ORIGINAL ARTICLE

http://dx.doi.org/10.21708/avb.2023.17.4.12083

Comparative study of the performance of africanized bees managed in thermal stress and thermal comfort in a semiarid region

Estudo comparativo do desempenho de abelhas africanizadas manejadas em estresse térmico e em conforto térmico em uma região semiárida

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ABSTRACT: In most of the Brazilian Semiarid region, the climate with high temperatures, low rainfall, and high levels of solar radiation are considered factors that can harm or compromise the thermal comfort of bees, especially if they are in a non-shaded environment, thus generating many losses and growth difficulties in the sector. This study aimed to compare the performance of Africanized bee colonies subjected to two distinct situations: thermal comfort and thermal stress. For this, standardized hives containing sister queens of the same age were monitored every 15 days to survey the combs regarding the areas occupied with pollen, honey, laying, open and closed brood until the queens died. Hives under thermal comfort conditions had a larger area of capped brood (20.66 ± 3.81%), significantly higher (p = 0.0106) than hives under thermal stress (15.76 ± 2.62%). In addition, the lifespan of queens managed under thermal comfort was longer (244 ± 71 days) than that of queens under thermal stress (193 ± 63 days), although we did not find a statistically significant difference. It can be concluded that the development of the colonies when they are in thermal comfort presents better performance, especially with regard to the growth of the brood area. Therefore, it is important to apply shading to hives in beekeeping in regions with hot and dry climates.

KEYWORDS: *Apis mellifera L.*; adverse environmental conditions; shading.

RESUMO: Na maior parte do Semiárido brasileiro, o clima com altas temperaturas, baixo índice pluviométrico e altos níveis de radiação solar são considerados fatores que podem prejudicar ou comprometer o conforto térmico das abelhas, principalmente se estiverem em ambiente não sombreado, gerando assim muitas perdas e dificuldades de crescimento no setor apícola. Este estudo teve como objetivo comparar o desempenho de colônias de abelhas africanizadas submetidas à duas situações distintas: conforto térmico e estresse térmico. Para isso, colmeias padronizadas contendo rainhas irmãs da mesma idade foram monitoradas a cada 15 dias para quantificação dos favos quanto às áreas ocupadas com pólen, mel, postura, cria aberta e operculada até a morte das rainhas. Colmeias sob condições de conforto térmico apresentaram maior área de criação operculada (20,66 ± 3,81%), significativamente maior (p = 0,0106) do que colmeias sob estresse térmico (15,76 ± 2,62%). Além disso, a expectativa de vida das rainhas manejadas sob conforto térmico foi maior (244 ± 71 dias) do que a das rainhas sob estresse térmico (193 ± 63 dias), embora não tenhamos encontrado diferença estatisticamente significativa. Pode-se concluir que o desenvolvimento das colônias quando estão em conforto térmico apresentam melhor desempenho, principalmente no que diz respeito ao crescimento da área de cria. Portanto, é importante aplicar o manejo de sombreamento sobre as colmeias na apicultura desenvolvida em regiões de clima quente e seco.

PALAVRAS-CHAVE: *Apis mellifera* L.; condições ambientais adversas; sombreamento.

INTRODUCTION

Brazilian beekeeping is in a wide development process, especially in the Brazilian Northeast, which has become an important honey-producing region and participates with the significance of Brazilian exports, especially when it refers to organic honey. The growth of the beekeeping sector is a consequence of the

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Received: 02/22/2023. Accepted: 10/16/2023

good characteristics of Africanized bees (*Apis mellifera L.*), such as high adaptability, resistance to diseases, and productivity, as well as the advancement of research and technical knowledge that collaborated considerably in the management of these bees (Schouten; Lloyd, 2019).

Although there have been advances and a growing diffusion of bee management techniques, many beekeepers are still frustrated with the low productivity of their colonies. The development of the swarm, as well as the performance of its individuals, are directly influenced by environmental factors because, depending on the edaphoclimatic conditions of a given place, organisms can undergo physiological or behavioral changes (Mcafee *et al.*,2020; Ribeiro *et al.*, 2018).

According to the literature, with a strong population and adequate nutrition, bees can maintain, through thermoregulation, an optimal microclimate inside the hive (Jarimi; Tapia-Brito; Riffat, 2020); however, physiological disarrangement can be observed if bees are managed under unfavorable climatic conditions, such as high temperatures (Abou-Shaara *et al.*, 2017; Kablau *et al.,* 2020; Mcafee *et al.*,2020; Medina *et al.*, 2018).

