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Tolerance of two honey bee races to various temperature and relative humidity gradients

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Abstract

There are various factors that can have an effect on honey bee colonies. Temperature and relative humidity, in particularly, have special importance for honey bee colonies. Relatively few studies have been conducted on the effects of temperature and relative humidity on honey bee races. Here, the effects of different levels of temperature and relative humidity on survival, tolerance and body water loss were investigated on two races, one adapted to harsh conditions (Yemeni honey bees) and the other adapted to normal conditions (Carniolan honey bees). Results showed that temperature had higher effect than relative humidity on workers survival and Yemeni honey bees were more tolerant to elevated temperature than Carniolan honey bees. Moreover, rates of body water loss for the two races were high under elevated temperature and low humidity conditions. In general, the response of the two races in the studied treatments was somewhat similar. However, under extreme conditions at elevated temperature or low humidity, Yemeni honey bees showed higher tolerance than Carniolan honey bees.

Key words: *Apis mellifera carnica, Apis mellifera jemenitica*, honey bees, humidity, temperature, tolerance, survival.
Abbreviations: RH, relative humidity.

Introduction

The importance of temperature and relative humidity for honey bees is well known, and all activities of honey bee colonies are under the control of these factors. Temperature, in particularly, is very important for internal as well as external activities of honey bee colonies. Maintaining a suitable range of temperature from 33 to 36 °C inside colonies is very important for honey bees (Petz et al. 2004). Deviation from this range can affect the developmental period of honey bee immature stages, emergence rate (Tautz et al. 2003), colour of emerged bees (DeGrandi-Hoffman et al. 1993), wing morphology (Ken et al. 2005), learning ability (Tautz et al. 2003), adult brain (Groh et al. 2004) and disease prevalence. Also, ambient temperature has a great effect on foraging activity, as high temperature has a negative effect on bee foraging (Cooper, Schaffer 1985; Al-Qarni 2006; Blazyte-Cereskiene et al. 2010). Moreover, very low temperature below 10 °C can prevent flight activity (Joshi, Joshi 2010).

On the other side, relative humidity has a particular importance within the colony where high humidity is mostly required for brood development (Human et al. 2006). Effect of humidity on egg hatching rate has been previously identified (Doull 1976) and a relative humidity about 75% within colonies could be considered as suitable for immature stages (Ellis et al. 2008). In the case of external activities, no clear direct impact of relative humidity on honey bees has been reported, including foraging activity (Joshi, Joshi 2010). Under low levels of relative humidity, within colonies, honey bee workers try to increase humidity by various means including nectar water evaporation and water collection (e.g. Human et al. 2006). Caged bees exposed to high temperature have been noticed to increase water uptake (Free, Spencer-Booth 1958). Therefore, the integration between temperature and relative humidity is very important for honey bee activity.

Not all honey bee races respond in the same way to thermal stress or even relative humidity levels. Hence, the success of honey bee races to occupy specific regions is the overall result of an adapted response to ecological stresses. Here, two honey bee races were studied (Yemeni honey bees, *Apis mellifera jemenitica*, bees adapted to harsh conditions of Saudi Arabia, and Carniolan honey bees, *Apis mellifera carnica*, bees adapted to normal conditions) to investigate the impacts of various temperature and relative humidity levels on survival, tolerance and body water loss for these two races as well as to identify differences in their tolerance ability.

Materials and methods

Material

The research was performed at the Bee Research Unit

laboratory, King Saud University, Saudi Arabia. The influence of temperature and relative humidity was investigated on forager bees (age above 21 days) of two honey bee races, Yemeni (*Apis mellifera jemenitica* Ruttner) and Carniolan (*Apis mellifera carnica* Pollmann) honey bees. The following experiments were conducted under controlled conditions of temperatures and relative humidity (RH) in Memeret incubators, Germany. These incubators allowed control of temperature and relative humidity, and have an internal glass door to allow treatment inspection without interrupting adjusted levels of temperature and relative humidity.

Workers survival

Three cages with dimensions 16 × 16 × 7cm and with one glass side and one wire mesh side (Fig. 1) per race were used per treatment. Fifty workers per each cage per race with a total of 300 workers for the two races were used per treatment. Caged bees were provided with 5 mL water, 5 mL 50% sugar syrup, and a 20 g pollen patty. Temperature levels of 35 °C, 40 °C and 45 °C at 75% constant RH, and RH levels of 15, 30, 50 and 75% at constant temperature of 35 °C were tested. These levels of temperature and relative humidity were selected to coincide with the existing harsh conditions, elevated temperature and low humidity, of Saudi Arabia. The cages were subjected to one of the treatments and daily inspection was made of the number of dead workers. Worker survival per each cage was calculated as the number of days at which all bees had died. Subsequently, the survival mean per treatment was calculated by dividing the total number of days at which 100% death was occurred in three cages by three (the number of cages per each treatment).

