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Effects of altitude and beehive bottom board type on wintering losses of honeybee colonies under subtropical climatic conditions

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Abstract

The effects of altitude and beehive bottom board types (BBBT) on the wintering performance of honeybee colonies were investigated in the South Aegean Region of Turkey: Experiment I (E-I), with 32 colonies, in 2010-2011, and Experiment II (E-II), with 20 colonies, in 2011-2012. Each lowland (25 m) and highland (797 m) colony was divided randomly into two BBBT subgroups, open screen floor (OSF) and normal bottom floor (NBF), and wintered for about three months. In E-I, the local genotype Aegean ecotype of Anatolian bee (AE) and Italian race (ItR) were used, while in E-II, only the AE genotype was present. In E-I, the effect of wintering altitudes on the number of combs covered with bees (NCCB), and the effects of BBBT on brood area (BA) and the NCCB were found to be statistically significant ($p < 0.05$), but the effects of genotype on BA and NCCB were statistically insignificant ($p > 0.05$). In the E-II, the effect of wintering altitude on beehive weight was found to be statistically significant ($p < 0.05$), while its effect on the NCCB was statistically insignificant ($p > 0.05$). The wintering losses in the highland and lowland groups in E-I were determined to be 25% and 62.5% respectively. In contrast to this result, no loss was observed in E-II for both altitudes. In E-I, the wintering losses for both OSF and NBF groups were the same (43.75%). In conclusion, under subtropical climatic conditions, due to variations from year to year, honeybee colonies can be wintered more successfully in highland areas with OSF bottom board type.

Additional key words: highland-lowland; open screen floor; brood area; honey consumption; beehive weight; Aegean ecotype of Anatolian bee.

Introduction

The wintering of honeybee colonies is an important issue affecting colony performance in the following seasons. Wintering losses can be increased by several factors such as worker population, age of queen, food reserves, diseases and varroa mite, and climatic conditions during wintering (Furgala & McCutcheon, 1987; Genç & Kaftanoğlu, 1997; Giray *et al.*, 2007; Genersch *et al.*, 2010).

Significant colony losses during wintering have been reported in the USA (30%), Europe (1.8-53%) and the Middle East (10-85%) since 2006 (Neumann & Carreck, 2010). In order to determine the main causes of colony losses in the USA in 2008 and 2009, Van Engelsdorp *et al.* (2010) conducted a survey among honeybee breeders. The US breeders pointed out climatic conditions, starvation and colony management as the main reasons for the losses (Van Engelsdorp *et al.*, 2010).

Giray *et al.* (2007) also emphasized the importance of climatic conditions and reported that losses were higher in the partly temperate regions of Turkey. Similarly, Topolska *et al.* (2008) investigated colony losses during wintering in Poland in 2007-2008, and stated that losses were higher in the region with a lower number of cold days in winter. Yildiz (2007) conducted a study to determine the effect of altitudes on colony losses during wintering in the south of Turkey. He observed that weight losses and reductions in the number of mature workers in colonies wintered at sea level were higher than those of the colonies wintered

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Abbreviations used: AE (Aegean ecotype of Anatolian bee); BA (brood area); BBBT (beehive bottom board types); BW (beehive weight); E-I (Experiment I); E-II (Experiment II); H (highland); ItR (Italian race); L (lowland); NBF (normal bottom floor); NCCB (number of combs covered with bees); OSF (open screen floor); RH (relative humidity).

at a higher altitude. In the same region (in Hatay), Muz *et al*. (2012) stated that sudden changes in weather conditions increased colony loses up to 30% during wintering.

The wintering performance of honeybee colonies is also affected by the beehive bottom board types (BBBT). Horn (1990) and Skowronek & Skubida (1995) studied the effects of BBBT on wintering. Skowronek & Skubida (1995) investigated the ventilation effects on wintering losses in Pulawy, Poland, by using three groups: one with standard conditions; a second with netting bottom boards; and a third with a 15-cm air space (air pillow) between the bottom board and the brood chamber. They reported that the highest wintering losses were observed in the colonies with a netting bottom board, and added that the reserves in winter were similar in all three groups. Horn (1990) stated that honeybee colonies with an open screen floor (OSF) had about 10-15% higher honey consumption during wintering than those with traditional beehive bottom floor types. In contrast to this, Harbo & Harris (2004) stated that OSF type did not have a significant effect on honey consumption during wintering.

