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Article

Effect of model and construction material of the brood box and brood comb coating on the thermoregulation and development of *Scaptotrigona mexicana* colonies

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Abstract:

In meliponiculture, the artificial division of *S. mexicana* bee colonies is one of the activities where the greatest loss of colonies is reported. One of the various causes of this mortality is the difficulty of the new colony to maintain the thermoregulation of its nest, given the traditional use of clay pots for this purpose, which render it difficult to maintain a stable temperature. The objective of this study was to analyze the interactions between the model of the box, its construction material and the brood-comb coating on the inner temperature of the nest and the development of colonies obtained by artificial division. The development of the nests was quantified based on their final and initial weight gain, the number of cells built, colony activity, and the capacity of the design to maintain the inner temperature of the nest. The results show that the best internal temperature ranges were achieved in nests transferred to Portugal-Araujo (P<0.05) and Ailton-Fontana (P<0.05) model rational boxes whose original designs were modified to include expandable polystyrene sheets. In addition, colony temperature and development were favored when the newly transferred combs were lined with *Apis mellifera L.* beeswax using a mold. The positive interaction between these factors provided an optimal temperature range (27.9 to 31.0 °C) for the development of the colonies, which obtained weight gains between 0.149 and 0.289 kg, and the number of brood cells constructed ranged between 3,511 and 4,956.

Key words: Thermoregulation, Cavity, Stingless bee, Artificial partitioning, Insulating material, Involvement substitute.

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Introduction

The artificial division of colonies is a method used in meliponiculture and consists in obtaining several daughter colonies over the years[1] from a mother colony of bees[2]. After the division, the survival of the daughter colony becomes critical if it is not provided with adequate conditions[3,4] because parts of the nest are destroyed[5], and the brood of the new colonies are exposed to external temperature fluctuations and do not regain stability until the nest structures are rebuilt[6-9]. The optimum nest temperature is between 31 and 35 °C[10,11]; in order to attain this temperature, the workers line the brood area with small sheets of cerumen known as involucrum[12,13], and subsequently the storage area, and they coat the peripheral walls with batumen (a mixture of cerumen produced by the bees and tree resin). Inadequate nest thermoregulation can have serious consequences for the colony[14], ranging from slow growth to death[15,16,17]. Under conditions of poor nest thermoregulation, the bees invest most of their energies in building insulation structures, and less effort in collecting nectar and pollen that are essential for colony development and survival[12,18].

Another key factor contributing to nest thermoregulation is the type of cavities in which *S. mexicana* nests are housed[12]; those commonly used in the traditional way are natural cavities such as logs and earthenware pots. The logs have thick walls (>10 cm) that allow them to adequately conserve the inner temperature of the nest[19]. However, in the rational boxes, there is a greater fluctuation in the inner temperature of the nest because they have a greater volume, height, and lower wall thickness than natural cavities[20].

Due to the importance of thermoregulation in the development of a colony, it is necessary to build rational boxes that will favor the maintenance of optimal temperature ranges for the development of the brood(21). The cavities commonly used for rearing *S. mexicana* are wooden boxes and earthenware(17,22). However, these materials do not have the best thermal insulating properties to help the nest maintain its optimum temperature, especially when the colonies are small.

Currently, rational wooden boxes have been designed to facilitate colony management and provide better thermoregulation of the nest(6,23,24). The most commonly used model for *S. mexicana* is the Portugal-Araujo(23), whereas the Ailton-Fontana model is used for *Tetragonisca angustula* and *Nannotrigona testaceicornis*(24). The latter box model can be modified to accommodate *S. mexicana* successfully. Both models can be greatly improved to provide greater thermoregulation to the stingless bee nest if an insulating material such as polyurethane(6) or expandable polystyrene foam is included in their design and construction.