Temperature tolerance

To assess heat tolerance for the two races, the method of Atmowidjojo et al. (1997) was adopted with some modifications. A total of 300 bees per each race were used in this experiment (50 bees per cage and six cages per race). The caged bees were equilibrated to room temperature before the incubator-programme started. The incubator was adjusted to a constant humidity (50%), while temperature was programmed to start at 30 °C and increase to 70 °C during 80 min. After the incubator heating program was started, the number of intolerant bees were recorded at each 0.5 °C step. The dorsal turning reflex was used to assess temperature tolerance. Bees unable to right themselves immediately were classed as intolerant to the given temperature. The temperature at which bees started to be intolerant and the percentage of intolerant bees per each temperature were recorded.

Body water loss

Rates of body water loss were estimated gravimetrically. Three plastic containers with an upper cover of aluminum foil with 100 pores were used per race per treatment. Ten bees were placed in each container (total of thirty bees per race per treatment) and were weighed by using GR 200 balance (A & D Company Limited, Japan) to the nearest 0.01 mg (W1). The experimental groups were maintained in incubators at 35 °C, 40 °C or 45 °C. Combinations of the temperatures and 10, 25 and 50% RH treatments for 2 h were employed, after which the bees were reweighed (W2). Rates of water loss were calculated as (W1) – (W2). To identify the water loss rate per bee, each weight was divided by 10 and the final results were expressed as mg per hour (mg h^{-1}).

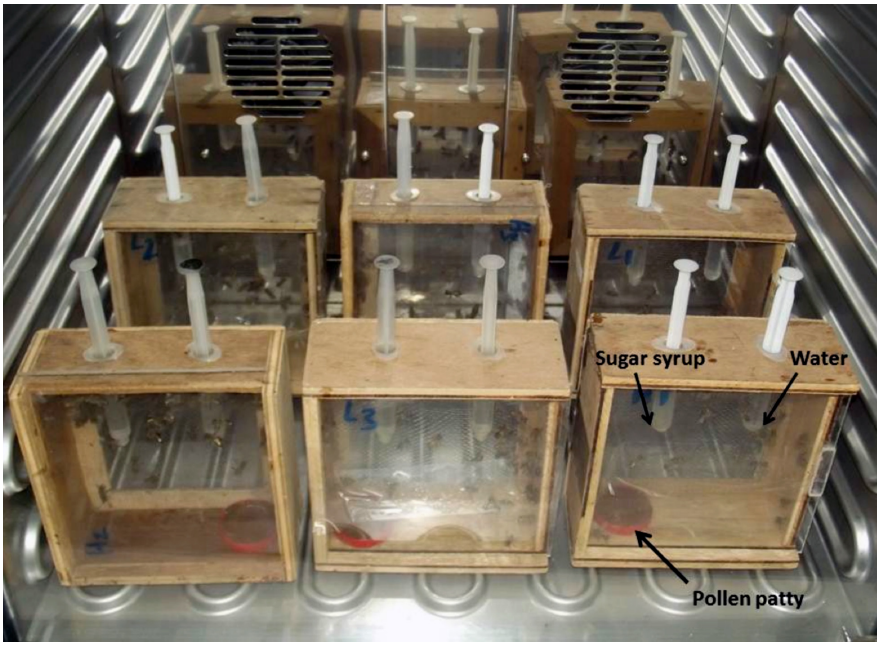

Fig. 1. Cages used for bee survival studies in the present experiments.

Statistical analysis

A completely randomized design was used for all the above-mentioned experiments. The obtained data were statistically analysed using analysis of variance (ANOVA) and means were compared by using the Least Significant Difference test ($LSD_{0.05}$) with the SAS 9.1.3 programme (SAS Institute 2004).

Results

Effect of temperature on worker survival

Honey bee races showed distinctive response to different temperature gradients (Table 1). At 35 °C, relatively long survival was found for Carniolan and Yemeni honey bees, while at 40 °C Yemeni honey bees survived longer than Carniolan honey bees by about 2.66 days. At 45 °C all workers of Carniolan and Yemeni honey bees had died within 24 h. Significant ($P < 0.05$) and a strong negative correlation ($r = -0.91$) was found between survival and temperature.

