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# Field and laboratory tests that associate heat with mortality of tracheal mites[1, 2]

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## SUMMARY

Twelve white and 12 dark (unpainted) hives were set up in a sunny location in Baton Rouge, Louisiana, USA, on 7 May 1992. Each hive received a uniform colony of 5 048 ± 125 (mean ± s.d.) honey bees (*Apis mellifera*) that had been established from a single, artificially mixed population; 38% of the bees were infested with tracheal mites (*Acarapis woodi*). During June and July, bees produced more brood and honey in white hives than in dark hives. On 5 August, dark hives contained fewer tracheal mites than white hives; mites were found in 1 dark and 10 white hives ($P < 0.001$). In summer, the temperature in the space between brood combs in the dark hives often exceeded 40°C; maximum temperatures in the brood areas of dark and white hives, were 45°C and 38°C respectively. Laboratory tests showed that heat can kill tracheal mites inside live bees. A single six-hour exposure of bees to 42°C, a condition comparable to the short periods of high temperature encountered in field colonies, significantly reduced mite populations. In a second test, three-day-old worker bees were kept at 34.5°C or 39°C for 48 h and evaluated four days later (both groups of bees were stored at 34.5°C during the last four days). Bees kept at 34.5°C (controls) contained 2.7 larval mites per adult mite; bees exposed to 39°C contained 0.01. Controls contained nearly twice as many eggs (2.8 *vs.* 1.5 eggs per adult mite). This suggests that existing eggs died or did not develop during the 48 h at 39°C and that viable eggs were not produced during that period.

**Keywords:** honey bees, *Apis mellifera*, tracheal mites, *Acarapis woodi*, mortality, heat, hive temperature, hive colour

[1]All editorial functions for this paper, including the selection of referees, have been undertaken by staff at IBRA headquarters

[2]Mention of a proprietary product does not constitute an endorsement by the United States Department of Agriculture

# INTRODUCTION

After testing stocks of honey bees (*Apis mellifera* L.) in small field colonies in 1991, none of the 15 colonies contained tracheal mites (*Acarapis woodi* (Rennie)) in mid-August (350 bees were examined) whereas 48% of the bees had been infested in May. The hives were made of unpainted wood that had weathered to a dark grey colour. Direct sunlight made these hives very hot on summer days. In trying to explain the absence of tracheal mites, I formed the hypothesis that heat may somehow eliminate tracheal mites from a population of honey bees.

Heat is already associated with the control of tracheal mites. Control with menthol is more effective when the weather is warm (Wilson *et al.*, 1988; Herbert *et al.*, 1987). This is attributed to an increase in evaporation of menthol as temperatures increase. Furthermore, menthol and similar treatments may generate heat in a colony by serving as irritants that cause bees to produce heat by their response to the irritant (running and fanning their wings). It may be the heat produced by the bees, in combination with an already warm ambient temperature, that produces temperatures in the thorax that are warm enough to kill tracheal mites.

The objectives of this study were (1) to measure the effect of hive colour on mite populations in field colonies and (2) to describe laboratory temperatures that can kill tracheal mites in honey bees without killing the host bee.

# MATERIALS AND METHODS

## Field test

White paint was chosen as the colour for the hives to be compared with dark coloured hives. Of the finishes tested (aluminium paint, a radiant barrier, and various colours of paint) white paint provided the coolest interior temperature in empty hives. Therefore, white paint was used to keep colonies cool. Kennedy (1986) also concluded that white paint provided the coolest hive temperatures. Although black paint provided the hottest hive interiors during sunny weather, the dark colonies in this experiment consisted of unpainted wood that had been dipped in paraffin and weathered to a dark grey colour. White hives were produced for this experiment by painting 12 of the paraffin-coated, dark hives and their wooden lids with two coats of oil-based, white paint.

Twenty-four single-storey Langstroth hives (12 dark and 12 white) were set up in a sunny location on 7 May 1992 in Baton Rouge, Louisiana, USA. Each colony received a caged queen, four preweighed combs (each 20 × 43 cm) that contained honey and pollen but no brood, and 5 048 ± 125 (mean ± s.d.) worker bees. Bees for the 24 colonies were established by subdividing a population of bees that had been collected into one large cage. These uniform populations of bees were then distributed as package bees to each of the 24 hives (Harbo, 1986).