The effects of OSF type on brood area (BA) and varroa population in the hives were also investigated by several researchers (Skubida & Skowronek, 1995; Pettis & Shimanuki, 1999; Ostiguy *et al*., 2000; Ellis *et al*., 2001; Sammataro *et al*., 2004). Skubida & Skowronek (1995) studied the ventilation effect on BA in Poland and found similar BA during wintering, but added that the highest BA was seen in the colonies wintered on a bottom board with a higher rate of ventilation from March to May.

Pettis & Shimanuki (1999) conducted research about the effects of bottom boards modified with wire mesh on BA and varroa mites in Maryland, USA, and found that the BA was higher in hives with bottom boards modified with wire mesh than in those with the normal bottom board type. They also found that the number of mites decreased in July and August in the hives with bottom boards modified with wire mesh; however, this decrease was not found to be statistically significant compared to hives with regular bottom boards.

The objectives of this research were to determine the effects of different altitudes, bottom board types and genotypes on colony losses of hives and worker populations during wintering under subtropical climatic conditions.

Material and methods

This study was conducted in the province of Aydin, located at the South Aegean region of Turkey, between 2010 and 2012. Wintering performances of honeybees were determined in lowland (N 37° 45.674’, E 27° 45.379’; 25 m asl) and highland (N 37° 40.384’, E 28° 18.607’; 797 m asl) sites by conducting two experiments in two years. The coordinates of the locations were determined using a GPS receiver (GPSmap 62S, Garmin), and the average temperatures of the locations were recorded using an Onset HOBO data logger at 30-min intervals during two wintering years.

Experiment I (E-I)

In the first year of the study in the winter of 2010-2011, as a breeding stock, the Aegean ecotype of Anatolian bee (AE) and Italian race (ItR) were used. AE and ItR queen bees were raised by grafting in April 2010. In the second week of May, the natural mated and egg-laying queen bees were placed in the colonies without a queen and with equal amounts of brood and mature honeybees. AE (16) and ItR (16) colonies were nursed and fed from June to September. A chemical drug was also used against *Varroa destructor* in all the colonies. Then, in October, all the colonies were equalized for mature honeybees, brood and feedstock. On November 2, 2010, both the AE and ItR colonies were randomly divided into two groups and 8 AE colonies and 8 ItR colonies were wintered in the highland (H), and 8 AE colonies and 8 ItR colonies were wintered in the lowland (L) areas. Before wintering, both groups were divided into two beehive bottom board types (BBBT), half of each group (4 colonies) was transferred into the OSF hives and the other half (4 colonies) into normal bottom floor (NBF) hives. The OSF hives were of 39-cm length and 28-cm width, with 3-mm diameter holes on the bottom board.

The averages of BA and the number of combs covered with bees (NCCB) before wintering were 4,909.2 ± 16.74 cm^2 and 8.5 ± 0.15 for the highland and 4,919.5 ± 16.74 cm^2 and 8.4 ± 0.18 for the lowland colonies respectively (Table 1). These two colony groups were wintered from November 25, 2010 to February 15, 2011.

Performances of all colonies were determined by measuring two axes of ellipsoidal brood areas (Fresnaye & Lensky, 1961) and by counting the NCCB of all colonies.

