. During the brood rearing season from February to October average temperature in brood cells located within the center of the brood nest is on average 1.6°C higher as compared to brood cells located at the very periphery of the brood nest (Table 1). Average temperature in brood cells located in the center of a peripheral comb is similar as compared to temperature in brood cells located at the edge of the middle comb. On average, temperature range in brood cells located at the periphery of the brood nest is about twice as large as in brood cells located in the center of the brood nest.

Table 1. Temperature and temperature range in different areas of the brood nest (February - October) 2000 - 800 h1.

	Average Temp. (°C)	Average Temp. range (°C)
Center of the middle comb	34.9 ± 0.4	1.5 ± 0.9
Edge of the middle comb	33.9 ± 0.7	2.7 ± 0.7
Center of the peripheral Comb	33.9 ± 0.4	1.7 ± 0.7
Edge of the peripheral comb	33.3 ± 0.7	3.1 ± 0.7

1 n=103 colonies with 721 - 2,884 measurements per colony.

The temperature curve within the brood nest tends to be dome-shaped (Fig. 1). Temperature in brood cells decreases with ambient temperature with the effect being far more pronounced in peripheral combs. At average ambient temperatures of -1.3°C brood cell temperature in the center of brood combs, all taken together, is on average 1.0°C lower as compared to brood cell temperatures at average ambient temperatures of 17.4°C. In the three middle combs the difference is on average 0.7°C, at the peripheral combs the difference is on average 1.8°C.



circle: lowest ambient temperature -3.9 °C / highest ambient temperature 6.9 °C
square: lowest ambient temperature 13.8 °C / highest ambient temperature 19.3 °C
n= 5 per group
all measurements conducted in brood chambers with seven brood combs brood combs always in the upper brood chamber in all used colonies ratio number of adult bees / number of brood cells 0.7-1.1

Fig. 1. Temperature distribution with the brood nest depending on position within the brood nest and ambient temperature.

Effect of bee race

At the end of February the size of the worker force and the size of the brood nest was similar in colonies of *Apis mellifera mellifera* and *Apis mellifera carnica* (Table 2). During the second half of March the size of the worker force was similar in colonies of both races but the *Apis mellifera carnica* colonies had on average a much larger brood nest and, therefore, a lower ratio of workers and brood cells. Temperature in all areas of the brood nest was on average lower in brood cells of *Apis mellifera carnica* colonies compared to *Apis mellifera mellifera* colonies, on average 0.3°C in the center of the brood nest and 1.3°C at the very periphery of the brood nest. During fall *Apis mellifera mellifera* colonies had on average a higher number of brood cells compared to *Apis mellifera carnica* colonies while the size of the worker force was similar. The temperature in brood cells located at the edge of the middle brood comb was lower in *Apis mellifera mellifera* colonies. Temperature in all other areas of the brood nest was similar in colonies of both races during fall.

Table 2. Comparative measurements in *Apis mellifera mellifera* and *Apis mellifera carnica* colonies1.