During the artificial division of stingless bee colonies, nest structures essential to provide adequate nest thermoregulation are destroyed during this activity. This renders the new daughter colonies vulnerable to sudden changes in ambient temperatures. A proposal to counteract this situation would be to design and build rational boxes with materials with thermal insulating properties and to line the rearing area with a material that will fulfill the functions of the enclosure. The objective of this research was to analyze, in small colonies obtained by artificial division, the effect on inner nest temperature and colony development of *S. mexicana*; covering their brood combs with a material that replaces the involucrum, and housing them in rational box models built with conventional materials and materials with thermal insulating properties.

Material and methods

The study was carried out in the Meliponarium located in the Permaculture area of the College of Postgraduates (Colegio de Postgraduados), Campus Córdoba, Amatlán de los Reyes, Veracruz, Mexico, located between the parallels 18° 46' and 18° 58' N; meridians 96° 49' and 96° 58' W, at an altitude of 600 m asl. The climate is warm humid (88 %) and semi-warm humid (12 %), with abundant rains in the summer, a temperature range between 20 to 24 °C, and a rainfall of 1,900 to 2,600 mm(25). The study period was from June to August 2016, once the experimental material was available and the experimental area was built, and seeking to avoid the rainy season, which in the case of Amatlán de los Reyes is from June to September. The experiment was planned for brief dry period that took place during this season and performed out under a canopy, in order to prevent fly infestation by

Pseudohyphocera spp. Another aspect to be considered for the study period was the availability of food to carry out the division; therefore, it had to be performed during the flowering period within the Permaculture area, where bees have access to flora that provided food (nectar and polen) during the months in which the division was carried out[3].

Thirty six (36) daughter colonies of *S. mexicana* were used in this experiment; they were obtained as follows: based on 107 colonies from two meliponariums of the College of Postgraduates (Colegio de Postgraduados), 36 mother colonies were established with an average weight of 5 kg per colony (range=4.52 to 5.86 kg). These were initially housed in earthenware pots, in order to facilitate handling. Only the brood and involucrum combs with an average weight of 265 g (range=118 to 384 g) were transferred to the Ailton Fontana rational box constructed of wood and polystyrene. The newly transferred colonies were fed *Apis mellifera* honey for 19 d, until the colonies were organized. After 84 d, the mother colonies were artificially divided[26] in order to obtain 36 daughter colonies, providing the same post-transfer management, and 35 d after the division they all had a fertilized queen.

Before placing the combs in the box, they were weighed on a digital scale (OHAUS®), and the initial weight of the combs in each daughter colony at the time of artificial division was recorded. This was within the range of 0.077 to 0.335 kg[27]. The new colonies were each composed of combs of young brood and cocoon brood, worker bees and a fertilized queen. The experimental design was carried out under a randomized complete block design (RCBD) with a full factorial arrangement in the treatments (Table 1) and initial colony weight as a covariate. The factorial arrangement consisted of three factors (2x2x3), the first of which was the Ailton-Fontana model (AFM) and the Portugal-Araujo model (PAM). The second factor was the material used in the construction of the rational cavities: boxes made of wood (BMW) and boxes made of wood on the outside, with a layer of expanded polystyrene in the middle and another layer of wood on the inside, joined together to form a sandwich (BMW+PS). The third factor was the type of material used to coat the newly transferred brood combs to the boxes, with three levels: uncoated transferred brood combs (UTBC) (control), transferred brood combs coated with *Apis mellifera* beeswax (TBCCAMBW) and expandable polystyrene foam-coated transferred brood combs (EPSFCTBC). The beeswax used was from *Apis mellifera L*.

Table 1: Factorial arrangement of treatments

Box model	Material of the box	Honeycomb material	Factorial arrangement of treatments	Code
AFM	BMW	UTBC	AFM- BMW - UTBC	T_1*
AFM	BMW	TBCCAMBW	AFM - BMW - TBCCAMBW	T_2
AFM	BMW	EPSFCTBC	AFM - BMW - EPSFCTBC	T_3
AFM	BMW +PS	UTBC	AFM - BMW +PS- UTBC	T_4
AFM	BMW +PS	TBCCAMBW	AFM - BMW +PS- TBCCAMBW	T_5
AFM	BMW +PS	EPSFCTBC	AFM - BMW +PS- EPSFCTBC	T_6
PAM	BMW	UTBC	PAM- BMW - UTBC	T_7*
PAM	BMW	TBCCAMBW	PAM - BMW - TBCCAMBW	T_8
PAM	BMW	EPSFCTBC	PAM - BMW - EPSFCTBC	T_9
PAM	BMW +PS	UTBC	PAM - BMW +PS- UTBC	T_{10}
PAM	BMW +PS	TBCCAMBW	PAM - BMW +PS- TBCCAMBW	T_{11}
PAM	BMW +PS	EPSFCTBC	PAM-BMW+PS- EPSFCTBC	T_{12}