Table 1. Colony performances before and after wintering (Experiment I)

	N	No. of colony losses	Before wintering		After wintering	
			BA (cm²)	NCCB	BA (cm²)	NCCB
Wintering place			NS	NS	NS	*
Lowland (L)	16	10	4,919.5 ± 16.74	8.4 ± 0.18	1,462.1 ± 388.42	2.9 ± 0.48[b]
Highland (H)	16	4	4,909.2 ± 16.74	8.5 ± 0.15	2,170.2 ± 305.94	4.3 ± 0.43[a]
Beehive bottom board type (BBBT)			NS	NS	*	*
Open screen floor (OSF)	16	7	4,916.3 ± 16.74	8.4 ± 0.18	2,428.9 ± 355.51[a]	4.3 ± 0.46[b]
Normal bottom floor (NBF)	16	7	4,912.5 ± 16.74	8.6 ± 0.17	1,203.3 ± 326.63[b]	2.9 ± 0.44[a]
Genotype			NS	NS	NS	NS
Italian (ItR)	16	6	4,923.0 ± 16.74	8.7 ± 0.17	1,853.6 ± 328.51	3.9 ± 0.44
Aegean (AE)	16	8	4,905.7 ± 16.74	8.2 ± 0.18	1,778.7 ± 365.75	3.4 ± 0.48

BA: brood area. NCCB: number of combs covered with bees. * Significant for $p < 0.05$. NS: not statistically significant.

On February 16, 2011, after wintering, the highland colonies were brought to the lowland area, next to the other group, and all the colonies were checked on the next day. The measurements of BA and NCCB were conducted on March 17, 2011.

Experiment II (E-II)

Because the differences found for BA and NCCB between the AE and ItR genotypes were insignificant, in the second year of the research, the experiment was conducted with only AE, the local genotype, in the same wintering locations as in the previous year. The same queen-rearing and colony-management practices were repeated, and 20 colonies were used in this experiment. In October, all the colonies were equalized and randomly divided into highland and lowland groups. Each group was also randomly divided into two subgroups for BBBT. One subgroup (5 colonies) was wintered in the OSF hives (39-cm length, 28-cm width with 3-mm diameter holes), and the other (5 colonies) were wintered in the NBF type.

The wintering was done from November 28, 2011 to February 24, 2012. Colony weight and NCCB were determined before (on November 23, 2011) and after (on March 15, 2012) wintering. Beehive colonies were weighed with a digital scale with 50 g sensitivity. At the beginning of the experiment, the empty beehives' tares were determined, and then the frames with comb and bees were transferred into the beehives and weighed again. The differences between these two weightings were accepted as the beginning weight of the colonies. The beginning weights of colonies were subtracted from their weights after wintering to determine the weight differences.

Evaluating the climatic conditions of the wintering locations

The average monthly temperature (°C) and relative humidity (RH, %) of both wintering locations are given in Fig. 1. The average monthly temperature and RH of the highland site for both wintering years were lower than those of the lowland location. As seen in Fig. 1, the average monthly temperatures for December, January and February in the E-I for both locations were higher than those of E-II. Except for January in lowland site, the same trend was seen for RH for both locations. Comparing the locations, it can be said that temperatures were higher in the lowland than in the highland site; however, the changes of the temperature in the highland were more stable than in the lowland. On most of the wintering days in the lowland location, maximum temperatures were over 20°C in all wintering months for both experiments, and reached 25°C in all wintering months in E-I. As the temperatures of the lowland site were monitored, it can be said that on most of the wintering days, the temperatures changed about 10°C between 11:00 and 16:00. In contrast, in the highland location, except for December 2010, the average maximum temperature was below 20°C for both experiments compared to over 20°C in the lowland site. For both locations, the average minimum temperatures dropped below

