Journal of Apiculture

2024.39(4) : 317-322
DOI: 10.17519/apiculture.2024.11.39.4.317

Original research article

Threshold Temperature for the Outdoor Flight Activity of Honey Bee, *Apis mellifera* under Overwintering Conditions

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Abstract Recent overwintering honey bee mortality in South Korea has been attributed to various factors, including abnormal temperatures and honey bee mite infestations. The emptying hive during overwintering was considered due to the outdoor flights of honey bees without return during warmer temperature. However, no experimental evidence of flight activities during winter relative to the temperature conditions is available. This study monitored flight activities during winter and related to the temperature profiles to figure out the threshold temperature for the flight within the shade house and open field conditions. Honey bee flight activities were recorded above 9.3°C and increased as temperature rose. Most flights occurred under the rain-cover house condition, but rarely under shading. Shading has significant effects on reducing temperature variability. Food consumption during winter was significantly lower under the shaded condition than open field or rain-cover conditions, which imply lower energy expenditure due to lower temperature variability. Based on these results, we propose season-long and nation-wide flight monitoring relative to the temperature during the winter season to figure out the winter mortality patterns and shading to mitigate the temperature variability during winter.

Keywords Climate change, Winter, Shading, Variability, Threshold temperature, Energy

INTRODUCTION

Honey bees contribute to income generation for beekeepers and play a crucial role as key pollinators in food production and ecosystem conservation (Lee *et al*., 2010; Jeong *et al*., 2016). However, honey bee health is threatened by various factors, including climate change, pests, diseases, pesticides, and nutritional deficiencies (vanEngelsdorp *et al*., 2009; Jung and Lee, 2018). Recently, the mortality of overwintering honey bees has been reported in South Korea (Jung and Bae, 2022; Kim, 2022; Kim and Lee, 2022; Lee *et al*., 2022). Such colony losses directly cause economic damage to beekeepers and, subsequently, reduce bee products and agricultural productivity due to a lack of pollination (Kim, 2022). The mortality of overwintering honey bee colonies in South Korea has been linked to various factors, including mite-related issues such as high-density infestations, pesticide resistance, and misuse of mite control agents. Abnormal weather conditions, including unusually low autumn temperatures and unseasonably high winter temperatures, have also been suggested as significant contributors (Jung and Bae, 2022; Kim, 2022; Kim and Lee, 2022; Lee *et al*., 2022). The overwintering failure rate in South Korea was reported to be approximately 17.4% (8.5−24.9%) from 2011 to 2013, but this percentage doubled to about 36% (22.6−67.7%) in 2022 (Jeong *et al*., 2016; Kang *et al*., 2024). In 2022, the primary observation in failed colonies was not the presence of large numbers of dead bees but rather empty hives with no bees inside (Kang *et al*., 2024). This finding suggests that honey bee flight activity may occur during the overwintering period.

The ecology, physiology, and activity of honey bees

http://journal.bee.or.kr/

Received 25 November 2024; Revised 29 November 2024; Accepted 30 November 2024
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are temperature-sensitive, and their behavior changes in response to temperature fluctuations (Jung and Bae, 2022). The supercooling point of honey bees is −8°C (Qin *et al*., 2019; Muhammad and Jung, 2021), and their minimum activity temperature has been reported to be between 6−7°C (Southwick and Heldmaier, 1987; Eskov and Toboev, 2011). Honey bees overwinter by clustering together to regulate heat, making weather conditions during the overwintering period critically important for environmental stability (Kim *et al*., 2021; Jung and Bae, 2022). Due to ongoing global warming in South Korea, weather variability has increased, and abnormal temperatures were recorded during the winter of 2021, coinciding with reported overwintering failures (Jung and Bae, 2022). When temperatures rise during overwintering, exposure to sunlight can cause significant temperature fluctuations inside and outside the colony. Some of these fluctuations may reach temperatures that allow bees to become active, potentially leading them to venture outside and not return.

Therefore, this study aimed to investigate the threshold temperature for honey bee flight activity during the overwintering period and evaluate the potential of shading as a strategy to minimize temperature fluctuations. The study sought to test hypotheses that above certain threshold temperature, honey bee would show external flight activities and increase as temperature increase, and shading would decrease the temperature variability resulting in less flight activities and food consumption during winter. By monitoring honey bee flight activity and temperature profiles, the research aimed to understand how environmental conditions, particularly temperature variations, affect honey bee behavior during winter and to explore shading as a potential method to mitigate these impacts, ultimately contributing to reducing overwintering mortality in honey bee colonies.

