Sociobiology
An international journal on social insects

RESEARCH ARTICLE - BEES

Colony Transport Affects the Expression of Some Genes Related to the *Apis mellifera* L. Immune System

Maurice F. Scaloppi[2], Samir M. Kadri[2], Daniel D. Mendes[2], Paulo Eduardo M. Ribolla[3], Ricardo O. Orsi[1]

1- Departamento de Produção Animal e Medicina Veterinária Preventiva, Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista, Botucatu-SP, Brazil

2- Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista – UNESP, Botucatu-SP, Brazil

3- Instituto de Biotecnologia, Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista – UNESP, Botucatu-SP, Brazil

Article History

Edited by
Evandro Nascimento Silva, UEFS, Brazil
Received 18 October 2021
Initial acceptance 12 October 2022
Final acceptance 03 December 2022
Publication date 28 December 2022

Keywords
Beekeeping, Colony transportation, Stress, Transportation Management, Welfare.

Corresponding author
Ricardo de Oliveira Orsi
Departamento de Produção Animal e Medicina Veterinária Preventiva
Faculdade de Medicina Veterinária e Zootecnia da UNESP, Campus de Botucatu
Rua Prof. Dr. Walter Maurício Correa, s/nº
CEP: 18618-681 - Botucatu, São Paulo, Brasil.
E-Mail: ricardo.orsi@unesp.br

Abstract

Migratory beekeeping can harm the bee colonies if not executed properly. Here, colonies of *Apis mellifera* were transported (for one or two hours) or not, following proper technical standards. To analyze gene expression (defensin-1, abaecin, and HSP70), forager bees were collected immediately, 24, and 72 hours after transportation. Bee mortality and population growth were measured before and after transportation. This study concludes that transporting honey bee colonies for 2 hours promotes immune system gene expression, although there are no significant changes in bee mortality and population growth of the colonies.

Introduction

Honey bees (*Apis mellifera* L.) (Hymenoptera: Apidae) have great importance for crop pollination (Potts et al., 2016), and beekeeping allows the production of several bee products and provides pollination service to many crops (Maderson & Wynne-Jones, 2016). Beekeeping can be divided into two main types: stationary and migratory. The first one consists of keeping the apiary permanently in the same place. Thus, colony production is limited by the supply of resources (nectar, pollen, resin, and water) determined by the local floristic potential (Crane, 1999). The second modality is based on the displacement of colonies to specific places where blossoms are distributed throughout the year to maximize honey production and the pollination of crops (Whynott, 1991).

However, colony transportation in beekeeping can be a stressor for the colonies, along with other factors such as pests, diseases, poor diet, and pesticide exposure (Even et al., 2012; Goulson et al., 2015). Colonies that undergo migratory beekeeping for commercial pollination in the summer showed a reduced capacity to perform thermoregulation during winter (Glenny et al., 2017). Colony transportation can also affect hypopharyngeal gland development, impairing the ability of nurse bees to feed future generations (Ahn et al., 2012). Moreover, it can reduce worker bees' life span, leading to increased levels of oxidative stress (Simone-Finstrom et al., 2016) and the prevalence and abundance of pests and diseases (Zhu et al., 2014; Alger et al., 2018).

Nevertheless, some procedures aim to minimize the stressful effects of colony transportation, such as the use of screened lids to promote ventilation; closing the hive

entrance at dusk, when the entire population is inside the hive, avoiding its exit; and performing the transportation during the night, with milder temperatures, preventing the colony from overheating.

Meanwhile, studies showing the impact of colony transportation on bees individually or at the colony level still need to be made available. A possible effect of this management may occur on the bees' immune system, affecting their ability to combat pathogens and pests (Mason et al., 2013). The bees' immune system is composed mainly of innate systems. The immune response against pathogenic organisms can be divided into two mechanisms that work together: cellular and humoral responses (Nation, 2015; Iwasaki & Medzhitov, 2015). The humoral response consists of a battery of antimicrobial peptides that are synthesized in the presence of pathogenic organisms or situations of stress (Yang & Cox-Foster, 2005; Antúnez et al., 2009).