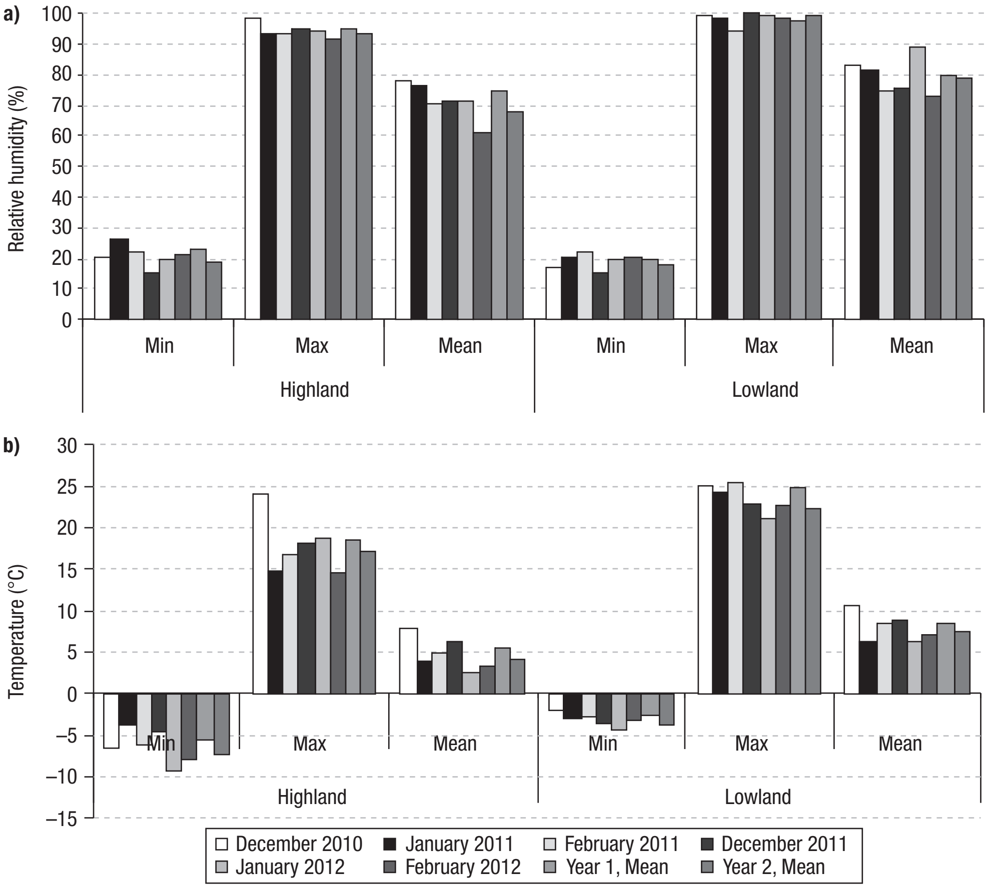

Figure 1. Relative humidity (a) and temperature (b) values of highland and lowland sites in this study.

0°C for all months for the first and second wintering years.

Statistical analysis

Data were analyzed using the GLM procedure of SAS and the differences between the means were considered to be statistically significant at $p > 0.05$ (2-tailed) based on Tukey's type I error rate. The statistical models used for both experiments are as follows:

$$y_{ijkl} = \mu + a_i + b_j + c_k + e_{ijkl} \qquad \text{[E-I]}$$

$$y_{ijk} = \mu + a_i + b_j + (ab)_{ij} + e_{ijk} \qquad \text{[E-II]}$$

where y_{ijkl} is the observation of BA or NCCB for the first experiment, y_{ijk} is beehive weight (BW) or NCCB for the second experiment, μ is the overall mean, a_i is the effects of wintering place (i = lowland or highland), b_j is the effects of bottom floor type (j = normal bottom floor or open screen floor), $(ab)_{ij}$ is the effects of wintering place × bottom floor type interaction, c_k is the effects of genotype (k = AE or ItR), e_{ijk} or e_{ijkl} are random errors.

Results

Wintering performances of the AE and ItR genotypes in two different altitudes in the Aydin province of Turkey in E-I are given in Table 1 and the performances of AE in E-II in Table 2.