Thus, handling bees exposed to environments with high temperatures and intense solar radiation, which are meteorological conditions found in the semiarid Brazilian northeast, keeps bees in a constant state of thermal stress (Domingos; Gramacho; Gonçalves, 2022) with diverse impacts on foraging activities, pollination services, physiology, reproductive capacity, growth and development of bees, causing many losses and generating enormous growth difficulties in the sector (Zhao *et al.*, 2021).

Under these conditions, the overheating of the colonies can be mitigated through cooling strategies (Stabentheiner *et al.*, 2021), as is the case of shading, which aims to prevent the penetration of direct solar radiation on the colonies. This simple strategy, in addition to keeping bees in thermal comfort (Domingos; Gramacho; Gonçalves, 2022), has the advantage of allowing better use of bees for essential tasks such as foraging instead of investing in the constant search for water and ventilation to maintain the thermal homeostasis of the colony. This fact can generate an energy saving favorable to obtaining a larger swarm population and improvement in the behavior and physiology of the colony, which, therefore, would reflect on increased production performance (Sombra *et al.*, 2023).

In other words, the use of shaded areas on the hives promotes thermal comfort and represents a saving of energy and time for the bees in the process of cooling the nest, leaving more "labor" for other tasks, such as foraging, thus increasing their productive capacity. Thus, the objective of this work was to perform a comparative study of performance (percentage of occupied area with honey, open brood, pollen, operculated brood, and egg) and longevity of Africanized bee colonies managed in thermal comfort and thermal stress.

MATERIAL AND METHODS

The study was developed in the beekeeping sector of the Federal Rural University of the Semi-Arid Region (UFERSA), geographically defined by the geographical coordinates 5º03'37" south latitude, 37º23'50" west longitude, with an altitude of 72 meters and semiarid climate, characterized by scarcity and irregularity of precipitation, with rainfall occurring in summer and strong evaporation due to high temperatures.

Africanized bee colonies were used, installed in Langstroth hives, and divided into two distinct experimental groups: hives exposed to direct solar radiation and hives protected from direct solar radiation by artificial shading. The purpose of shading was to provide an environment of thermal comfort for the bees, which was obtained through protection against solar radiation. On the other hand, keeping the hives without any direct sun radiation protection allows the bees to enter a state of thermal stress. Domingos; Gramacho; and Gonçalves (2022) state that hives exposed to direct solar radiation can be called bees in thermal stress, while bees protected from solar radiation can be called bees in thermal comfort. Therefore, the same terms will be used here.

To reduce the effect resulting from the genetic variability of the colonies, before the assembly of the experiment were introduced in each hive, sister queens of the same age. For this, Africanized bee queens were produced according to the method of Doolittle (1899), and the larvae were transferred from offspring frames of a single matrix colony. After birth, the sister queens were marked with non-toxic white paint on the upper chest and introduced into nuclei of fertilization for nuptial flight and natural fertilization. In the process of introduction, the queens were held individually in wire cages containing Candy paste (a mixture of honey and sugar) as food. The Candy was placed over the cage opening so that it would only be opened a few days after the introduction, after the queen and the workers had eaten some of the food, giving the queen passage and gradually releasing her. After 15 days of introduction, the nuclei were checked to confirm whether the queens were fertilized and begin the laying of eggs.

After the beginning of oviposition, ten random queens that presented a normal posture were captured from the nuclei of fertilization, had one of the wings clipped (to avoid a posterior escape), and then were divided into two groups and introduced into colonies previously orphaned (five hives under conditions of thermal comfort and five hives under thermal stress). The hives also had several breeding frames and previously standardized foods, so the experiment was started with similar biomass conditions for each group of colonies. Each colony had approximately five frames of brood, three frames with food and two with honeycomb wax sheets.

After two months of the assembly of the experiment, enough time for the bee population has already be renewed (offspring of the new queen); then the reviews of the colonies *in loco* every 15 days for five months (August to December) to survey the combs as the areas occupied with pollen, honey, egg, open and capped brood, until the queens die, as the objective of evaluating the performance of the colonies, as well as the longevity of the queens. In the inspections, the colonies of both groups were inspected, and the queens were captured, trapped in wire cages, and left at the bottom of each hive. This was a measure to prevent the queen from being killed during the manipulation of the frames since, to prevent the occurrence of looting, the revisions were carried out at night, and the lack of adequate lighting combined with intensive manipulation could result in the queen's death by crushing. After imprisoning the queen, all the beehive frames were then removed and transported to the laboratory, where the development of the colonies was evaluated in general, using the method of Al-Tikrity *et al.* (1971). For this procedure, a device with a wooden frame having, on one of its sides, wire wires dividing the area into 36 quadrants proportionally identical. The device allows you to determine the percentage of area occupied with honey, pollen, eggs, and open and capped brood of each Langstroth model frame placed inside it. In this case, the tables were classified according to the percentage of occupied area for each item analyzed.