A significant difference ($P < 0.05$) was found between survival of Yemeni and Carniolan honey bees 40 °C while no significant differences were found for the other treatments ($LSD_{0.05}$ values were 6.54, 2.62 and 0 for treatments 35 °C, 40 °C and 45 °C, respectively).

Effect of relative humidity on worker survival

At a fixed temperature of 35 °C, humidity had effect on worker survival (Table 1). The best survival for the two races was at relative humidity of 75% followed by 50%, 30%, and then 15%. Mean values of worker survival were higher for Carniolan honey bees than Yemeni honey bees at all humidity treatments except at 15% where Yemeni honey bees had higher survival than Carniolan honey bees. In general, no large differences in survival were found between Yemeni and Carniolan honey bees at all humidity treatments, as difference in survival between the two races was only 1, 0.66, 0.34 and 1 days for treatments of 15, 30, 50 and 75%, respectively. Significant ($P < 0.05$) and moderate positive correlation ($r = 0.79$) was found between survival and humidity.

No significant differences ($P > 0.05$) were found between mean survival of Yemeni and Carniolan honey bees at all humidity treatments ($LSD_{0.05}$ values were 5.70, 4.43, 5.23 and 6.54 for treatments 15, 30, 50 and 75% RH, respectively).

Effect of temperature and relative humidity on worker survival

Results of temperature and relative humidity treatments are shown in Table 1. Temperature seemed to have higher effect on worker survival than humidity, as a noticeable reduction in workers survival was found with elevated temperature. On the other hand, no major difference in workers survival was observed between humidity treatments, especially between 30 and 50% as well as between 50 and 75%. The highest reduction in worker survival was observed at temperatures 40 and 45 °C, as well as at relative humidity 15%. However, the reduction rate of survival in the case of temperature was higher than that of humidity.

No significant differences ($P > 0.05$) were detected between survival at 40 and 45 °C in the case of Carniolan honey bees, while significant differences ($P < 0.05$) were found between survival in all heat treatments for Yemeni honey bees. Also, no significant differences were found between survival at humidity treatments 15 and 30% as well as 30 and 50% in the case of Carniolan honey bees, while no significant differences were found between survival among humidity treatments 75, 50 and 30% as well as 50, 30 and 15%. In general, significant differences were found between the overall mean survival on heat treatments while no significant differences were detected between the overall mean of some humidity treatments (30 and 50% as well as 30 and 15%).

It was clear that temperature had higher effect on worker survival than humidity. Yemeni honey bees showed higher survival under elevated temperature and very low humidity conditions than Carniolan honey bees while Carniolan

Table 1. Mean survival (days) for two honey bee races at different temperature and relative humidity gradients. Means followed with the same letter in the same column within each treatment category are not significantly different ($P > 0.05$)

Type of treatment	Temperature (°C) / humidity (%)	Survival mean (days) ± SE		Overall mean ± SD
		Carniolan honey bees	Yemeni honey bees	
Heat treatments	35 / 75	13.67 ± 1.45 a	12.67 ± 1.85 a	13.16 ± 2.64 a
	40 / 75	2.67 ± 0.67 b	5.33 ± 0.67 b	4.00 ± 1.78 b
	45 / 75	1.00 ± 0.00 b	1.00 ± 0.00 c	1.00 ± 0.00 c
Humidity treatments	35 / 15	5.33 ±1.20 c	6.33 ± 1.67 b	5.83 ± 2.32 c
	35 / 30	8.33 ± 0.66 bc	7.67 ± 1.45 ab	8.00 ± 1.79 bc
	35 / 50	9.67 ± 0.67 b	9.33 ± 1.76 ab	9.50 ± 2.07 b
	35 / 75	13.67 ± 1.45 a	12.67 ± 1.85 a	13.16 ± 2.64 a
	$LSD_{0.05}$ (temperature)	3.19	3.94	2.26
	$LSD_{0.05}$ (humidity)	3.44	5.51	2.68

honey bees had higher survival than Yemeni honey bees under suitable conditions, especially at temperature 35°C and humidity above 30%.

Temperature tolerance

After exposing honey bee workers to various temperatures from 30 to 70 °C, Carniolan honey bee workers began to be intolerant at 57.5 °C while Yemeni honey bee workers began to be intolerant at 61 °C (Fig. 2). All Carniolan honey bee workers had died at 66 °C, while all Yemeni honey workers had died at 68 °C. The majority of intolerant Carniolan honey bees were observed at 62.5 and 66 °C while for Yemeni honey bees the majority occurred at 64 to 68 °C. In general, Yemeni honey bees had higher ability to tolerate temperature than Carniolan honey bees.