AFM= Ailton-Fontana model, PAM= Portugal-Araujo model, BMW= box made of wood, BMW+PS= + expandable polystyrene, UTBC = unlined transferred brood combs, TBCCAMBW = transferred brood combs lined with *Apis mellifera* wax, EPSFCTBC = transferred brood combs covered with expandable polystyrene. * Control treatments: T1 for Ailton-Fontana Model (AFM) and T7 for Portugal-Araujo Model (PAM).

Because the initial colony weight was highly variable for all split colonies, it was introduced as a covariate to the statistical model. The statistical model of the experiment was a mixed-effects model with analysis of covariance, represented as follows:

$$\mathbf{y_{ijkl}} \mu + \text{block}_l + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + \delta\left(x_{ijkl} - \bar{x}_{\dots}\right) + e_{ijkl} \quad \text{with } i = 1,2; \quad j = 1,2; \quad k = 1,2,3 \text{ and } l = 1,2,3$$

Where:

$\mathbf{y_{ijkl}}$ is the response variable of the ith effect of the box model with the jth type of building material and the kth type of brood comb coating in the lth block;

$\boldsymbol{\mu}$ is the overall mean; block_l is the random effect of the lth block with mean 0 and variance σ^2_{block};

$\boldsymbol{\alpha_i}$ is the fixed effect of the ith fixed effect of the box model;

$\boldsymbol{\beta_j}$ is the fixed effect of the box construction material;

$\boldsymbol{\gamma_k}$ is the fixed effect of the kth type of brood comb coating;

$(\boldsymbol{\alpha\beta})_{ij}$, $(\boldsymbol{\alpha\gamma})_{ik}$, $(\boldsymbol{\beta\gamma})_{jk}$, **and** $(\boldsymbol{\alpha\beta\gamma})_{ijk}$ are the interactions of the fixed effects;

$\boldsymbol{\delta}(\mathbf{x_{ijkl}} - \bar{\mathbf{x}}_{...})$ is the covariate whose linear regression coefficient is δ with respect to the initial weight x_{ijkl};

$\mathbf{e_{ijkl}}$ is the random experimental error, which is assumed to be independent and identically normally distributed with mean zero and variance σ^2.

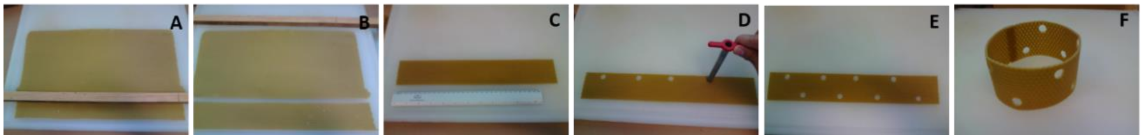
The multiple comparison analysis of means was performed using the GLIMMIX procedure of the SAS version 9.4 statistical package. The means were compared using Fisher's LSD test.

The response variables (temperature) analyzed under this design were classified as follows: daytime ambient temperature (DAT), nighttime ambient temperature (NAT), average ambient temperature (AAT), inner nest temperature in the daytime (INTD), inner nest temperature in the nighttime (INTN) and average internal temperature (AIT). With respect to colony size, the following measurements were made: initial colony weight (ICW), final colony weight (FCW), colony weight gain (CWG), number of brood cells built (NBCB), number of total pots built (NTPB). Finally, in order to observe the relationships between the response variables, a Pearson correlation analysis was performed.