Reviews also recorded the presence of queen cells, as well as possible swarming. The colonies were followed up through reviews and frame monitoring until the death of each colony's queen when their lifespan was recorded, as well as the probable cause of death or disappearance, or both. To verify possible additional factors that could influence the performance of the worker bees and the development of the colonies, the occurrence of disease, looting, predation, or other unexpected external events was also recorded and evaluated. The site of the experiment had water approximately 200 meters from the hives, as well as the availability of bee pasture.

The data of all parameters evaluated in the colonies had normality verified by the Kolmogorov test and the Shapiro-Wilk test, and, subsequently, the means of the treatments were compared by the T-Student Test at the level of 5% probability (Bilateral test - two independent samples). All analyzes were performed with the help of the software ASSISTAT version 7.7 beta (Silva; Azevedo, 2002).

RESULTS AND DISCUSSION

Although Figure 1 shows that the colonies in thermal comfort performed better, the statistical analyses showed no differences between the treatments for the variables open brood, egg, honey, and pollen. Only the operculated brood variable showed a significant difference between bees in thermal comfort and bees in thermal stress, with the first group showing the best performance. The overall averages for the percentage of area occupied by open brood, eggs, honey and pollen were 9.39 ± 4.90%, 7.39 ± 4.59%, 12.57 ± 6.20%, 7.73 ± 5.48%, respectively, for the bees in thermal comfort, and 8.77 ± 4.45%, 6.94 ± 3.82%, 13.27 ± 6.67% and 8.93 ± 6.10%, respectively, for the bees in thermal stress.

The operculated brood variable showed different responses to the other variables. Specifically, in the second month of observation, the colonies that were in thermal comfort had an average percentage of area occupied by operculated brood that was statistically higher than that of the bees that were in thermal stress, this percentage being 20.66 ± 3.81% for the bees in comfort and 15.76 ± 2.62% for the bees in thermal stress. This larger brood area is a result that can be directly reflected in the expansion of the swarm, providing a population with more workforce. Depending on the number of individuals and the demand for activities in the colony (Mckinnon, 2023), the workers can be induced to perform other functions, and in this work, the bees that were in a condition of thermal comfort were possibly better directed towards the tasks of feeding the brood and the queen. In turn, the queen, well cared for and fed by the brood, lays more eggs, and, consequently, her colonies will have a larger brood area, a strong population, and, therefore, greater productivity.

The fact that the difference between the two groups was only evident in the second month of the experimental period does not diminish the importance of providing the bees with a thermally comfortable environment provided by shading; on the contrary, this positive response highlights the importance of this simple management for the development of the colonies. The other months may have been influenced by other climatic factors, which may have affected the bees' temperature control, even though they were in thermal comfort. Given the results and based on other studies, this possibility cannot be ruled out. For example, Sombra *et al.* (2023) also assessed the development of colonies under conditions of stress and thermal comfort in the semi-arid Northeast. The author reported that the use of a shaded area significantly improved the development and production parameters of the colonies and concluded that this must have occurred due to the greater saving of time and energy by the bees in the thermoregulatory processes of the hive. Lopes *et al.* (2011) also found better colony performance under shaded conditions when compared to hives exposed directly to the sun. Sombra *et al.* (2023) reported estimates of a 45% increase in honey production for colonies in shaded areas.

Regardless of the treatment evaluated in this study, a sharp decrease in colony biomass was observed, especially from October onwards, with the reduction peaking in December. This was also the period in which the bees invested in migratory swarming (even if it was unsuccessful due to the queens having one of their wings clipped). These findings are similar to those found by Sombra *et al.* (2023) in the same place as this study. The author observed that during this period, there