Honey bees have six antimicrobial peptides, including defensin-1 and abaecin. Defensin-1 is a cysteine-rich cationic peptide, is the primary defense system of many organisms. It acts mainly against Gram-positive bacteria, permeating its cytoplasmic membrane, and is up-regulated upon bacterial infection (Evans, 2004; Randolt et al., 2008). In turn, abaecin is a proline-rich peptide and has a broad spectrum of action against bacteria (Casteels et al., 1990; Lourenço et al., 2018). Its expression is up-regulated very quickly in response to bacterial infection (Richard et al., 2012; Aronstein & Saldivar, 2005).

In addition to antimicrobial peptides, other proteins act in stressful situations. Hexamerins are highly conserved molecules that act as molecular chaperones in biotic or abiotic stresses, such as overheating, the presence of toxic compounds and pathogens (Sahebzadeh & Lau, 2017). Constituted by proteins of different molecular masses, including HSP70, they perform functions in cell differentiation and regulation and embryonic development (Elsik et al., 2014).

Stress caused by colony transportation can have effects both on antimicrobial peptides and proteins that compose the bees' immune system and also on bee mortality and the colony's population growth. Therefore, this work aims to evaluate the effects of honeybee colony transportation for different time lengths on immune system gene expression and bee mortality, and population growth of the colonies.

Material and Methods

Colony preparation and transportation

Twenty-one honey bee colonies kept in standard Langstroth nuclei were used, standardized in the number of brood and food frames. The colonies were submitted to three treatments of 7 colonies each: Treatment 1 (T0), control, where the colonies were not transported; Treatment 2 (T50), where the colonies were transported for 1 hour, which corresponded to about 50 km; Treatment 3 (T100), where the colonies were transported for 2 hours, corresponding to approximately 100 km of distance.

The colonies were transported during the night, once, on May 25th, 2018. For transportation, a screened transport lid was placed, allowing the hive to be ventilated. The entrance was closed with foam at the time of the transportation and the colonies were placed side by side in the back of a truck. The colonies left an apiary located at the geographical coordinates 22°49'14.9" S and 48°23'23.8" W at an average altitude of 502 m; circulated by local roads for the specified time lengths, and arrived at an apiary located at the geographical coordinates 22º50'28" S and 48º25'42" W and at an average altitude of 730 m, where the control colonies already were. The distance between the two sites was approximately 4.6 kilometers. The colonies were placed on individual stands, the entrances opened, and the transport lids were replaced by conventional lids. The region's climate is characterized as Cfa by the Köppen classification (da Cunha & Martins, 2009).

Gene expression analysis

For gene expression analysis, before transportation, a frame containing capped brood was removed from the colonies of each treatment, placed in a screened tissue, returned to the original colony, and kept inside the hive for 24 hours. Then, approximately 100 newly emerged bees were marked on the dorsal part of the thorax (pronotum) with a non-toxic marker and reintroduced into their original colonies. After 22 days, the colonies were transported in their determined time lengths. At 0, 24, and 72 hours after transportation, 10 marked bees were collected from each colony to evaluate the effects of transportation as foragers. The same protocol was used for the control colonies, and the bees were collected in the same periods described above.

The collected bees were immediately anesthetized on ice and subsequently frozen at -80 ºC. Total RNA was extracted from a pool of 5 heads (Schlüns & Crozier, 2007) following the methodology proposed by Scharlaken and collaborators (2008) using 500 µl of TRIzol® Reagent (Life Technologies, Carlsbad, CA), according to the manufacturer's instructions. The investigated genes were defensin-1, abaecin, and HSP70. The oligonucleotides sequences and details are described in Table 1. The extraction product was visualized on a 1% agarose gel and quantified using a NanoDrop device (Spectrophotometer ND-1000). Then, cDNA synthesis was prepared with SuperScript® III Reverse Transcriptase kit (Invitrogen, Carlsbad, CA), according to the manufacturer's protocol.