For this study, 18 AFM boxes with a volume of 5.3 L and 18 PAM boxes with a volume of 4.2 L were built. In the AFM model, nine of the boxes were made entirely of wood (2.54 cm thick), and nine, of wood (2.54 cm thick) and expandable polystyrene. As for the PAM model boxes, they were built under the same conditions as described for AFM.

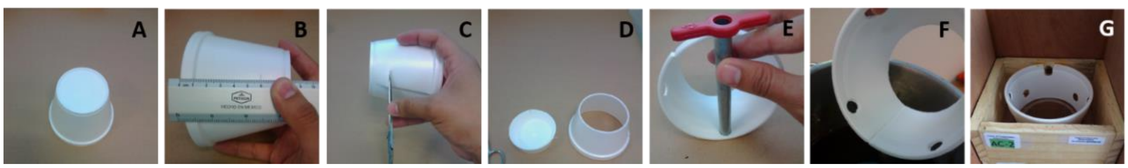
On the other hand, the brood combs transferred to the 36 boxes built with the aforementioned characteristics were lined with the following types of materials: an *Apis mellifera* wax mold (TBCCAMBW) (Figure 1) with a diameter of 12 cm by 6 cm high and a thickness of 0.2 mm, 4 ventilation holes, a diameter of 1 cm on each of the edges, and an expandable polystyrene foam mold (EPSFCTBC) (Figure 2) that had similar characteristics to TBCCAMBW and also included nests without this treatment as a control.

Figure 1: Preparation of the *Apis mellifera* wax coating for transferred brood combs (TBCCAMBW)



A) Measurement tracing on a stamped European honeybee *Apis mellifera L.* wax sheet B) Cutting of sheets. C) Size adjustment (6 x 30 cm). D) Production of ventilation holes with punch with a diameter of 1 cm. E) Finished sheet with ventilation holes. F) Cylinder for placing the newly transferred brood inside the cylinder. Source: Developed by the authors. Photo: H.R. Salazar-Vargas.

Figure 2: Processing of the coating for transferred brood combs using an expandable polystyrene container



A) View of the bottom of the expandable polystyrene container B) Plotting measurements on the container. C) Cut with scissors from the lower part of the container. D) The lower part of the container is removed. E) Production of ventilation holes with punch with a diameter of 1 cm. F) Finished coating with ventilation holes. G) Transferred brood honeycombs lined with expandable polystyrene (EPSFCTBC). Source: Developed by the authors. Photo: H.R. Salazar-Vargas.

Table 1 shows the 12 treatments (three replicates each) resulting from the combinations of three factors (box model, construction material of the boxes, nest-coating materials) and their respective levels. In order to facilitate the identification of the combination, a key was assigned to each combination.

During 41 d prior to the start of the experiment, periodic revisions were made where all colonies were fed with honey and pollen and control measures were taken: cleaning of the box and use of internal traps with the use of attractant (5% acetic acid), in colonies that were positive for vinegar flies (*Pseudohypocera spp.*). When the colonies showed similar behavior, they were placed in the Meliponarium of the Permaculture Area of the Córdoba-CP Campus.

These were randomly distributed in three blocks of 12 colonies with each of the combinations, the first block was placed at a distance of 2 m from the floor; the second, at a distance of 1.5 m, and the third, at a distance of 1 m from the floor. Nest entrances were oriented to the north. In order to evaluate the interaction of box pattern, box construction material, and nest coating material on internal temperature, a digital thermometer sensor was placed on the last brood disk of each colony (VA-DT-1H Avaly®). For 12 wk, the inner nest temperature and ambient temperature were recorded every 4 h. Temperature data were collected during the day at 0900 h, 1300 h, and 1700 h, while during the night, the times were 2100 h, 0100 h and 0500 h. In order to estimate the weight increase of the daughter colony, its initial and final weights were quantified. A portable scale (EQB-100 Torrey®) was used for this purpose. In addition, the number of brood cells was calculated, indirectly, by measuring the height and diameter of the nest, relating it to the estimated number of cells per square centimeter according to a previous count, and the number of total pots was visually counted[5].