The initial bee populations for each colony were calculated by weighing small cages before and after they received bees from the big cage. Subsamples were weighed to calculate the number of bees. Thirty bees from each of the four subsamples were examined for the presence of tracheal mites.

Brood production was estimated on 28 May, 10 July, 27 July and 5 August by measuring the area of capped brood on the combs with a wire grid (6.5 $cm^2$/square) and calculating 3.7 cells per $cm^2$.

Temperatures were monitored hourly with thermocouples located between frames of brood in one dark and one white hive. Hourly readings were collected throughout the experiment with a Datalogger.

Weight gain during the first brood cycle was estimated by removing combs and adhering bees from each colony on 28 May and weighing them to the nearest gram in a preweighed box. This avoided the variability caused by the weight of moisture in the other hive parts. Unlike the final evaluation, colonies were open during this procedure which provided minimal disturbance of the 4-frame colonies. The difference between this weight and the sum of the initial weights of combs and bees provided an estimate of colony weight gain in May. A fifth comb was added to each colony on 29 May.

The final bee populations were estimated on the last day of the experiment (5 August) by screening the entrance of each colony after sundown (when all the bees were inside) and then weighing each colony the next morning. After a colony was weighed, the weight of the bees was established by brushing the bees from the frames and hive and then reweighing the equipment without bees. A sample of bees was then collected to estimate numbers of bees per gram and populations of tracheal mites. Thirty bees were examined from each colony for the presence of tracheal mites. Combs were weighed in the laboratory.

The average lifespan of workers was calculated from measures of brood production and the final adult population (Harbo, 1988). Each measure of capped brood in July provided an estimate of the daily emergence of adult bees into each colony during the next 12 days. The average lifespan of bees from each colony equalled the number of days (counting backwards from 5 August) until the accumulated daily emergences of adult bees equalled the population of adult bees on 5 August. The purpose of this was not to get an exact measure of lifespan of the bees but to determine if low mite populations were associated with bees that had short lifespans.

Queens for the test were from two different stocks to create a 2 × 2 factorial design. Factors were stock

**TABLE 1. Comparing 12 colonies in dark unpainted hives with 12 colonies in white hives. Data are in mean ± s.d.**

| Characteristic measured | White colonies | Dark colonies | $P^2$ |
|---|---|---|---|
| Initial adult bee population | 5 014 ± 171 | 5 075 ± 131 | 0.35 |
| Wt gain (g), 8 May–28 May[1] | 1 483 ± 276 | 1 674 ± 430 | 0.22 |
| Wt gain (g), 8 May–5 Aug[1] | 975 ± 631 | 30 ± 456 | 0.0002 |
| Capped brood (cells), 28 May | 5 046 ± 559 | 4 848 ± 687 | 0.48 |
| Capped brood (cells), 10 July | 3 668 ± 672 | 2 972 ± 831 | 0.02 |
| Capped brood (cells), 27 July | 2 753 ± 613 | 1 601 ± 492 | 0.0001 |
| Capped brood (cells), 5 Aug | 3 445 ± 1 002 | 1 741 ± 961 | 0.0002 |
| Adult bee population, 5 Aug | 6 495 ± 1 207 | 5 410 ± 1 295 | 0.016 |
| Adult bee lifespan (days) | 24.5 ± 4.6 | 28.8 ± 8.0 | 0.009 |
| Bees with *A. woodi*, 5 Aug | 8.6%(31/360) | 0.6%(2/360) | 0.0001[3] |
| Bees with *A. woodi*, 26 Oct | 25.2%(83/330) | 2.1%(7/330) | 0.0001[3] |

[1] This weight included pollen, honey, brood and bees.
[2] Probabilities that the means for dark and white colonies represent the same population. Except for the last two lines, each value in this column is part of a separate 2 × 2 factorial analysis of variance (see table 2).
[3] These probabilities are from Fisher's exact test (SAS, 1979).

type and hive colour. Fisher's exact probability test was used to compare mite populations in the dark *vs.* white hives (SAS, 1979) (table 1).

## Laboratory tests

**General design** Three incubators were set at 42, 39, and 34.5°C (50–70% RH). Bees were collected into cages (8 × 8 × 9 cm) that contained a comb, honey, water and pollen. The cages were screened on one side and the bottom. Temperatures and humidities in each incubator were monitored with hygrothermographs.