	Apis mellifera mellifera	*Apis mellifera carnica*	p-value;Wilcoxon matched pair signed ranks test
A. February 19 -29, n= 5 pairs, average ambient temperature - 0.9 ± 3.4 °C			
number of workers	9,440 ± 3,053	8,768 ± 4,462	n.s.
number of brood cell	5,539 ± 1,750	4,388 ± 2,120	n.s.
average ratio of workers per brood cells	1.7 ± 0.3	2.0 ± 0.7	n.s.
temperature within brood cells (°C)			
center middle comb	34.7 ± 0.2	34.6 ± 0.3	n.s.
edge middle comb	33.6 ± 0.6	33.2 ± 0.9	n.s.
center peripheral comb	34.2 ± 0.2	34.4 ± 0.3	n.s.
edge peripheral comb	33.2 ± 0.8	32.8 ± 0.9	n.s.
B. March 18 -22, n= 5 pairs, average ambient temperature 4.7 ± 2.9 °C			
number of workers	10,560 ± 3,725	11,312 ± 3,179	n.s.
number of brood cells	7,440 ± 1,840	15,360 ± 3,100	0.04
average ratio of workers per brood cell	1.4 ± 0.1	0.7 ± 0.09	0.04
temperature within brood cells (°C)			
center middle comb	34.9 ± 0.2	34.6 ± 0.2	0.04
edge middle comb	34.1 ± 0.3	32.9 ± 0.7	0.04
center peripheral comb	34.8 ± 0.2	34.2 ± 0.2	0.04
edge peripheral comb	33.7 ± 0.4	32.4 ± 1.1	0.04
C. September 29 - October 25, n= 13 pairs, average ambient temperature 12.4 ± 2.9 °C			
number of workers	6,658 ± 3,861	5,931 ± 2,698	n.s.
number of brood cells	6,840 ± 1,840	3,720 ± 1,175	0.01
average ratio of workers per brood cell	1.0 ± 1.5	1.6 ± 1.7	n.s.
temperature within brood cells (°C)			
center middle comb	34.9 ± 0.4	34.9 ± 0.5	n.s.
edge middle comb	33.7 ± 0.5	34.0 ± 0.5	0.03
center peripheral comb	34.3 ± 0.6	34.4 ± 0.7	n.s.
edge peripheral comb	33.1 ± 0.6	33.3 ± 0.6	n.s.

[1] n.s.=statistically not significant.

During spring also, colonies of the race hybrid Buckfast had a clearly lower ratio of workers and brood cells compared to colonies of *Apis mellifera mellifera* (Table 3). Numbers of brood cells were similar in colonies of both races but Buckfast colonies had on average a lower number of workers. In all areas the average temperature in brood cells was clearly higher in colonies of Apis mellifera carnica. During spring the colonies of the race hybrid Buckfast had on average a higher number of workers and a higher number of brood cells but a lower ratio of workers and brood cells than *Apis mellifera mellifera* colonies. Temperature was lower in brood cells of Buckfast colonies in all areas of the brood nest, statistically significantly lower within cells at the edge of the peripheral comb and the center of the middle comb.

Effect of ratio of numbers of workers and brood cells upon brood nest temperature

In colonies with 50% of the brood cells removed, differences in average brood cell temperatures were $\leq 0.2°C$ for all areas of the brood nest as compared to measurements conducted before removal of the brood cells (Table 4). After removal of 50% of the worker force in all the colonies average brood cell temperature was lower in all areas of the brood nest. The effect of worker removal is significant for each brood nest area ($p<0.01$; Dixon and Mood sign test). Obviously the ratio of worker bees and brood cells clearly affects brood nest temperature but only at a low ratio.

Table 3. Comparative measurements in *Apis mellifera mellifera* /*Apis mellifera carnica* and Buckfast colonies1.

	Apis mellifera mellifera	Race hybrid Buckfast	p-value;Wilcoxon matched pair signed ranks test
April 30 - May 3, n= 5 pairs, average ambient temperature 7.5 ± 0.8 °C			
number of workers	10,604 ± 5,233	3,658 ± 49	0.04
number of brood cells	13,695 ± 3,556	15,092 ± 4,905	n.s.
average ratio of workers per brood cell	0.8 ± 0.2	0.2 ± 0.04	0.04
temperature within brood cells (°C)			
center middle comb	35.3 ± 0.2	34.9 ± 0.3	0.04
edge middle comb	34.4 ± 0.2	33.3 ± 0.7	0.04
center peripheral comb	34.8 ± 0.4	34.3 ± 0.4	0.04
edge peripheral comb	33.6 ± 0.3	32.9 ± 0.2	0.04
	Apis mellifera carnica	Race hybrid Buckfast	p-value;Wilcoxon matched pair signed ranks test
April 8- 12, n= 5 pairs, average ambient temperature 3.2 ± 3.9 °C			
number of workers	4,760 ± 413	7,808 ± 413	0.04
number of brood cells	8,208 ± 1,683	16,656 ± 898	0.04
average ratio of workers per brood cell	0.58 ± 0.06	0.47 ± 0.05	0.04
temperature within brood cells (°C)			
center middle comb	35.1 ± 0.2	34.8 ± 0.4	0.04
edge middle comb	34.4 ± 0.4	34.1 ± 0.3	n.s.
center peripheral comb	34.7 ± 0.4	34.3 ± 0.5	n.s.
edge peripheral comb	34.0 ± 0.5	33.4 ± 0.6	0.04