Results

During the study period, the average daytime ambient temperature was 27.3 °C (range= 26.7 to 28.4 °C) and the average nighttime ambient temperature was 21.2 °C (range= 20.8 to 22.4 °C). The interaction between box model, box construction material and nest coating material for daytime internal nest temperature (DINT) shows significant difference (P<0.05), but not for the nighttime internal nest temperature (NINT) (P>0.05). For the DINT between treatments, the highest adjusted mean corresponds to T11 with an adjusted mean of 31.5 °C (Table 2), and the lowest, to T4. The treatments with the highest adjusted mean for the variable TINN were T1, T5 and T11, and the lowest was T4. No significant differences (P>0.05) were observed between the control treatment T1 and T5. However, were significant differences (P>0.05) were found between treatments T7 and T11.

Table 2: Adjusted means (±SD) for the variables DINT, NINT and NIT of the nest of *S. mexicana*

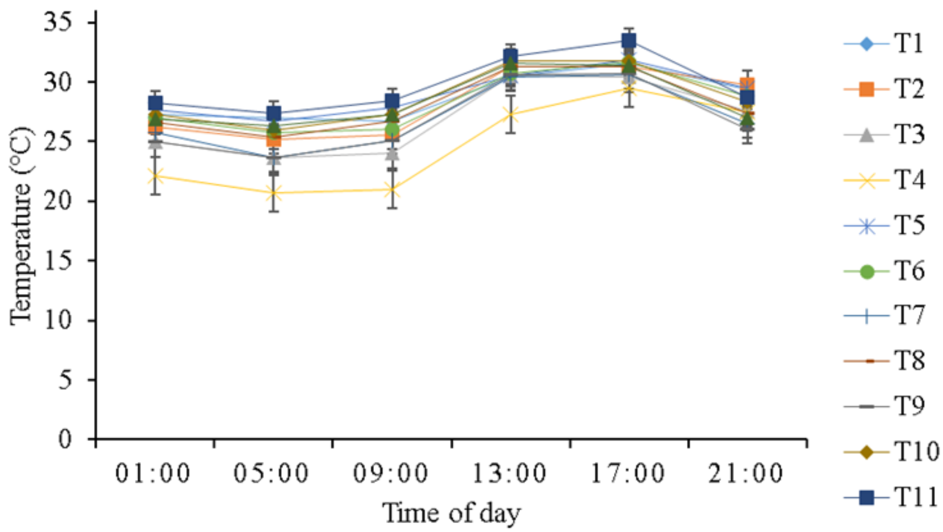
Treatment	Nest inner temperature (°C)		
	In the daytime (DINT)	**At night (NINT)**	**Mean (NIT)**
T_1	29.598 ± 0.715 abc	27.896 ± 1.012 a	28.745 ± 0.837 ab
T_2	29.391 ± 0.726 abc	27.058 ± 1.03 ab	28.222 ± 0.851 ab
T_3	28.361 ± 0.714 c	25.386 ± 1.011 ab	26.872 ± 0.836 bc
T_4	25.929 ± 1.024 d	23.395 ± 1.504 b	24.655 ± 1.223 c
T_5	30.132 ± 0.69 abc	27.973 ± 0.972 a	29.052 ± 0.807 ab
T_6	29.497 ± 0.697 abc	27.178 ± 0.984 ab	28.339 ± 0.815 ab
T_7	28.736 ± 0.69 bc	25.323 ± 0.971 ab	27.029 ± 0.806 bc

T_8	29.771 ± 0.741 abc	26.484 ± 1.054 ab	28.13 ± 0.87 ab
T_9	28.811 ± 0.73 bc	24.895 ± 1.037 ab	26.855 ± 0.856 bc
T_{10}	30.292 ± 0.698 ab	27.198 ± 0.985 ab	28.746 ± 0.816 ab
T_{11}	31.358 ± 0.761 a	28.137 ± 1.088 a	29.75 ± 0.896 a
T_{12}	30.148 ± 0.772 abc	26.741 ± 1.106 ab	28.448 ± 0.91ab

[abcd] Different letters indicate significant difference between treatments ($P<0.05$).