Both thoracic tracheae were removed from freshly killed bees and were immediately examined for mites by dissecting the tracheae and viewing at × 60 magnification. Movement was used to classify adults as dead or alive. Immobile mites were prodded with a pin if they appeared as though they could be alive, and any feeble twitch classified an adult mite as alive. Dead adults tended to be golden brown in colour and dorso-ventrally flattened. When the survival of eggs or larvae was included in the analysis (experiments 2 and 3), all stages of mites were evaluated three or four days after heat treatment. Plump, clear eggs and larvae were classified as alive. A four-day delay caused dead eggs and larvae to appear as flattened hulls that could be differentiated from the immatures that may be alive. No attempt was made to count dead eggs or larvae or to differentiate between larvae and phaerate adults (sometimes called nymphs).

**Experiment 1** This test monitored the survival of adult mites in bees that were kept in incubators at 34.5, 39, or 42°C. Worker bees of unknown ages were collected at random from a colony infested with tracheal mites. Bees were removed from the three incubators at 24-h intervals and immediately evaluated for live and dead adult mites. This method was abandoned because it did not provide information about adult fecundity following treatment, delayed mortality of adults, or mortality of immatures. Since all mites (and most bees) died during or shortly after a 24-h exposure to 42°C statistical analyses compared only the 34.5 and 39°C treatments at each of the daily intervals ($\chi^2$; SAS, 1979).

**Experiment 2** The objective of this experiment was to measure the effect of a short period of heat on the survival of mites. Groups of sister bees (0–24 h old) were marked with a dot of paint on the abdomen and then inoculated with mites by putting them into an infested colony for three days. On the fourth day these marked workers were put into five different cages. Two cages were placed immediately into 39°C or 42°C incubators for 6 h (experiment 2a); two were kept at 34.5°C for seven more days and then exposed to 39°C or 42°C for 6 h (experiment 2b). The fifth cage provided the controls. The bees in this experiment were highly infested with mites on the fourth day, averaging 6.1 foundress females per bee (four samples of 10 bees) when they were retrieved from the colony and put into cages.

Each part of this experiment (2a and 2b) consisted of a randomized complete block design, with four blocks (replicates in time) and three treatments: 6 h at 39°C, 6 h at 42°C, and control (only 34.5°C). Relative humidity was 60 ± 10%. Mite populations were evaluated three days after treatment by counting all live immature mites and the live and dead adult mites in a sample of 10 bees per treatment.

Bees treated at four days of age were not compared with bees treated at 11 days of age. Evaluations

were different for the two groups because older bees contained eggs, larvae and adult mites that had developed in the bee; 7–8-day-old bees also contained eggs and larvae, but the only adults were females that had originally infested the bee. Therefore, the early treatment is called experiment 2a (table 3) and the later experiment is called 2b (table 4).

**Experiment 3** The objective of this experiment was to measure the effect of exposing mites to 39°C for 48 h. As in experiment 2, groups of sister bees emerged as adults in an incubator, were marked with paint on the thorax, and were then placed into a colony that was infested with tracheal mites. Three days later, when four days old, the marked, sister bees were divided into two groups (paired *t* design); one group was put into a cage that was kept at 39°C while the other (control) was put into a cage that was kept at 34.5°C. At this time, the mites in their tracheae consisted of adult females and a few eggs that some of these females had laid. Forty-eight hours later, the cage of bees in the 39°C incubator was moved to be with the control bees at 34.5°C.

Mite populations in the two treatments were compared four days later when the bees were 9–10 days old, nine days after the first possible inoculation of mites. The number of mites (eggs, larvae, and adults) were counted in all tracheae that were examined. Data for each treatment were based on progeny of 21–35 live, adult mites in a sample of 10 or more bees. Therefore, each replicate of this experiment consisted of data from about 60 adult mites.

This experiment was repeated three times, each time different colonies were used as the source for test bees and as the source for inoculating the test bees with mites. Results were analysed with a paired *t* test (2 d.f.) (SAS, 1979).

# RESULTS

## Field test

During the first brood cycle, colonies in white and dark hives were not different in weight gain or brood production. This was a period of moderate temperatures and hive colour seemed to have little effect on the colonies. This equality during the first brood cycle supports the contention that the two groups were uniform at the beginning of the experiment. However, during June and July, bees produced more brood and honey in the white hives than in the dark hives (table 1).