Body water loss

Table 2 shows body water loss for the studied races under different levels of temperature and relative humidity. The highest body water loss was found when honey bee workers were exposed to 10% RH and temperatures of 40 and 45 °C, as well as 25% RH and 45 °C, while the least loss was obtained when honey bee workers were exposed to 50% RH and temperature 45, 40 and 35 °C. In general, Carniolan honey bee workers lost more body water than Yemeni honey bees, especially at high temperatures 40 and 45 °C and low humidity 10 and 25%.

Significant differences, in general, were found in water loss between all temperature and relative humidity treatments except between temperature treatments of 35 and 40 °C at all humidity treatments for Carniolan honey bees, also between treatments of 35 and 40 °C at 10% RH as well as 35 and 40 °C at 25% RH for Yemeni honey bees. Moreover, significant differences were found between Carniolan and Yemeni honey bees for all treatments ($P < 0.05$), except at 50% RH and 35 °C.

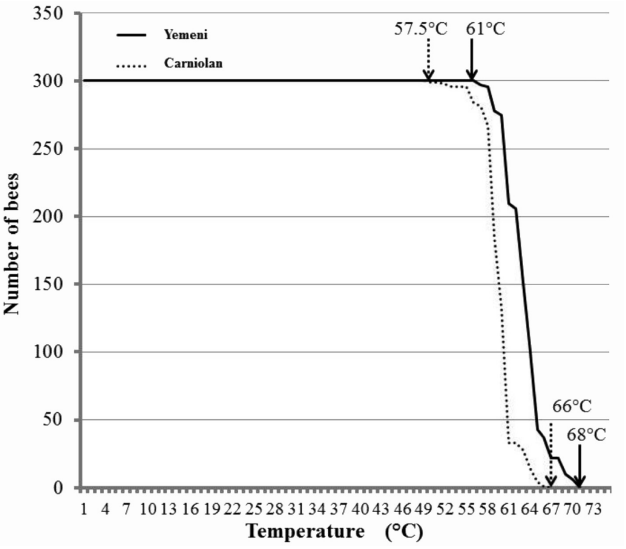

Fig. 2. Mortality of Yemeni and Carniolan honey bees with elevated temperature.

Discussion

Effect of temperature and relative humidity on workers survival

High temperature had negative effect on worker survival for the two races. This result is in accordance with Remolina et al. (2007) who exposed honey bee workers to 42 °C till death and found the range of life span mean was from 31 to 91 h (about 1.29 to 3.79 days). Also, Mardan and Kevan (2002) found that adult workers of giant honey bees, *Apis dorsata*, exposed to 38 and 45 °C had died within 5 days and 48 h, respectively which supports the idea that temperature has negative effect on worker survival.

The highest mean worker survival was at temperature treatment of 35 °C, observed by Clinch and Faulke (2012) who found that the least mortality rates of honey bee workers were at temperature treatment of 35 °C. Yemeni honey bees showed higher survival at temperature 40 °C than Carniolan honey bees. However, no differences were found between survival of the two races at 35 and 45 °C. Thus, Yemeni honey bees can be used for beekeeping purposes at regions with higher ambient temperature than Carniolan honey bees.

Higher relative humidity treatments was associated with increasing worker survival. However, the effect of humidity was not high and no differences in survival mean were detected between the two races. Moreover, Carniolan honey bees had higher survival than Yemeni honey bees for all treatments except at 15% RH, which indicated the high performance of both Carniolan than Yemeni honey bees under normal conditions, while under stress conditions Yemeni honey bees seemed to have better response than Carniolan honey bees.

Generally, high humidity is better for enhancing survival. Unfortunately, there are not many studies that have considered effect of humidity on honey bee workers. However, the effect of humidity seemed to be rather low, in accordance with the findings of Joshi and Joshi (2010) who investigated honey bee flight activity.