The graph of the adjusted mean NIT of the treatments over a 24 h period (Figure 3) shows similar oscillations between treatments. The highest mean oscillations correspond to T11 and T5, and the lowest, to T4.

Figure 3: Adjusted mean temperature (±SD) of *S. mexicana* nests



T1(AFM-BMW-UTBC), T2(AFM- BMW-TBCCAMBW), T3(AFM- BMW- EPSFCTBC), T4(AFM- BMW +PS- UTBC), T5(AFM BMW +PS- TBCCAMBW), T6(AFM- BMW +PS- EPSFCTBC), T7(PAM- BMW-UTBC), T8(PAM- BMW-TBCCAMBW), T9(PAM- BMW- EPSFCTBC), T10(PAM- BMW +PS- UTBC), T11(PAM- BMW +PS- TBCCAMBW), and T12 (PAM- BMW +PS- EPSFCTBC), during 24 h.

Table 3 shows the results of the adjusted means for the final colony weight (FCW), colony weight gain (CWG), as well as the number of built breeding cells (NBBC) and the total number of built pots (TNBP). Significant differences were observed for the variables analyzed: FCW (P=0.0224), CWG (P=0.0224), NBBC (P=0.0036), but not for TNBC (P=0.1509).

Table 3: Adjusted means (±SD) of the ICW, FCW, and CWG of *S. mexicana* nests after 12 weeks of the experiment

Treatments	Weight of the colony (kg)		
	Initial weight (ICW)	**Final weight (FCW)**	**Weight gain (CWG)**
T_1	0.2021± 0.0293	0.308 ± 0.0515 abc	0.1499 ± 0.0515 abc
T_2	0.2112 ± 0.0293	0.294 ± 0.0524 abc	0.1358 ± 0.0524 abc
T_3	0.2015 ± 0.0293	0.368 ± 0.0514 ab	0.2099 ± 0.0514 ab
T_4	0.3352 ± 0.0293	0.17 ± 0.0768 c	0.0113 ± 0.0768 c
T_5	0.1653 ± 0.0293	0.308 ± 0.0495 abc	0.1491 ± 0.0495 abc
T_6	0.1344 ± 0.0293	0.307 ± 0.05 abc	0.1481 ± 0.05 abc
T_7	0.1594 ± 0.0293	0.188 ± 0.0494 c	0.0295 ± 0.0494 c
T_8	0.0953 ± 0.0293	0.233 ± 0.0537 bc	0.0748 ± 0.0537 bc
T_9	0.1026 ± 0.0293	0.195 ± 0.0528 c	0.0369 ± 0.0528 c
T_{10}	0.1333 ± 0.0293	0.254 ± 0.0501 bc	0.0953 ± 0.0501 bc
T_{11}	0.083 ± 0.0293	0.448 ± 0.0554 a	0.2893 ± 0.0554 a
T_{12}	0.077 ± 0.0293	0.258 ± 0.0563 bc	0.0991 ± 0.0563 bc

[abc] Different letters indicate significant difference between treatments (P<0.05).

Comparisons between treatments revealed that for the FCW and CWG variables, T3 (AFM-BMW-EPSFCTBC) and T11 were the highest (Table 3), and T4 (AFM-BMW- EPSFCTBC) were the lowest. With respect to the NBBC variables, the highest values correspond to T5 and T11, and for TNBP, the highest values correspond to treatments T4, T5 and T11 (Table 4). There is a significant correlation between BIT and NBBC ($P<0$.0001), between ICW and TNBP ($P<0$.0001), and between FCW with NBBC ($P<0$.0001) and TNBP ($P<0$.0001). T3 and T11 had higher FCW and CWG compared to the other treatments (Figure 3).