Experiment I

In this experiment, a total of 14 out of 32 colonies died, a wintering loss of 43.75%. In the lowland group, 10 out of 16 colonies died; however, in the highland group wintered in the mountains, only four out of 16 colonies died. The wintering losses in the highland and

Table 2. Beehive weight (BW) and number of combs covered with bees (NCCB) before wintering and after wintering (Experiment II)

	N	Beehive weight (kg)		No. combs covered with bees	
		Before	**After**	**Before**	**After**
Wintering place (WP)		NS	*	NS	NS
Lowland (L)	10	22.2 ± 0.37	16.1 ± 0.49[a]	8.7 ± 0.18	3.9 ± 0.23
Highland (H)	10	21.4 ± 0.37	18.5 ± 0.49[b]	8.7 ± 0.18	4.3 ± 0.23
Beehive bottom board type (BBBT)		NS	NS	NS	NS
Open screen floor (OSF)	10	22.5 ± 0.37	17.4 ± 0.49	8.8 ± 0.18	4.3 ± 0.23
Normal bottom floor (NBF)	10	22.1 ± 0.37	17.2 ± 0.49	8.6 ± 0.18	3.9 ± 0.23
WP*BBBT		NS	NS	NS	NS
L*OSF	5	21.9 ± 0.52	16.6 ± 0.69	8.7 ± 0.25	4.4 ± 0.33
L*NBF	5	22.4 ± 0.52	15.5 ± 0.69	8.6 ± 0.25	3.4 ± 0.33
H*OSF	5	21.0 ± 0.52	18.1 ± 0.69	8.8 ± 0.25	4.2 ± 0.33
H*NBF	5	21.8 ± 0.52	19.0 ± 0.69	8.6 ± 0.25	4.3 ± 0.33

* Significant for $p < 0.05$. NS: not statistically significant.

lowland groups were determined to be 25% and 62.5% respectively.

As seen in Table 1, if the wintering losses were compared for BBBT, they were the same for both OSF and NBF groups; seven colonies from each group were lost and the wintering losses were 43.75% for both bottom board types.

The wintering loss in the ItR genotype was lower than in the AE genotype. Half of the AE colonies (8) were lost in the first experiment and the number of losses in the ItR genotype was 6 (37.5%).

The effect of wintering locations on NCCB was found to be statistically significant ($p < 0.05$); however, its effect on the BA was statistically insignificant ($p > 0.05$). In the lowland group, BA and NCCB decreased in the wintered colonies from 4919.5 ± 16.74 cm^2 and 8.4 ± 0.18 to 1462.1 ± 388.42 cm^2 and 2.9 ± 0.48 respectively. The numbers for the highland colonies were 4909.2±16.74 cm^2 and 8.5±0.15 to 2,170.2 ± 305.94 cm^2 and 4.3 ± 0.43 respectively. As seen in Table 1, although the mean brood areas of both location groups were the same before wintering, the decrease in the lowland colonies (3,457.4 cm^2) after wintering was higher than that of the highland colonies (2,739.0 cm^2), but the difference between these two groups was statistically insignificant ($p > 0.05$). A similar situation was seen for the NCCB. The decrease in the NCCB in the lowland group was higher than that of the highland group ($p < 0.05$). The losses of NCCB were 5.5 and 4.2 respectively for the lowland and highland colonies.

The effects of beehive floor type on BA and the NCCB were also determined to be statistically significant ($p < 0.05$). As seen in Table 1, despite the fact that the BA and NCCB were almost the same for OSF and NBF types before wintering, the differences between the floor types were statistically significant ($p < 0.05$) after wintering. During wintering, the decreases in BA and NCCB were higher in NBF than those of the OSF. The decreases were 3,709.2 cm^2 and 5.7 in NBF compared to 2,487.4 cm^2 and 4.1 in OSF. After wintering, BA and NCCB in OSF were 1225.6 cm^2 and 1.4 higher than those of NBF.

The effects of genotype on BA and NCCB after wintering were statistically insignificant ($p > 0.05$), even though colony losses were lower in the ItR race. However, BA and NCCB were higher in the ItR genotype after wintering than those of the AE genotype. For the ItR genotype, BA (1,853.6 ± 328.51 cm^2) and NCCB (3.9 ± 0.44) were 74.9 cm^2 and 0.5 higher than those of AE genotype (Table 1), but these differences were statistically insignificant ($p > 0.05$).