Whereas table 1 lists the probabilities that are of primary interest to this study, table 2 includes a more complete analysis. This includes the effect of stock type and the interaction between stock type and hive colour. Stocks differed with regard to capped brood on 10 July, summer weight gain, adult lifespan and number of adults at the end of the experiment.

**TABLE 2. Factorial analysis of variance to evaluate the effects of stock type and hive colour on the characters described in table 1 (experiment 1).**

| Source | d.f. | *F* value | *P* > *F* |
|---|---|---|---|
| **Wt gain, 8–28 May** | | | |
| Stock type | 1 | 0.91 | 0.35 |
| Hive colour | 1 | 1.6 | 0.22 |
| Stock × colour | 1 | 0.0 | 0.96 |
| Error | 20 | | |
| **Wt gain, 8 May–5 August** | | | |
| Stock type | 1 | 1.0 | 0.34 |
| Hive colour | 1 | 21.6 | 0.0002 |
| Stock × colour | 1 | 6.0 | 0.02 |
| Error | 20 | | |
| **Capped brood, 28 May** | | | |
| Stock type | 1 | 0.03 | 0.87 |
| Hive colour | 1 | 0.51 | 0.48 |
| Stock × colour | 1 | 2.57 | 0.13 |
| Error | 17 | | |
| **Capped brood, 10 July** | | | |
| Stock type | 1 | 7.55 | 0.012 |
| Hive colour | 1 | 6.48 | 0.02 |
| Stock × colour | 1 | 0.48 | 0.50 |
| Error | 20 | | |
| **Capped brood, 27 July** | | | |
| Stock type | 1 | 0.80 | 0.38 |
| Hive colour | 1 | 24.49 | 0.0001 |
| Stock × colour | 1 | 0.08 | 0.79 |
| Error | 20 | | |
| **Capped brood, 5 August** | | | |
| Stock type | 1 | 1.05 | 0.32 |
| Hive colour | 1 | 19.79 | 0.0002 |
| Stock × colour | 1 | 3.06 | 0.10 |
| Error | 20 | | |
| **Adult population, 5 August** | | | |
| Stock type | 1 | 13.78 | 0.0014 |
| Hive colour | 1 | 6.97 | 0.016 |
| Stock × colour | 1 | 0.27 | 0.61 |
| Error | 20 | | |
| **Adult lifespan** | | | |
| Stock type | 1 | 48.09 | 0.0001 |
| Hive colour | 1 | 8.47 | 0.009 |
| Stock × colour | 1 | 3.96 | 0.06 |
| Error | 20 | | |

Since neither stock had many tracheal mites when occupying dark hives, genetic resistance to mites could only be detected in white hives, wherein the two stocks were about equally infested (0.72 and 0.57 tracheal mites per bee, respectively).

**TABLE 3. Populations of tracheal mites in experiment 2a where 3–4-day-old worker bees were treated for 6 h at 42°C or 39°C and evaluated 3 days later. Controls were kept at 34.5°C.**

| Characteristic | Treatment | | Control | P[1] |
|---|---|---|---|---|
| | 42°C | 39°C | | |
| % live adult mites | 39.5a[2] | 88.5b | 93.8b | 0.004 |
| Eggs per adult mite[3] | 0.71a | 1.79b | 2.16b | 0.0016 |
| Larvae per adult mite[3] | 0.27a | 0.78ab | 1.10b | 0.029 |

[1] Probability that none of the three treatment means are different (based on *F* test with d.f. = 2,3).
[2] LSD mean separation was used to detect significant effects among the three treatment means in each row. Means followed by different letters were different at the 0.05 level.
[3] Adult mites included the sum of live and dead adult mites in all the bees that were dissected. Each observation was ≥ 34 adult mites.

**TABLE 4. Populations of tracheal mites in experiment 2b where 10–11-day-old worker bees were treated for 6 h at 42°C or 39°C and evaluated 3 days later. Controls were kept at 34.5°C.**

| Characteristic | Treatment | | Control | P[1] |
|---|---|---|---|---|
| | 42°C | 39°C | | |
| Dead adult females/bee[2] | 3.8a[3,4] | 1.6b | 1.2b | 0.01 |
| Live adult females/bee[2] | 3.1a | 9.0b | 14.0b | 0.006 |
| Males per bee | 2.0a | 4.0b | 4.7b | 0.04 |
| Eggs per bee | 2.5a | 4.4ab | 5.25b | 0.06 |
| Larvae per bee | 3.7a | 10.3ab | 12.5b | 0.04 |

[1] Probability that none of the three treatment means are different (based on *F* test with d.f. = 2,3).
[2] Worker populations averaged 6.1 foundress female mites per bee.
[3] LSD mean separation was used to detect significant effects among the three treatment means in each row. Means followed by different letters were different at the 0.05 level.
[4] Each observation consisted of all the mites in 10 bees.