Table 4: Adjusted means (±SD) of NBBC and TNBP of *S. mexicana* after 12 weeks of the experiment

Treatments	Number of built breeding cells (NBBC)	Total number of built pots (TNBP)
T_1	2372.1 ± 819.66 bc	32.801 ± 8.6603 abc
T_2	3029.8 ± 829.78 abc	26.189 ± 8.7897 bc
T_3	2158.8 ± 819.01 bcd	18.558 ± 8.6521 c
T_4	-496.44 ± 1110.6 d	36.3 ± 12.286 abc
T_5	3511.5 ± 797.54 ab	43.688 ± 8.3764 ab
T_6	1950.6 ± 803.82 bcd	31.724 ± 8.4572 abc
T_7	1293.7 ± 796.98 cd	21.184 ± 8.3692 c
T_8	2189.1 ± 843.39 bcd	31.932 ± 8.9632 abc
T_9	1245.2 ± 833.41 cd	25.555 ± 8.8359 bc
T_{10}	2739.2 ± 804.48 bc	30.551 ± 8.4657 bc
T_{11}	4956.8 ± 862.5 a	52.006 ± 9.2057 a
T_{12}	1784.7 ± 872.76 bcd	32.846 ± 9.3355 abc

[abcd] Different letters indicate significant difference between treatments (P<0.05).

Discussion

In this study, the best treatments providing average nest inner temperature (NIT), DINT and NINT suitable for the development of *S. mexicana* broods were the following treatments: T5 (AFM- BMW+PS- TBCCAMBW) and T11 (PAM-BMW+PS- TBCCAMBW). This is partly explained by the positive interactions resulting from adding a material with thermal insulating properties (expandable polystyrene) in the construction of rational boxes(28), and coating the newly transferred brood combs with a material that mimics the function of the involucrum, such as *Apis mellifera L*. beeswax. The interactions of these combinations had a positive impact on the inner temperature of the nest, which in turn influenced the development of the colony by improving the following aspects: NBBC, TNBP, FCW, and CWG. As in the case of *Melipona subnitida*(29), which was provided with the most favorable conditions, such as high temperature and food, which led to an increase in brood cells. It has also been observed that *Nannotrigona testaceicornis*, housed in artificially heated boxes during the winter period, maintains brood production and reduces the thermoregulatory activities of the bees during the day or night(30).

When comparing the results obtained in the nest temperatures (DINT, NINT, NIT) in treatments T5 (29.052 ± 0.807) and T11 (29.75 ± 0.896), although there were no significant differences (P>0.05) between these two treatments, T11 showed the best results in terms of nest inner temperature and colony development expressed in a higher number of brood cells

(4,956 ± 862) and storage pots (52 ± 9). These results confirm that internal nest thermoregulation is important for social insects, since the development and survival of the colony depends on it[8,9]. The temperature range attained (28.137 to 31.35 °C) with this treatment was attributed to the fact that the PAM rational box was designed from the beginning for *S. mexicana* and has the appropriate volume (4.3 L) for the species. The interaction between the internal volume of the PAM, the material with thermal insulating properties, and the protection of the brood using a wax mold of *Apis mellifera L.* render this treatment one of the best for breeding *S. mexicana*. The temperature range for good brood development in native bees is 31 to 32.3 °C[11], and the optimal temperature is 35 °C[10]. The nest temperature result obtained with this treatment lies within the range that has been calculated as adequate for the development of this species.

Including a material with insulating properties (expandable polystyrene foam) in the rational box models (AFM, PAM) and covering the transferred brood combs is crucial for providing an adequate temperature range for the development of the small-sized colony obtained by artificial division. The interaction between these two factors plays an important role in nest thermoregulation. This is clearly verified in the results obtained in treatments T7 (27.029 ± 0.806 °C) and T11 (29.75 ± 0.896 °C); where the rational box model PAM was the same for both treatments. The difference is that, in T7, the rational box does not include a material with insulating properties in its design, nor is the brood covered with any type of material. These differences in the treatments resulted in significant differences in the internal nest temperature (P<0.05). Other stingless bee species respond in a similar way, as is the case of *Melipona colimana*, which maintains a homogeneous temperature regardless of the ambient temperature, if the conditions inside the box are favorable[31].