Experiment II

Wintering performance of AE in the second experiment is given in Table 2. The effect of wintering place on BW after wintering was found to be statistically significant ($p < 0.05$), but its effect on NCCB was statistically insignificant ($p > 0.05$). Similarly, the effects of beehive floor type and the interaction between wintering place and beehive floor type on BW and NCCB after wintering were also statistically insignificant ($p > 0.05$).

Unlike in the first year of the experiment (E-I), no colony loss was seen in E-II. In the lowland colonies, BW and NCCB decreased from 22.2 ± 0.37 kg and 8.7 ± 0.18 to 16.1 ± 0.49 kg and 3.9 ± 0.23, respectively. The same figures for highland colonies were 21.4 ± 0.37 kg and 8.7 ± 0.18 to 18.5 ± 0.49 kg and 4.3 ± 0.23, respectively (Table 2).

Discussion

In order to determine the effects of wintering location and BBBT on wintering losses in honeybees in the subtropical climatic conditions of the Aegean region of Turkey, two studies were conducted in two consecutive wintering years between 2010 and 2012. In the first experiment (E-I) in this study, the higher colony losses found in wintered colonies in both wintering locations (L and H) should not be accepted as a usual circumstance. In the literature, many reasons are indicated for wintering losses in honeybee hives, such as queen failure, starvation, weakness of the colonies in autumn, *V. destructor* mite (Van Engelsdorp *et al.*, 2008), bacterial and parasitic pathogens (Muz *et al.*, 2012), colony collapse disorder (CCD), and some viruses (Johnson *et al.*, 2009; Van Engelsdorp *et al.*, 2009; Genersch, 2010).

Because the colonies were controlled for queen egg laying, fed and struggled against *V. destructor* mite in the fall, queen failure and weakness of worker bee population in autumn and *V. destructor* could not be the main reasons for the high death rate in the first year of the experiment. In the dead colonies, bacteriological and viral examinations were not done, but after wintering there were no notable diseases in these colonies either. So, in the first year of the experiment, starvation and a decrease in the number of workers caused by mild climate in winter could be the main reasons for colony losses in the lowland and highland regions.

The overall temperature differences during wintering between E-I (in H 5.5°C, in L 8.5°C) and E-II (in H 4.1°C, in L 7.4°C) were 1.4°C in H and 1.1°C in L. If the mean monthly temperatures are compared between the years (experiments), the temperature differences are even higher (Fig. 1). For example, the difference between the average temperatures for December was 1.6°C in H and 1.8°C in L between the years. The temperature differences between the wintering locations (H and L) were, on the other hand, about 2.5°C in December and January, and about 4°C in February. However, this cannot be the only reason for the difference in colony losses observed between the wintering locations and between the experiments; other factors could play a role in the colony losses.

Visual observation of dead colonies in E-I indicated that some had consumed their honey stock completely, but some had died because of a critical decrease in worker population despite the fact that some honey remained in the hives. The higher temperatures in the lowland region in November and December encouraged an increase in forager flights leading to more rapid physiological aging. In response to insufficient nectar sources and feeding, food stocks in the hive also ran out fast in this region. On the other hand, it was observed that nectar sources in highland region, such as *Marchalina hellenica* in November, became richer than those of the lowland region during wintering, and this could be another reason of the different death rates between the wintering locations.

Despite the fact that colony losses were found to be higher in E-I, the death rate found in E-I in this study is in agreement with Giray *et al*. (2007, 2010), Topolska *et al*. (2008) and Muz *et al*. (2012). They all report that wintering losses could increase as the wintering temperature increases. Topolska *et al*. (2008) indicated a higher colony loss in the region with a low number of cold days during winter. Giray *et al*. (2007, 2010) also reported a higher wintering loss in the temperate regions of Turkey, and added that the losses were over 40% in the 2006-2007 wintering season. A similar result was reported by Muz *et al*. (2012), with about 30% losses in the 2010-2011 wintering season in the subtropical region (Hatay).