Gene expression determination was performed by real-time polymerase chain reaction (RT-PCR) in triplicates, using the actin gene as an endogenous control (Scharlaken et al., 2008). Negative control was used for each reaction, consisting of a mixture of reagents and water. They were performed on ABI 7500 FAST device (Applied Biosystems, Foster City, CA) using a SYBR® Green PCR Master Mix kit (Applied

Table 1. Oligonucleotides initiating immune system genes (defensin-1, abaecin, and HSP70) and constituent (actin) of honeybees submitted to different transportation time lengths.

Gene	Number Gene Bank	Primer sequence 5'-3'
Actin	AB023025	TGCCAACACTGTCCTTTCTG AGAATTGACCCACCAATCCA
Defensin-1	U15955	CTGCACCTGTTGAGGATGAA GCGCAAGCACTGTCATTAAC
Abaecin	AF442147	CAGCATTCGCATACGTACCA GACCAGGAAACGTTGGAAA
HSP70	Martins et al., 2008	CAAAGTTGTAAGCGACGGCGGAA TGTCTCCGGCTGTGGAGCGCA

Biosystems, Foster City, CA) under the following conditions: one cycle at 50 ºC for 2 minutes; one cycle at 94 ºC for 10 minutes, followed by 40 cycles of 94 ºC for 15 seconds and 60 ºC for 1 minute. The dissociation curve was obtained as follows: 95 °C for 15 seconds, 60 °C for 30 seconds, and 95 °C for 15 seconds.

Oligonucleotides efficiency (E) was calculated using a standard curve from four dilutions of the cDNA samples: 1: 5, 1:25, 1:125, and 1:625, using the formula E = 10 (-1/slope). Genes relative quantification (R) was determined by the ratio of the expression of the target gene to the endogenous gene, in which the CP (crossing point) is defined as the point at which the detected fluorescence is appreciably above the background fluorescence, according to the equation below (Pfaffl, 2001):

$$R = \frac{E_{target}^{\Delta CPtarget}}{E_{ref}^{\Delta CPref}}$$

Etarget being the efficiency of the target gene; Eref the efficiency of the reference gene; ΔCPtarget is the CP of the target gene of the control treatment - CP of the target gene of the transported treatments; ΔCPref is the CP of the control treatment - CP of the reference gene of the transported treatments.

Bee mortality evaluation

To assess bee mortality after transportation, under basket-dead bee boxes (Accorti et al., 1991) were installed in all experimental colonies. The boxes were positioned immediately after transportation and maintained for 7 days, with bee mortality being assessed daily and expressed in the number of dead bees per box.

Population growth evaluation

For population growth assessment, open and closed broods of the central frame of each experimental colony were evaluated. The evaluation was carried out one week before transport, one day before transport, and four weeks after transport, according to the methodology adapted from Al-Tikrity and collaborators (1971). The pictures were positioned in a structure in which its sides are composed of a 2 x 2 cm grid. In this way, the two sides of the selected frame were photographed, and the areas (cm²) were measured using CorelDRAW® X8 (Corel, Ottawa, ON). For the proposed assessment, the results were grouped into two periods: before and after transportation.

Statistical analysis

The data obtained for population development and gene expression were first tested for normality (Anderson-Darling test) and homogeneous variances (Levene's test). When significant deviations ($p < 0.05$) from these assumptions were detected, the data were compared using the non-parametric Mann-Whitney test, and the median and interquartile intervals (Q1_Q3) were presented. When no significant deviations from normality or homoscedasticity were detected, the data were analyzed with one-way ANOVA, and the mean ± standard deviation values were presented. P-values below 0.05 were considered significant. All statistical analyses were performed using the statistical software Minitab.

Results

The results of the relative expression analysis of defensin-1, abaecin, and HSP70 genes are shown in Graphics 1A, 1B, and 1C, respectively. For the defensin-1 gene, up-regulation was observed in treatment T50 right after transport, with a significant difference compared to the control and treatment T100. Then, T50 showed down-regulation 24 hours after transport, returning to expression values similar to the beginning 72 hours after. For treatment T100, down-regulation was observed 0, 24, and 72 hours after, with a significant difference, compared to the period immediately after transport. Down-regulation observed 72 hours after transport in T100 also showed a significant difference compared to the treatment T50 in the same period (Figure 1A).