MATERIALS AND METHODS

1. Experimental design

The honey bees (*Apis mellifera*) used in the experiment were from colonies reared without exposure to pesticides at the experimental apiary of Andong National University, located in Songcheon-dong, Andong-si, Gyeongsangbuk-do. To apply shading treatments to overwintering honey bee colonies, two types of greenhouse-style beehouses (Rain-cover house, Shaded house) were constructed: one covered with shading screens to block sunlight and another allowing sunlight to pass through while providing rain protection (Fig. 1). For this experiment, 27 colonies were placed in a shaded house, another 27 colonies in a rain-protected but unshaded house, and 10 colonies were placed in the open field. The experiment was conducted in two phases. The first phase was carried out from January 4th to February 4th, while the second phase was conducted from February 5th to February 17th, after the completion of the wintering period. To investigate the effect of shading on temperature variations outside the colony, data loggers (HOBO U23 Pro v2, Onset, USA) were installed around the colonies in both the shaded and rain-cover house, recording external colony temperatures at 10-minute intervals.

Fig. 1. Photographs of the shaded house (left), rain-cover house (right) used in the experiment and the open field.

2. Flight activities

1) The flight activity of honey

bees under shaded and unshaded conditions were investigated using five colonies of similar strength randomly selected from each beehouse. The flight activity of honey bees was observed from 10:00 to 16:00 at 2-hour intervals. Honey bee flight activity was counted by counting the number of bees leaving the colony and returning to the colony for one minute per colony, and this was repeated three times per colony. Temperature data at the time of honey bee flight activity were extracted to analyze the correlation between external colony temperature and honey bee flight activity.

3. Food consumption

To estimate food consumption, the weight of the colonies placed in each overwintering environment was measured. The weight of the colonies was measured on November 15th, 2023, at the start of overwintering, and again on February 5th, 2024, at the end of overwintering. The food consumption was estimated by subtracting the colony weight at the end of overwintering from the weight at the beginning.

4. Statistical analysis

To compare temperature variability between the rain-cover and shaded houses, the average temperature, daily mean maximum temperature, and daily mean minimum temperature during each observation period were analyzed. After testing for normality, a two-sample t-test was performed for data with normal distributions, while the Wilcoxon rank-sum test was used for non-normal data. The flight activity of honey bees under shaded and unshaded conditions was described using descriptive statistics for flight activity per colony. Linear regression analysis was conducted to examine the relationship between honey bee flight activity and temperature. Food consumption under different overwintering conditions did not follow a normal distribution and was therefore compared using Kruskal-Wallis analysis and the multiple comparisons Dunn test. All statistical analyses were performed using R Studio software (Ver. 4. 3. 2).

RESULTS

1. Temperature variation

Variabilities between the rain-cover and shaded houses were analyzed with daily average, maximum and minimum temperatures. There was no substantial difference between two conditions enough to affect the honey bees. t (1st phase: Two-sample t-test, $p > 0.05$; 2nd phase: Two-sample t-test, $p > 0.05$ for each).

2. Flight activities

The flight activity of honey bees during the wintering period primarily occurred at 14:00 and 16:00. During the first investigation period (January 4th to February 4th), honey bee colonies under the rain-cover house exhibited an average of 27 incoming flights and 12 outgoing flights (Fig. 2). In contrast, colonies placed in the shaded house showed an average of 0.2 incoming flights, with no outgoing flights observed. During the second phase of the study, from February 5th to February 17th, the colonies in rain-cover house showed more flight activity, like the first phase. Moreover, a higher level of flight activity was observed compared to the first phase.

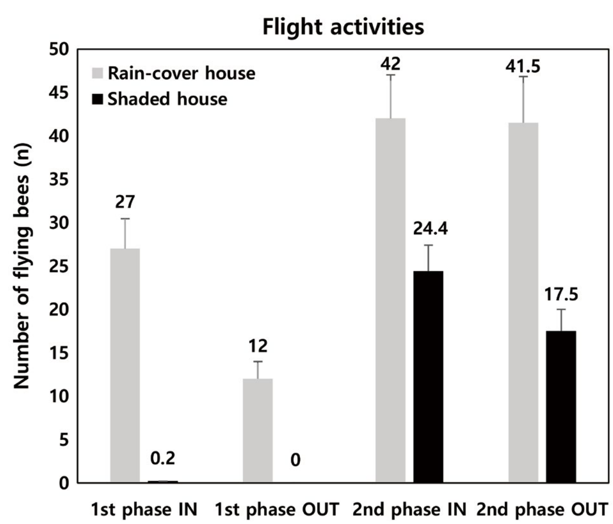

Fig. 2. Honey bee flight activity under rain-cover house and shaded house: the 1st phase was conducted from January 4th to February 4th, and the 2nd phase from February 5th to February 17th.