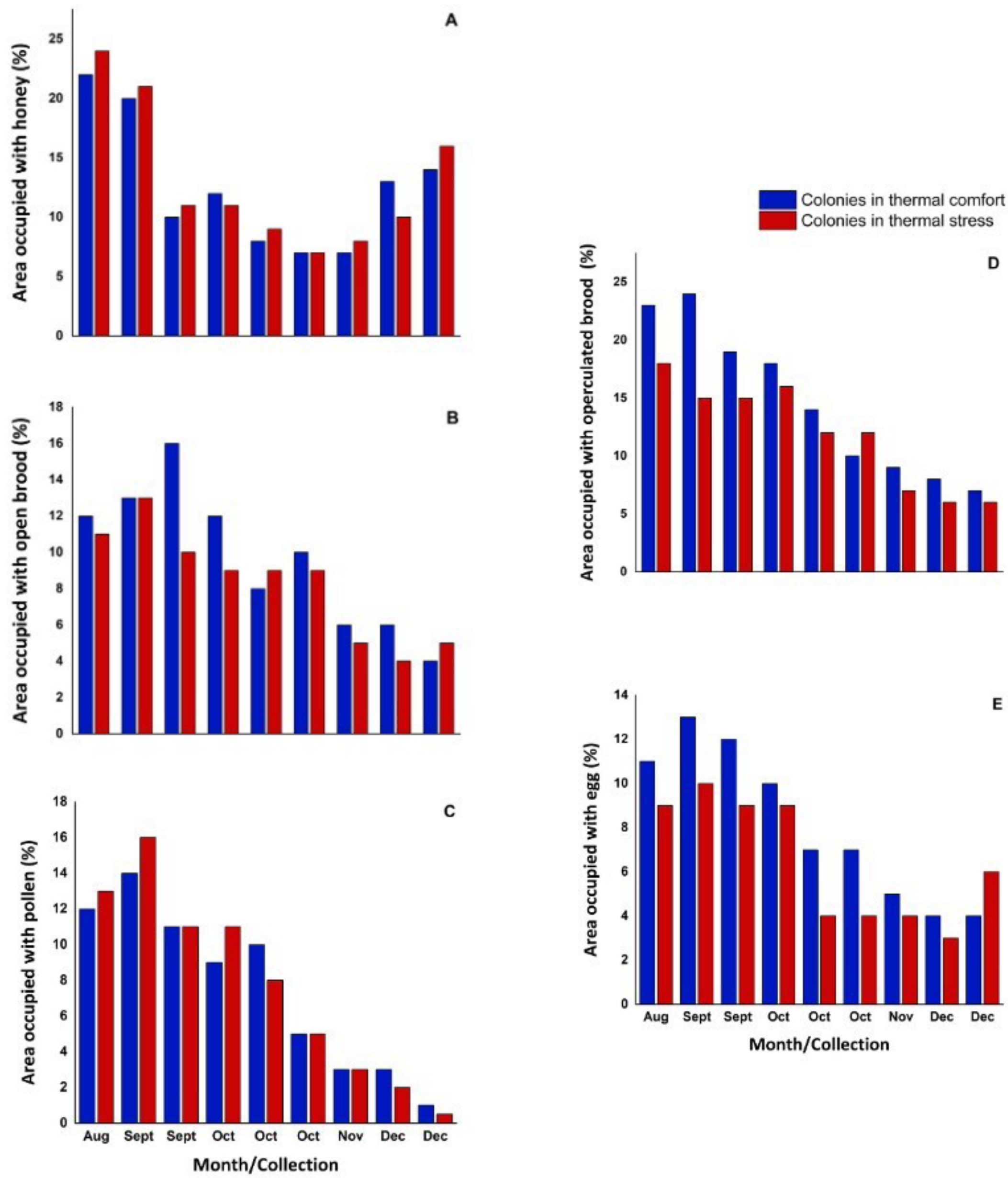

Figure 1. Shows the percentage of area occupied by honey (A), open brood (B), pollen (C), operculated brood (D), and eggs (E) in colonies in thermal comfort and thermal stress during the months of observation. Percentage of the area occupied by honey (A), open brood (B), pollen (C), operculated brood (D), and eggs (E) in colonies of Africanized bees (*Apis melífera* L.) under conditions of thermal comfort and thermal stress in the Brazilian semi-arid region.

was a decline in the availability of flowers in the wild, and, as a consequence, there was a consumption of stored food and a reduction in queen laying and brood areas in the combs, as well as an increase in hive abandonment.

The results of the decline in biomass in the colonies were also due to the aging of the queens. They were produced in May, so, taking into account the longevity of 6 to 8 months, the queens were at the end of their lives when the production of the colonies fell to the lowest levels, and this time interval was also where the highest occurrence of queen deaths was observed. Therefore, the reduction in the production variables evaluated occurred under the combined influence of the

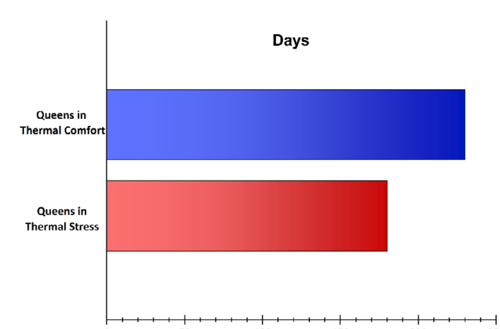

Figure 2. Longevity of Africanized queens in colonies under conditions of thermal comfort and thermal stress in the Brazilian Semiarid.

scarcity of resources in nature and also due to the weakening of the queens' activities.