[1] n.s.=statistically not significant.

Ratio of numbers of workers and brood cells in dependence of season and bee race.

The ratio of workers per brood cell decreased rapidly during spring (Fig. 2). At the end of February and the beginning of March the average ratio of workers per brood cell was slightly higher in *Apis mellifera carnica* colonies compared to *Apis mellifera mellifera* colonies, but during March the ratio decreased much faster in *Apis mellifera carnica* colonies. During May the number of workers was

very low compared to the size of the brood nest. Buckfast colonies had similar ratios of workers per brood cell as *Apis mellifera carnica* colonies. During September and October the ratio of workers per brood cell reached high values again with *Apis mellifera carnica* colonies on average reaching higher values compared to *Apis mellifera mellifera* colonies.

Table 4. Repeated measurements within the same colony after removal of brood and worker bees1.

	Full size colony	Brood removed	p-value; Dixon & Mood sign test
May 3 - 15, n= 10, average ambient temperature 6 - 14°C			
number of workers	20,453 ± 2,779	20,453 ± 2,779	n.s.
number of brood cells	19,562 ± 2,216	10,360 ± 996	0.01
average ratio of workers per brood cell	1.0 ± 0.1	2.0 ± 0.1	0.01
temperature within brood cells (°C)			
center middle comb	35.1 ± 0.2	35.3 ± 0.2	n.s.
edge middle comb	34.2 ± 0,2	34.4 ± 2.9	n.s.
center peripheral comb	34.7 ± 0.6	34.5 ± 0.2	n.s.
edge peripheral comb	33.3 ± 0.1	33.1 ± 0.1	n.s.
	Full size colony	Worker bees removed	p-value; Dixon & Mood sign test
May 3 - 15, n= 10, average ambient temperature 6 - 14°C			
number of workers	21,146 ± 2,883	13,333 ± 754	0.01
number of brood cells	23,160 ± 3,080	23,160 ± 3,080	n.s.
average ratio of workers per brood cell	0.9 ± 0.2	0.6 ± 0.1	0.01
temperature within brood cells (°C)			
center middle comb	35.4 ± 0.2	35.2 ± 1.2	0.01
edge middle comb	34.4 ± 0.5	33.9 ± 0.5	0.01
center peripheral comb	34.5 ± 0.1	34.3 ± 0.1	0.01
edge peripheral comb	33.1 ± 0.2	32.4 ± 0.3	0.01

[1] n.s.=statistically not significant.

Population growth of Varroa jacobsoni in differentially managed colonies

On average, 24 (12%) of the introduced mites were found on the bottom boards during October and November, within a 6 week period after inoculation of the experimental colonies with 200 mites each. From December until end of February average numbers of mite fall never exceeded 3 mites per colony per two week interval. After the beginning of March, numbers of dead mites on bottom boards increased quickly. Mite fall in colonies of the LT group was on average higher than the HT group. When mite down fall was determined for the last time before the start of the acaricide treatment, average numbers of dead mites on bottom boards of the LT colonies exceeded those of the HT colonies by 95%.