Table 1. The average, maximum, and minimum temperatures, as well as the daily maximum and minimum average temperatures of the unshaded and shaded houses were analyzed. The first phase covered the period from January 4th to February 4th, while the second phase spanned from February 5th to February 17th.

Temperature (°C)	1st phase		2nd phase	
	Rain-cover house	Shaded house	Rain-cover house	Shaded house
Average ± SD.	−0.5 ± 5.42	−0.1 ± 6.39	1.9 ± 5.84	2.4 ± 6.86
Maximum average ± SD.	8.2 ± 4.19	10.1 ± 6.42	12.5 ± 3.28	15.5 ± 2.97
Minimum average ± SD.	−6.1 ± 3.97	−5.9 ± 3.70	−3.7 ± 3.28	−3.6 ± 2.97

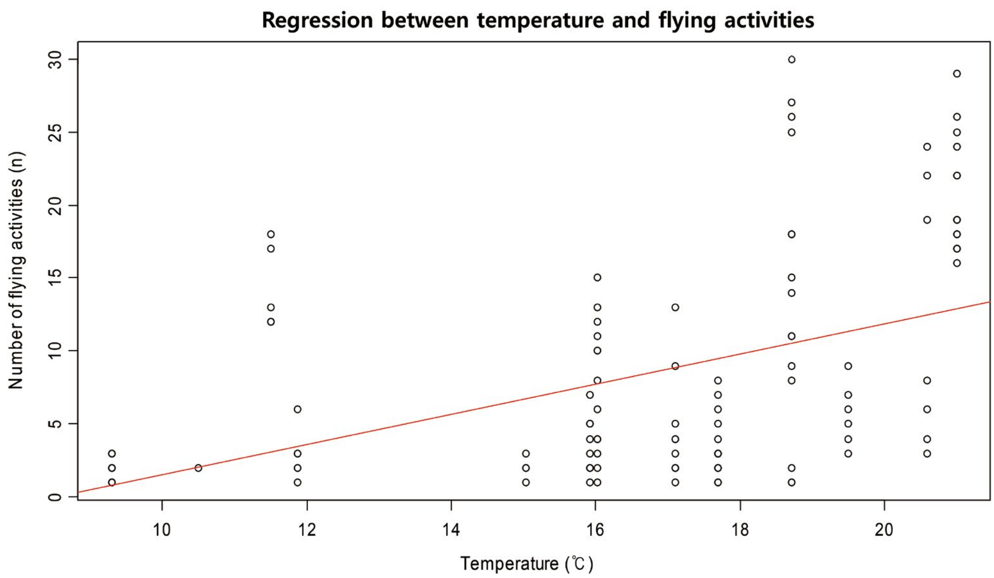

Fig. 3. The regression analysis of flight activity is related to the external colony temperature during winter in 2023 (Regression analysis. $p < 0.05$, $R^2 = 0.25$, $y = 1.0311x - 8.7979$).

3. Relationship between flight activities and temperature

In this study, the lowest temperature at which honey bee flight activity occurred was 9.3°C, while the only temperature at which flight activity was observed in the shaded apiaries during the first phase was 22.5°C. Regression analysis was conducted to examine the relationship between the external temperature of the colony and the number of flight activities. A significant linear relationship was revealed by the regression of temperature and the number of honey bee flying activities (Regression analysis, $p < 0.05$, $R^2 = 0.25$, $y = 1.0311x - 8.7979$; Fig. 3).

4. Food consumption

The food consumption of honey bee colonies placed in each overwintering environment (Open field, Rain-cover house, Shaded house) was estimated based on weight measurements. Colonies in the Open field exhibited the highest food consumption at 4.1 kg, which was statistically significantly higher compared to colonies in the Shaded house (Kruskal-Wallis test, $p < 0.05$; Fig. 4). Colonies in the Rain-cover house consumed 2.0 kg of food, while those in the Shaded house consumed 1.7 kg.