**TABLE 5. Populations of tracheal mites in experiment 3 where 3–4-day-old worker bees were treated for 48 h at 39°C and evaluated 4 days later. Controls were kept at 34.5°C.**

| Characteristic | Treatment at 39°C | Control | *t* | P[1] |
|---|---|---|---|---|
| % live adult mites | 81.1 | 90.5 | 1.54 | 0.20 |
| Eggs per live adult mite | 1.46 | 2.78 | 4.82 | 0.04 |
| Larvae per live adult mite | 0.01 | 2.69 | 36.4 | 0.0008 |

[1]Probability that the treatment means are not different (d.f. = 2).

Midday temperatures were often 5–6°C warmer in dark hives than in white hives; temperatures were equal at night and on rainy days. Many times in June and July, the interior of dark hives exceeded 40°C, with maximum temperatures of 45 and 38°C recorded in the brood areas of dark and white hives, respectively. Figure 1 is an example of hive temperatures on a hot, sunny day in Louisiana (16 June 1992).

On 5 August, when 30 bees from each hive were checked for tracheal mites, mites were found in 10 of the white but in only one of the dark hives ($P < 0.01$). The percentage of bees infested in the white hives was 8.6%, and 0.6% in the dark hives (table 1); 38% (45/120) of the bees had been infested at the beginning of the experiment (7 May). On 26 October, mites were found in all 11 white hives and in six of the 11 dark hives ($P = 0.035$). At both dates, the two groups also differed in numbers of infested bees (table 1).

## Laboratory tests

Experiment 1 showed high mortality of adult mites when kept at 42°C for 24 h, significant mortality when kept at 39°C for 3 days or more, and no apparent mortality when kept at 34.5°C (fig. 1). By day 5, immature mites appeared dead and shrivelled in the 39°C treatment and healthy in the 34.5°C treatment. There was no mortality of bees kept at

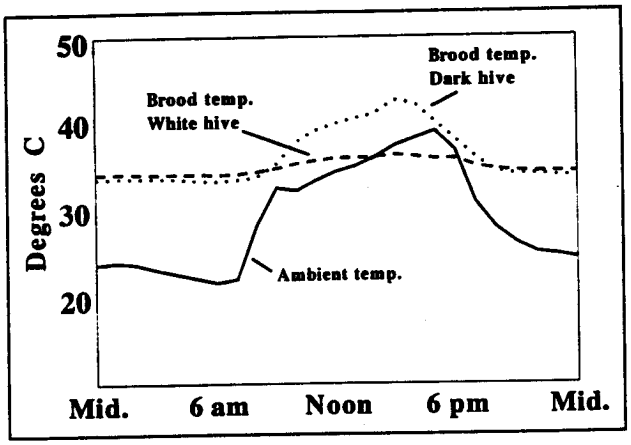


**FIG. 1. A comparison of temperatures in the brood area of white and dark coloured hives on a hot, sunny day in Louisiana (16 June 1992). Colonies contained 5 combs and about 7 000 adult bees. Temperatures were measured every 10 minutes and an average temperature was recorded at the end of every hour.**

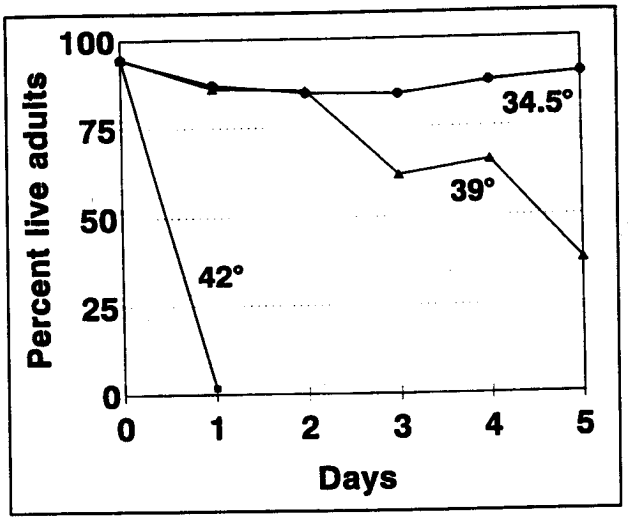


**FIG. 2. The effect of temperature on the survival of adult mites in the tracheae of worker bees (experiment 1).**

either 34.5°C or 39°C. However, most of the bees died after exposure to 42°C for 48 h, and lethal effects may occur much sooner.