Temperature tolerance

Presently, the bees adapted to harsh conditions (Yemeni honey bees) had higher tolerance ability than the adapted bees to normal conditions (Carniolan honey bees). This result is in agreement with the findings of Atmowidjojo et al. (1997), who observed that tolerance of the feral honey bees, more adapted bees to harsh conditions, as higher than domestic honey bees of the Arizona region. In general, Yemeni and Carniolan honey bees were less tolerant to very high temperature, which is supported by the study of Mardan and Kevan (2002) who found that temperature from 26 to 36 °C did not affect survivorship of workers

of giant honey bees, *A. dorsata*, survivorship while at 45 °C workers had died within 48 h. Thus, honey bees have less tolerance ability to endure high temperatures for a long time. The ability of honey bee races to survive under high temperatures before death can be explained by the presence of heat shock proteins which have been identified previously in honey bee larvae (Chacon-Almeida et al. 2000) and adults (Severson et al. 1990). Also, the differences between Yemeni and Carniolan honey bees in their thermal tolerance could be attributed to differences in their body size as Yemeni honey bees are smaller than Carniolan honey bees (Abou-Shaara, Al-Ghamdi 2012).

In the present study, the maximum tolerance for honey bee workers was found at 57.5 and 61 °C while the maximum tolerance was found at 49.1 °C by Kafer et al. (2012) study and at 42.8 and 50.7 °C by Atmowidjojo et al. (1997). Thus, the present results differ from those in other studies. These differences in maximum tolerance can be attributed to study conditions and honey bee race used: Kafer et al. (2012) investigated Carniolan honey bees and a temperature range from 25 to 53 °C at an increasing rate of 0.25 °C per minute. Atmowidjojo et al. (1997) used a temperature range from 30 to 60 °C at an increasing rate of 0.5 °C per minute for domestic and feral honey bees of the Arizona region. In the present study a temperature range from 30 to 70 °C at an increasing rate of 0.5 °C per minute and RH 50% were tested on Carniolan and Yemeni honey bees. No data about relative humidity were provided in the Kafer et al. (2012) and Atmowidjojo et al. (1997) investigations.

Body water loss

As compared with Yemeni honey bees, Carniolan honey bees lost more body water under the studied treatments. This result is in accordance with Al-Qarni (2006), who found that mean weight loss was higher for Carniolan honey bees than Yemeni honey bees, after subjecting the bees to air temperature during season for two hours in conditions of the Riyadh region, Saudi Arabia. Also, rates of body water loss increased with elevated temperature and with low humidity. This is in accordance with Roberts and Harrison (1999), who observed that water vapour loss increased at air temperature values above 33 °C.

Moreover, Atmowidjojo et al. (1997) recorded the highest rate of body water loss at 35 °C and 0% relative humidity while the least mean body water loss was at 25 °C / 75% RH and at 30 °C / 100% RH. Additionally, in a study by Heinrich (1980) honey bees at ambient temperature of 15 °C to 25 °C were found to maintain head temperature above the ambient temperature by about 7 °C while at ambient temperature of 46 °C mean of head temperature was about 43 °C, which implies that honey bees under high temperature decrease their body temperature mainly by body water loss or other means.

Table 2. Mean ± SE of body water loss (mg h^{-1}) of honey bee workers under different levels of temperature and relative humidity

Race	Relative humidity / temperature									$LSD_{0.05}$
	10%			25%			50%			
	35 °C	40 °C	45 °C	35 °C	40 °C	45 °C	35 °C	40 °C	45 °C	
Carniolan	2.67 ± 0.16 b	3.50 ± 0.28 b	7.16 ± 0.33 a	1.33 ± 0.17 b	2.33 ± 0.16 b	6.00 ± 0.50 a	1.00 ± 0.28 b	1.83 ± 0.16 b	3.17 ± 0.33 a	RH 10% (0.94)
										RH 25% (1.10)
										RH 50% (0.94)
Yemeni	1.33 ± 0.17 b	1.67 ± 0.16 b	4.17 ± 0.17 a	0.67 ± 0.16 b	1.17 ± 0.16 b	3.17 ± 0.17 a	0.50 ± 0.00 c	1.00 ± 0.00 b	1.67 ± 0.16 a	RH 10% (0.58)
										RH 25% (0.58)
										RH 50% (0.33)
$LSD_{0.05}$	0.65	0.92	1.03	0.65	0.65	1.46	0.80*	0.46	1.03	

Conclusions

Temperature and relative humidity had high effect on honey bee adults. Elevated temperature had negative effect on worker survival while relative humidity had positive effect on worker survival. Differences between the studied races in their heat tolerance ability were observed and the most adapted bees to harsh conditions, Yemeni honey bees, showed higher tolerance than Carniolan honey bees. Also, rates of body water loss of the two races increased with temperature and lower humidity. In general, Yemeni honey bees showed less body water loss than Carniolan honey bees. It is reasonable to believe that Carniolan honey bees have higher performance than Yemeni honey bees under normal conditions while under harsh conditions, elevated temperature and low humidity, Yemeni honey bees are more suitable than Carniolan honey bees.

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