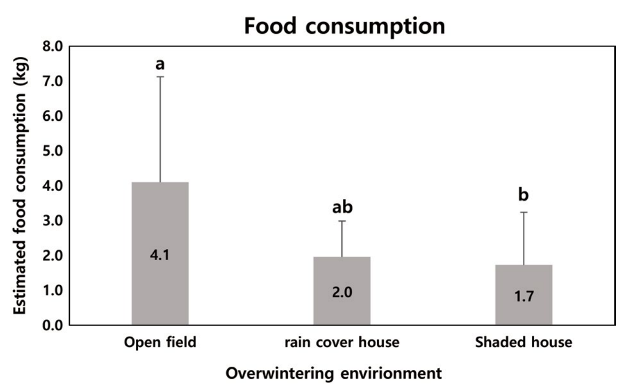

Fig. 4. Food consumption of honey bee colonies under different overwintering conditions (Open field, Rain-cover house, Shaded house).

DISCUSSION

The temperatures in the shaded house were higher than those in the rain-cover house, but the differences were not statistically significant. The flight activity of overwintering honey bees predominantly occurred in colonies placed in the rain-cover house, whereas colonies in the shaded house exhibited only a single flight event, which occurred at a temperature of 22.5°C. Honey bee flight activity showed a positive correlation with temperature, and the food consumption of overwintering colonies was highest in the open field environment.

In this study, the lowest temperature at which honey bee flight activity was observed was 9.3°C. Based on the temperatures during the first investigation period, the frequency of temperatures exceeding the honey bee activity threshold of 9.3°C, as identified in this study, was 317 instances in the shaded house and 113 instances in the rain-cover house. However, honey bee flight activity predominantly occurred in the rain-cover house. Previous studies have reported that the activity level of honey bees in mango orchards is more strongly influenced by changes in light intensity than by temperature (Park *et al*., 2014). Additionally, the foraging activity of honey bees has been shown to exhibit a positive correlation with light intensity (takeoff: $r=0.577-0.849$; landing: $r=0.201-0.803$), though the correlation is slightly lower than that with temperature (Choi, 2015). Since solar radiation varies seasonally (Kim *et al*., 2009), further research is needed to investigate the solar radiation conditions under which honey bee flight activity occurs during the overwintering period.

This study found no significant difference between the number of bees exiting and entering the hive. The presence of empty hives with no bees inside has been reported as a primary symptom of honey bee colony mortality during the overwintering period in South Korea (Kang *et al*., 2024). Although this study attempted to identify the patterns of mortality symptoms through partial observations conducted every 2 hours from 10 a.m. to 4 p.m., it was unsuccessful. Therefore, a comprehensive investigation covering the entire overwintering period is necessary to discern patterns of these symptoms. Furthermore, research is required to elucidate the underlying causes of colony mortality and the conditions under which it occurs.

The food consumption of honey bee colonies varied according to overwintering environments, with colonies in the open field showing the highest consumption. By the end of the overwintering period, 55.6% of the colonies in the open field had already initiated brood rearing, which may explain the increased food consumption (rain-cover house: 35.3%; shaded house: 17.4%). Honey bees form clusters to survive the winter; however, if the external temperature exceeds 12°C for an extended period, the queen may initiate egg-laying (Winston, 1987; Jung and Bae, 2022). When brood rearing occurs during the overwintering period, winter bees undergo physiological changes, transitioning to a state similar to summer worker bees, such as nurse or forager bees (Doke *et al*., 2015; Jung and Bae, 2022). Additionally, these workers experience a reduced lifespan due to increased energy demand for heat production, feeding, and brood care activities (Page *et al*., 2001). Therefore, strategies to prevent brood rearing during the overwintering period are essential for effective colony management.

CONCLUSION

This study aimed to determine the threshold temperature for honey bee flight activities during winter and to assess the influences of shading on the bee hives mitigating the extreme temperature variability. The threshold temperature was determined to be 9.3°C, with flight activity increasing as temperatures rose. Shading was found to effectively reduce honey bee flight activity. Food consumption was higher in colonies located in open field or rain-cover condition than the shading conditions. This finding must be confirmed by repeated measurements from various circumstances, However, here we propose season-long and nationwide monitoring of honey bee flight activity relative to the temperature and overwintering environments to better figure out the cause and result relationship of winter losses from climate change.

ACKNOWLEDGEMENTS

This study was carried out with the support of the National Institute of Agricultural Sciences project (Development of smart management technology for honey bees in response to abnormal climates, RS-2023-00232847).

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