In experiments 2a and 2b, a 6-h exposure to 42°C caused significant mortality of all stages of mites (tables 3 and 4); significant differences were not detected between controls and those given a 6-h exposure to 39°C. However, with these data (d.f. = 2,3) it would be premature to conclude that 6 h at 39°C had no effect on the mite populations. In experiment 2b, some new adults had been produced by the time of their 6-h exposure to heat and many more were present three days later at evaluation time.

Experiment 3 showed that a 48-h exposure to 39°C caused mites to produce fewer progeny (table 5). As in experiment 1, exposure to 39°C for 48 h did not cause significant mortality of adult mites. However, it did inhibit the production or survival of immatures, because bees kept at 39°C had fewer immature mites (eggs and larvae) than did bees kept at 34.5°C.

## DISCUSSION

During summer in Louisiana, bees in white hives were more productive than bees in dark hives. However, it is possible that the opposite could occur during cooler seasons in Louisiana and in hives located in cooler climates. Hive colour probably has no effect when hives are not placed in direct sunlight.

The field test showed that mite populations were smaller in dark hives than in white hives. Mite populations may have declined because they were unable to migrate to another host, they may have been unable to reproduce in the tracheae, or they may have been killed inside the tracheae. Inability of mites to migrate is not likely because mites migrate to new hosts mostly at night (Pettis *et al.*, 1992), and no differences were found between dark and white colonies at night. Insufficient time to produce adult progeny in the tracheae also seems unlikely because bees in dark colonies seemed to live as long or longer than bees in white hives (table 1). For these reasons, and because of results in subsequent laboratory tests, I conclude that heat probably killed mites in the tracheae of live bees.

Therefore, when selecting bees for resistance to tracheal mites, bees should probably be kept in white hives or in shady locations to minimize the effects of heat.

Laboratory experiments showed that temperatures that are not lethal to bees can kill tracheal mites. This study did not define the exact limits of temperatures that are harmful to bees or mites, nor did it define the ambient temperature in the tracheae where the mites were located. It showed that exposure of bees to temperatures as moderate as 39°C can kill adult and immature mites in those bees, it can slow mite reproduction, and there is no apparent harm to adult bees. Higher temperature (42°C) killed mites sooner, but adult bees showed high mortality in the days after being exposed to this temperature; 6 h at 42°C did not seem to harm populations of 50 bees (however, 42°C can kill larger populations in only a few hours). Other combinations of temperature and humidity (which was always 60 ± 10% RH in laboratory experiments) may be more effective in controlling mites. In general, higher temperatures seem to kill mites in a shorter time, but time and temperature need to be adjusted so as to enhance the survival of honey bees and the death of tracheal mites.

Based on observations in experiment 1 and data in experiment 3, egg and larval stages of the tracheal mite seem to be more susceptible to exposure at 39°C than do adult mites. In experiment 3, I concluded that existing eggs died during the 48 h at 39°C and that either (1) the adult females did not lay eggs during the hot period or (2) the eggs laid during this period were non-viable. Adult mites were still fertile after this period and resumed oviposition after being moved to 34.5°C. Therefore, immatures may provide a weak link in the life cycle of tracheal mites.

I conclude that any stimulus that generates excessive heat in a bee is a potential control measure for tracheal mites. Such stimuli may be natural or artificial. The response by the bee is to fly or fan its wings and thereby produce heat with wing muscles that are located in the thorax next to the mites. Thus, tracheal mites are trapped near the source of the heat which is probably much warmer than the ambient temperature. If the ambient temperature is already elevated, the temperature in the tracheae could become lethal to mites. Excess heat in a hive may serve as a stimulus for producing more heat within an individual bee because the act of cooling a hive probably raises the internal temperature of worker bees.

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