At test end, colonies of the LT group contained on average a 117% larger mite population as compared to colonies of the HT group (Fig. 3). The difference

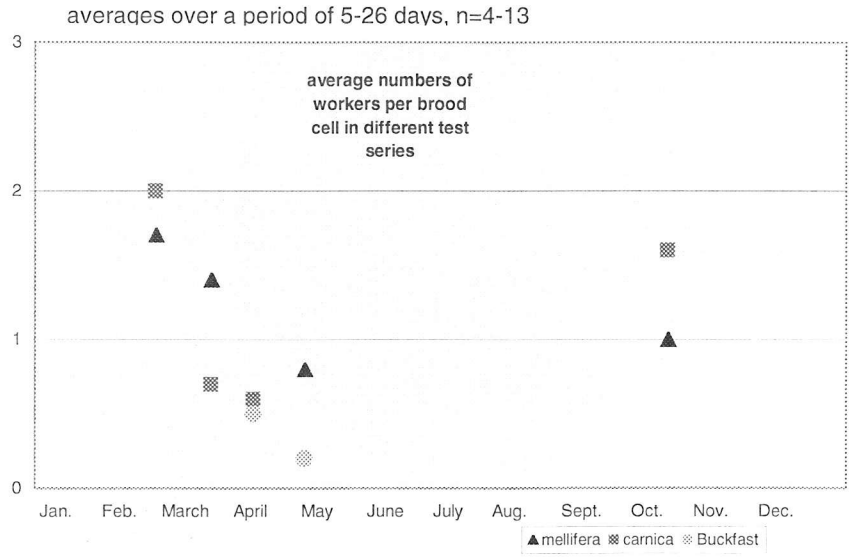


Fig. 2. Workers per brood cell in colonies of different races (1995 and 1996).

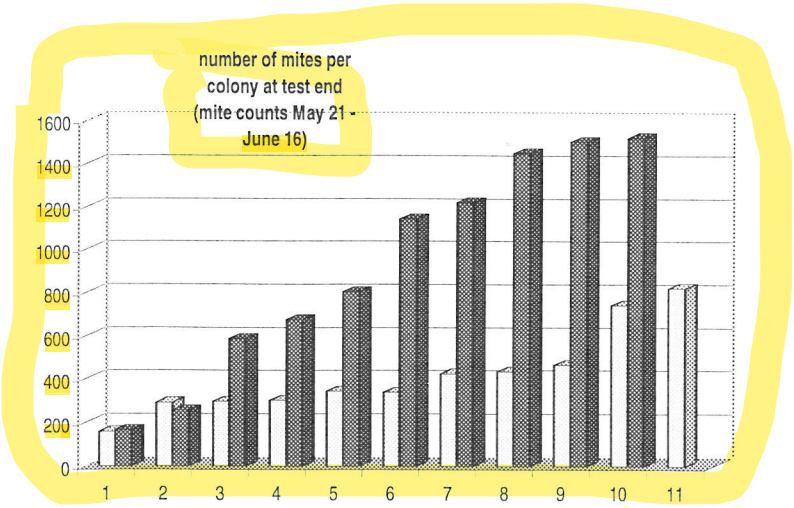
is statistically significant. Within-group variance of mite populations was lower in the HT group as compared to the LT group (Levene's test for equality of variances, p=0.001).

During October, when the experimental colonies were inoculated with mites, most of the colonies did not contain any open brood. The mites were thus not able to reproduce. We assume in our calculation that the mites started reproduction with the beginning of March. From inoculation of the colonies until February 27, on average 34 mites per colony were found on the bottom boards. The average mite population per colony at the start of the reproductive period was 166. Within 109 days, at the end of the acaricide treatment June 16, the average mite population per colony was 741. Given a population size N_0 of 50 mites and a population size Nt at the time t, determined by counting dead mites after treatment with fluvalinate, the increase R per elapsed time p is determined as follows:

$$R = \sqrt[t]{\frac{N_t}{N_0}}$$

t represents the number of elapsed periods p. (Emmel 1976). The intrinsic rate of population growth is 1.11 per week in the experimental colonies.