Regarding abaecin gene, treatment T50 presents up-regulation immediately after transport, with a significant difference compared to the control. Then, there is down-regulation 24 hours after transport, with a significant difference concerning the 0 hour period. Finally, 72 hours after transport, there is a new up-regulation with a difference between the T100 treatment for the same period. In treatment T100, up-regulation is also observed right after transport, with a significant difference in relation to the control and the period 72 hours after, which shows down-regulation (Figure 1B).

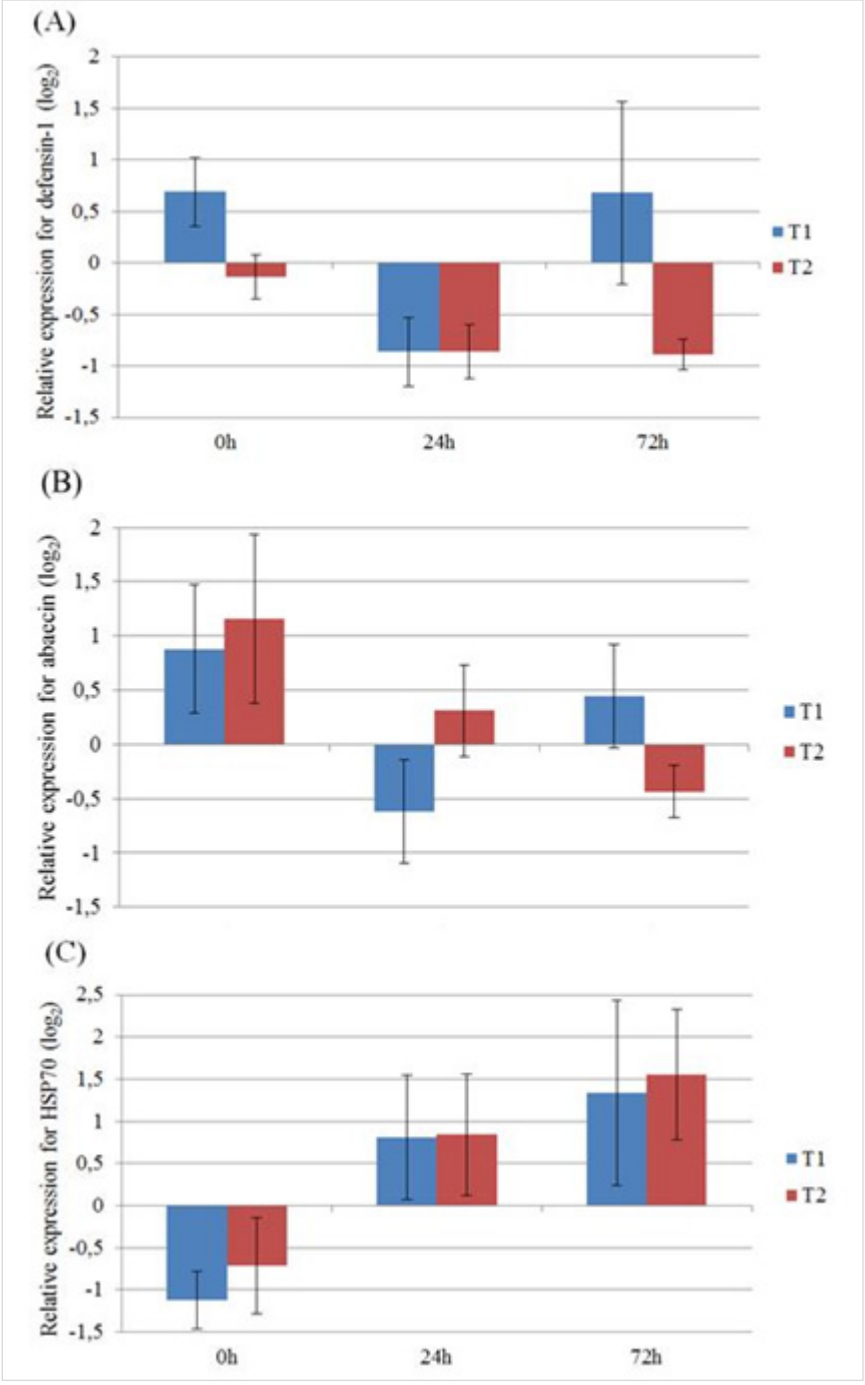

Fig 1. Relative expression of defensin-1 (A), abaecin (B) and HSP70 (C) genes in honey bees submitted to transport for 1 hour (T50) and 2 hours (T100) and collected at 0, 24 and 72 hours after. *represents difference in relation to the control; distinct letters represent differences between the means: upper case between the treatment T50; lower case between treatment T100.