Yorgancioglu (2001) stated that as temperatures increase, the wintering cluster gets loose and becomes wider. As the temperature drops suddenly, the honeybees choose a place in which food is insufficient to form a cluster again, and this situation was reported to be the main reason for the wintering losses (Yorgancioglu, 2001).

The insignificant difference in wintering performance found between the ItR and AE genotypes in this study is in agreement with a similar study conducted by Ucak-Koc & Karacaoglu (2013). In their study, in addition to the reproductive features (Ucak-Koc & Karacaoglu, 2011), wintering performance and whole-year colony development such as BA and NCCB were also found to be similar in the ItR and AE genotypes under subtropical climatic conditions (Ucak-Koc & Karacaoglu, 2013).

In the second experiment (E-II), all the colonies in H and L were wintered successfully, but the weight losses in the L group were determined to be higher than those of the H group, despite the similar bee population and food reserve before wintering for both groups. The higher honey consumption found in the L group could result from greater activity in the colonies during wintering. This could be the main reason for higher honey consumption in the L group than the H group. In subtropical regions similar to the lowland area in this study (25 m), because there are very few cold days during winter, the honeybees do not form a tight cluster, becoming more active and consuming more honey than those of the colonies that do form clusters (Crane, 1990). In addition, the greater weight loss found in L than in H is in agreement with Yildiz (2007).

On the other hand, in E-II, the higher BA and NCCB in the OSF group (2,428.9 ± 355.51 cm^2 and 4.3 ± 0.46) than in the NBF group (1,203.3 ± 326.63 cm^2 and 2.9 ± 0.44) in E-I, and a higher NCCB in OSF (4.3 ± 0.23) than in NBF (3.9 ± 0.23) show that choosing OSF as a bottom board type gave an advantage in the performance of the colony during wintering compared to the NBF bottom board type. The result of this study is in agreement with Pettis & Shimanuki (1999), who found a higher BA in OSF colonies than that in NBF colonies after wintering in April in Maryland (USA).

Based on the results found in this study, it can be said that using an OSF bottom board enables the colonies to reduce the stress caused by high temperatures in summer in the region where this study was conducted. One of the positive effects of OSF could also be the decrease of the varroa population. In addition, the use of OSF bottom boards could also decrease the colony losses caused by insufficient ventilation during transportation observed in traditional beehive types in migratory beekeeping.

With subtropical climatic conditions, the south Aegean region of Turkey has many sunny days in winter that allow honeybees to fly. The higher temperature differences between day and night in winter, and the temperature fluctuations over a short time in the day, could cause the cluster become wider and narrower. On sunny winter days, some honeybees may separate from the cluster and go out for nectar collection. Then, when the temperature decreases rapidly, they cannot get back to their colonies. Moreover, because of this higher level of physiological activity during wintering, those honeybees that do make it back to the colony age quickly and finish the food stock faster than those in a tight cluster.

Neukirch (1982) reported a reverse relationship between the longevity of honeybees and daily flight numbers, and added that as the energy reserves of the bees decreased or were exhausted, they were unable to fly and could not return to the colony. On the other hand, Esch (1988) and Goller & Esch (1990) reported that as the thorax heat of worker bees decreases to 9-11°C, they are not able to activate their wing muscles for a long time. Remolina *et al*. (2007) state that chronological ageing starts at 50 days in worker bees and because of the physiological stress caused by starvation and higher temperatures, the younger foragers are more durable than older ones.

In conclusion, under subtropical climatic conditions, the wintering of honeybee colonies is more successful in highlands than in lowlands. In addition, the use of OSF bottom boards would offer some advantage in reducing colony losses. Considering global warming, it can be said that under subtropical climatic conditions, honeybees become inactive in the cluster in colder highland locations than in lowlands, and this reduces colony losses, significantly. However, if wintering takes place in the lowlands (at lower altitudes), depending on the climatic conditions of that year, it is necessary to observe the honeybee flights frequently, so that supplementary feeding can be provided if required to reduce colony losses in these places.

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