Given this same intrinsic rate of population growth, the mite populations in the colonies would have reached the following levels by the end of September, when most beekeepers apply measures of Varroa treatment: LT group 7,495 ± 4,091 mites per colony, HT group 3,457 ± 1,540 mites per colony.



left column: high brood nest temperature group (n=11)
right column: low brood nest temperature group (n=10)
average numbers of mites per colony:
LT group 1032 ± 563
HT group 476 ± 212
U-test Wilcoxon, Mann and Whitney, a=0.04

Fig. 3. Final varroa mite population in honey bee colonies after differential beekeeping management during Spring.

DISCUSSION

Effect of bee race, season, position within the brood nest, number of worker bees and brood cells upon temperature within honey bee brood cells

Our study and earlier studies conducted in honey bee colonies in the field (Gates 1914, Hess 1926, Himmer 1927, Dunham 1929, Büdel 1960) as well as studies conducted under laboratory conditions (Koeniger 1978, Kronenberg & Heller 1982, Ritter 1982) showed a dependence of brood nest temperature on ambient temperature. Ambient temperature and position within the brood nest were the factors which caused the most pronounced effects. Average temperature differences of up to 2°C were found between brood cell positions, and similar temperature differences were found for cells in the center of peripheral combs with varying ambient temperatures. Temperature distribution within the brood nest is dome-shaped with the peripheral combs being clearly more dependent on ambient temperature as compared to combs located within the brood nest center.

Repeated measurements within the same colony showed that among the factors of size of the worker force, size of the brood nest and ratio of workers to brood cells, the ratio influences brood nest temperature the most. This result also explains differences in brood nest temperatures of *Apis mellifera mellifera*, *Apis mellifera carnica* and Buckfast colonies. During spring colonies of *Apis mellifera carnica* increase size of the brood nest faster than colonies of *Apis mellifera mel-*

lifera and the size of the brood nest decreases faster during fall (Lunder 1953, Otten 1991). We found that especially during March *Apis mellifera carnica* colonies increased the size of their brood nest very quickly. During fall the average difference in brood nest size and ratio of workers per brood cell was not as pronounced in our study as reported by Otten (1991). Also, Buckfast colonies are known to increase the size of the brood nest comparably early during spring. We found similar ratios of workers per brood cell in Buckfast and in *Apis mellifera carnica* colonies. Colony development also varies with climate, population and bee management techniques. Studies dealing with brood nest temperature in colonies of the *Apis* species *mellifera, cerana, florea* and *dorsata* revealed no clear differences (Rosenkranz *et al* 1992). Comparisons of thermoregulation in European bee races and Africanized bees (hybrids of *Apis mellifera scutellata* and European *Apis mellifera* races) were conducted in order to estimate the capability of Africanized bees to survive cold winters. Villa *et al.* (1987) and Rosenkranz & Engels (1994) found no differences in average brood nest temperature of *Apis mellifera carnica* colonies and colonies of Africanized bees at low ambient temperatures. Unfortunately the authors do not provide data on numbers of workers and brood cells per colony. The temperature differences, therefore, can not be clearly attributed to race specific physiological differences. Núñez (1979) reported only small differences in the temperatures of the brood nest of European bees and Africanized bees at high and low ambient temperatures, but behavioral differences were obvious. We found no indication that the brood nest temperature differs in colonies of different *Apis mellifera* races as long as the colonies are of comparable size. Another experiment (Kraus & Velthuis, unpublished results) supports this conclusion: even when 10 small colonies of each race with 1,000 - 1,500 worker bees and 4,000 - 5,000 brood cells were kept at low temperatures of 4 - 8°C for 5 h, no significant differences in brood nest temperatures were found. Only in Spring, and to some degree in late Fall, the ratio of worker force and brood nest size varies with race. This causes race specific differences in brood nest temperature during spring. Spring is, because of low ratios of worker force and brood nest size and low ambient temperatures, the season, where temperature in brood cells can reach levels close to the reproductive optimum of Varroa jacobsoni (Velthuis & Kraus, this volume).