For the HSP70 gene, treatment T50 is down-regulated right after transport, with a significant difference concerning the control and the periods 24 and 72 hours after, which have up-regulation. In treatment T100, down-regulation is also observed right after transport, but with a significant difference only 72 hours after. In both treatments where transportation occurred, up-regulation observed 72 hours afterward presents a significant difference compared to the control (Figure 1C).

The results of bee mortality are shown in figure 2. There was no significant difference between treatments T50 and T100 compared to the control ($p = 0.6149$). The average mortality was obtained during seven evaluation days, observing a similar pattern between treatments.

The results of population growth are shown in figure 3. For the closed brood area, a reduction in all treatments is observed, being significant only for the control ($p = 0.0809$ and $p = 0.3938$, for T50 and T100, respectively). Evaluating

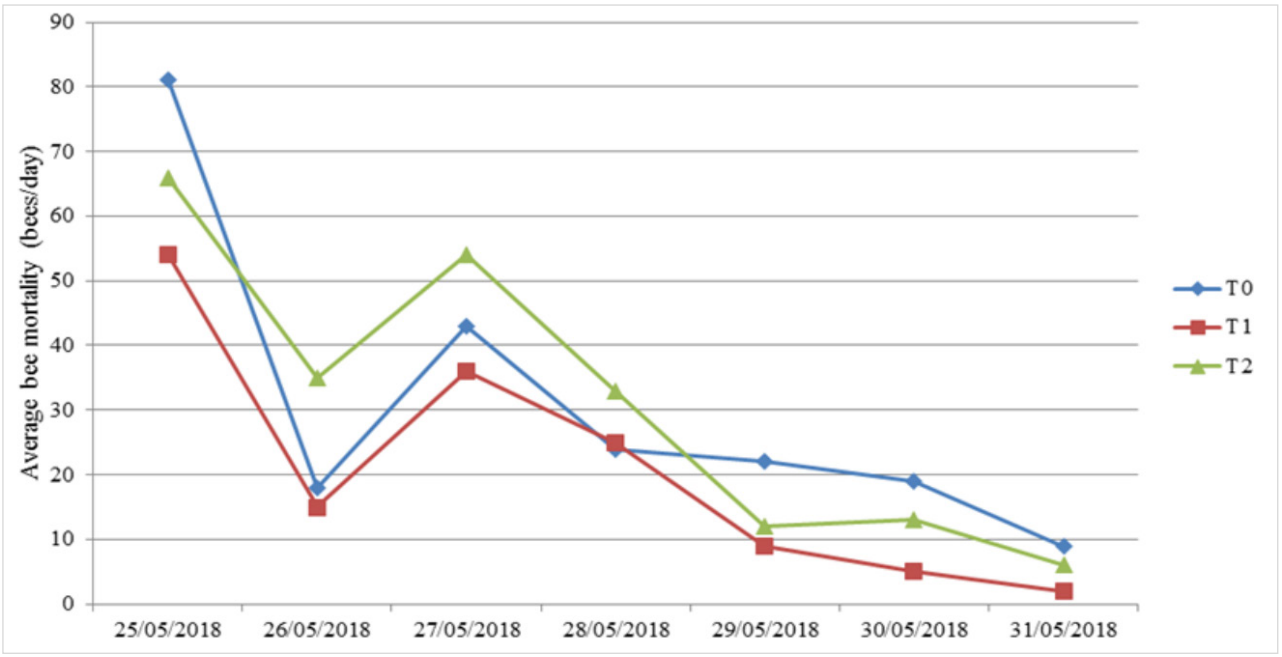

Fig 2. Average daily mortality of honey bees not transported (T0) and submitted to transport for 1 hour (T50) and 2 hours (T100).