In the case of the AFM rational box, which was designed for the species *T. angustula* and *N. testaceicornis*[24], its dimensions were modified in order to adapt it to the behavior of *S. mexicana*. Its final volume due to the modifications was 5.3 L, slightly higher than that of the PAM box. Due to its larger volume, the results showed that the modified AFM works adequately to house *S. mexicana*, but the transferred brood combs must be coated with an *A. mellifera L.* beeswax mold. The modified, all-wood, AFM rational box also provided adequate nest temperature when the brood combs were coated with beeswax.

The treatment with the lowest nest NIT was treatment T4 (AFM- BMW+PS- UTBC). The NINT was 23.395 ± 1.504 and had a negative effect on the NBBC (-496.44 ± 1110.6). An inadequate internal temperature range causes the bees to spend most of their energies on building insulation structures, and less time collecting nectar and pollen that are essential for colony development and survival[11,32]. In the case of *Scaptotrigona depilis*, temperatures between 26 and 34 °C do not affect brood survival; however, temperatures below 22 °C or above 38 °C cause brood mortality; therefore, it is very likely that small colonies will be lost under these conditions[14].

It is important to note that the Ailton-Fontana Model (AFM) was designed for *T. angustula* and *N. testaceicornis* (small-sized species), although in this study the model was modified by adding expandable polystyrene foam sheets to its original design. However, this action was not sufficient to provide an optimal temperature for the development of small-sized colonies of *S. mexicana* obtained by artificial division. Due to the larger volume size (5.3 L) of the AFM box, in the case of *S. mexicana* the brood combs must be coated with beeswax in order to obtain better results, as was observed in the T5 treatment (AFM- BMW+PS-TBCCAMBW) where one of the best NITs was achieved.

Colonies housed in the AMF modified with expandable polystyrene foam sheets and without comb coatings had the lowest values for the following variables: final brood weight, brood weight gain, and number of built breeding cells. This is attributed to the relationship that these variables have with nest thermoregulation. As for the total number of pots, this variable was similar to the rest of the treatments; this is explained by the fact that, during the study period (spring-summer), the food supply is not affected because the nectar flow is abundant and is favored by the ambient temperature during the day. In other studies with *Trigona carbonaria*, it was observed that, for these colonies, low temperatures in the nest produced a good weight gain; due to the excessive construction of involucrum or batumen; however, there was little brood development(33).

The results showed that the AMF rational box constructed exclusively of 3.74 cm (2.54 cm + 1.2 cm) wood provides adequate temperature ranges (27.896 to 29.598 °C) for the brood housed inside it. In the case of *M. colimana*, internal temperature control is better with greater wall thickness within the box(22). The combination of using the AMF and coating the combs with the *Apis mellifera L.* wax mold further improves the internal temperature range of the nest.

The combinations of treatments T3, T6, T9 and T12 are not recommended because the bees reject the covering of the brood combs with expandable polystyrene foam molds: at the end of the study period, bites were observed on this material, which the bees were trying to manipulate in order to carry out the passive thermoregulation of the nest.

Conclusions and implications

The modified Portugal Araujo model box (PAM) provides a suitable temperature range (25,323-28,736°C) for the development of small-sized colonies of *S. mexicana* obtained by artificial division. However, colony development and temperature range is slightly improved when the brood combs are coated with *Apis mellifera L.* beeswax. The modified PAM brood box built with wooden walls and expandable polystyrene foam sheets provided a better temperature range (27.198-30.292 °C). The Ailton-Fontana model box (AFM), although not designed for breeding *S. mexicana*, showed that the modified model provides adequate temperature ranges (27.896-29.598 °C) for the development of this species. This temperature range was improved when the AFM box was modified by adding polystyrene foam sheets to its design and coating the brood combs with European beeswax (T5). The use of modified AFM rational brood boxes with expandable polystyrene is not recommended unless a coating of *Apis mellifera L.* beeswax is applied to the newly transferred brood combs, because their volume makes it difficult for the bees to maintain an adequate temperature range for their development.

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