Concerning the lifespan of the queens determined in the two groups, there was no significant difference between the means at the 5% probability level, possibly due to the reduced sample size. However, one fact stood out. The queens in the colonies in thermal comfort had an average lifespan of around 244 ± 71 days (approximately eight months), while the queens in the colonies under thermal stress had an average lifespan of around 193 ± 63 days (approximately six months) (Figure 2). In practical terms for beekeeping, this represents a difference of approximately two months more life for queens that are managed in thermal comfort. The authors suggest that an experiment with a larger sample size is necessary to prove the difference in longevity between queens from colonies under shaded conditions and queens from colonies exposed to direct solar radiation.

Longevity was calculated from birth, so in the semi-arid conditions observed, the life expectancy of Africanized bee queens is around six to eight months, and this difference may be related to the thermal environment. These results can be compared to those found in other regions of Brazil. Based on this, a periodic queen replacement program should not use periods longer than those mentioned. Knowing the appropriate period for replacement is very important (Bieńkowska; Loś; Węgrzynowicz, 2020) because due to the aging of the queen, which progressively reduces its performance, even if it is genetically "good", it becomes unsuitable to rule the colony, dying naturally or being replaced by its daughters.

In temperate zones, bee colonies stop laying during the winter and resume at the end of this period to build up a sufficient workforce to exploit floral resources next spring (Nürnberger; Härtel; Steffan-Dewenter, 2018). On the other hand, in warm climates, bee colonies do not go through this period of hibernation, during which the queens stop laying; on the contrary, oviposition is continuous, although it can vary in intensity according to the successive flows of nectar secretion. Therefore, it is worth pointing out that the results found in this study are not similar to those observed for European breed bees in temperate climates, which are not in line with the tropical reality, but the results are reported in most of the literature on this subject with *A. mellifera*. Medina *et al.* (2018) evaluated the effect of heat stress on the longevity of Africanized bees and found that it was reduced, as well as changes in the phenotype and behavior of these bees.

The results presented in the present work may differ from other findings not only due to the high genetic variability of the Africanized bee but also motivated by other factors such as geography, period of the year, conditions in which the queens were produced, and, mainly, by the environmental factors of each location. Taking into account the longevity of six to eight months for the management of Africanized queens in the semi-arid region, it is therefore advisable that the queens are produced around two months before the large nectar flows so that the colonies do not change queens in the middle of the high productivity season.

When beekeepers allow queen replacement to occur naturally, in addition to losing control over the quality of their bees and suffering from a higher incidence of swarming problems, they also run the risk of their colonies producing a new queen to replace the old one in the middle of the flowering season. This leads to a breakdown in food stocks, mainly due to the delay in natural replacement.

All the colonies in this study had their queens introduced in the middle of the flowering period, and most of them died before the rainy season began. This reinforces the need to replace the queens before the start of the year's main flowering season so that the bees don't naturally change during the harvest period, interrupting development and seriously compromising the production of the colonies. To avoid the migratory swarming of bees, the use of shaded areas is the minimum that beekeepers should provide for colonies in the semi-arid region. The reduction in the biomass of the colonies and the attempts to abandon them during the most critical period make this clear. This behavior (swarming by abandonment) (Stabentheiner *et al.*, 2021) often occurs with great intensity in Africanized bees and, although it is so fundamental to the propagative success and dispersal of bees in the wild, it has been one of the main reasons for losses in the beekeeping sector in hot regions.

Given the above, ways to mitigate these negative effects should be increasingly investigated, especially in regions where temperatures are very high, where the internal temperature of the colony becomes uncontrollable by the bees, to the point of causing serious physiological and behavioral damage to the individuals and even frequent abandonment of the hives.

CONCLUSIONS

Africanized bees (*Apis mellifera* L.) are sensitive to climatic factors in the semiarid region of northeastern Brazil. The development of colonies, under thermal comfort, presents better performance than that of hives under thermal comfort, especially with regard to the growth of the brood area. Shading hives in the hot and dry climate of the Brazilian Semiarid region is a simple but practical and valid tool to be applied by beekeepers in order to increase beekeeping productivity.

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