Population growth of Varroa jacobsoni in differentially managed colonies

Calculations of Varroa mite populations in colonies based on natural death rate are known to be very unreliable, but mite fall gives some indications on the parasite's population dynamics (Fuchs & Koeniger 1984, Maul 1984, Liebig *et al.* 1984, Rademacher 1985). The low levels of mite fall after inoculation of the experimental colonies show that the parasites were successfully introduced into the colonies. Given the number of mites found on bottom boards, average mite mortality in our study was 17%, which is close to results of a study conducted by Moosbeckhofer (1991). Considering factors like undetected mite losses caused by bees dying outside the hive and mites on dead bees being removed by workers, mite mortality might be much higher (Fries *et al.* 1991, Korpela *et al.* 1992). The mite population in spring 1997 was thus most likely overestimated and population growth underestimated. Reproduction of Varroa jacobsoni was furthermore underestimated because a high proportion of mites was killed at the beginning of the final Apistan® treatment and, therefore, excluded from reproduction for the duration of the four treatment weeks. The mite population sizes expected

in 1997 represent very conservative estimates. However, the calculated intrinsic rate of population growth is close to results of earlier studies (Camazine 1988, Kraus & Page 1995). The high variability of mite population size within the LT group might be caused by the fact that reproductive success of *Varroa jacobsoni* decreases with increasing temperature ranges within brood cells (Velthuis & Kraus, this volume). If thermoregulation within the hive becomes too inefficient and brood cell temperature too instable, population growth of the mite decreases, even if the average temperature is within the optimum range.

The results of this study demonstrate, that beekeepers can substantially slow down population growth of *Varroa jacobsoni* by supporting bee colonies in maintaining high average brood nest temperatures. According to our calculation 40% of the colonies of the LT group would have reached levels of more 10,000 mites before Fall treatment, an infestation level where the colonies most likely collapse (Fries *et al.* 1995). None of the HT group colonies would have reached levels above 6,800 mites. From a practical point of view, early spring is the most important phase concerning impact of temperature upon reproductive success of the parasite. March 3 all the colonies contained capped brood cells. The group specific difference in mite populations is thus not due to earlier availability of brood cells for reproduction of the parasite in the stimulated colonies. The average number of 9,000 brood cells per colony, estimated on March 12, also indicates that by the beginning of March in both groups sufficient brood cells were available for the reproduction of *Varroa jacobsoni*, given the low numbers of mites per colony at that point in time.

Temperature within the brood nest of Apis cerana colonies

Studies by Kraus *et.al.* (1998) showed that *Apis cerana indica* is less capable of maintaining high average temperatures within the periphery of the brood nest as compared to *Apis mellifera*. Average ambient temperature is about 27°C in Sabah, Malaysia. At the edge of the peripheral comb, where the workers mostly build drone brood cells (Tewarson *et al.* 1992), temperature within worker brood cells is about 33.5°C at that ambient temperature.

Temperature in drone brood cells of *Apis cerana* was on average about 0.4°C lower as compared to temperature in worker brood cells. Average temperature in drone brood cells of *Apis cerana indica* colonies in Sabah, Malaysia, is thus about 33°C throughout the year. This result offers a simple explanation for the fact that *Varroa jacobsoni* reaches its reproductive optimum at a temperature of 33°C in worker brood of *Apis mellifera* (Velthuis & Kraus, this volume), which is mostly kept at temperatures between 34 and 35°C: the cell capping period of Apis cerana worker cells is to short for mite reproduction. *Varroa jacobsoni* therefore specialized on drone cells of its original host with its comparably low temperature. Our experiments demonstrate, that beekeepers, using *Apis mellifera* colonies, could profit from this specialization of the mite by applying techniques that help their colonies in maintaining their brood nest at the preferred temperature of 35°C.

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