the treatments among themselves in each of the bee collection periods, control colonies presented a closed brood area larger than treatment T100 before transport. After transport, the areas were not statistically different in any of the treatments ($p = 0.4325$).

Open brood areas did not show significant differences in any of the treatments before and after transport ($p = 0.7634$; $p = 0.8999$; $p = 0.6009$, for T0, T50, and T100, respectively). There was also no significant difference between these areas before ($p = 0.1088$) and after transport ($p = 0.3181$).

As for the food areas (nectar, honey, and pollen), there was an increase in all treatments after transport, but there was no significant difference between treatments ($p = 0.3195$, $p = 0.0733$, before and after transport, respectively). However, there was a significant difference in the evaluation of treatments between T0 and T50 ($p = 0.1671$ for T2).

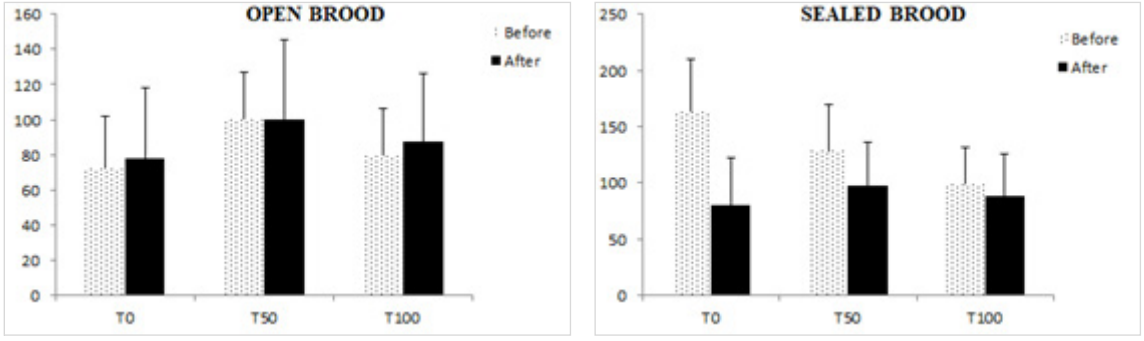

Fig 3. Open brood and Sealed brood (cm²) before and after transport for not transported (T0) and submitted to transport for 1 hour (T50) and 2 hours (T100).

Discussion

The results obtained in the experimental conditions of this work show that colony transportation promotes defensin-1, abaecin, and HSP70 gene modulation. Relative gene expression analysis of the antimicrobial peptides defensin-1 and abaecin are valid indicators of effects on the bees' immune system (Siede et al., 2012). Colonies transported for 1 hour showed similar patterns in the expression of these two genes, with up-regulation right after transport, followed by down-regulation after 24 hours, and up-expression again 72 hours later, at a level close to the one observed in the collection made shortly after transportation. For colonies transported for two hours, the defensin-1 gene is down-regulated in all evaluated periods and the abaecin gene 72 hours after transport.

These results suggest that there is a suppressive effect on the bees' immune system at the time lengths considered for

transport, with the 24 hours being the most vulnerable for colonies transported by one hour, where there is an immunosuppressive effect for both genes (Casteels et al., 1990; Lourenço et al., 2018). For colonies transported for two hours, this occurs more intensely 72 hours later, when there is down-regulation for the two antimicrobial peptides. However, this is also observed for defensin-1 24 hours after transport. Although colony transport can be considered acute stress by lasting less than four hours (Even et al., 2012), its effects lasted for at least 72 hours.

Thus, transport in both treatments caused up-regulation in antimicrobial peptide genes defensin-1 and abaecin, as well as down-regulation, indicating an immunosuppressive effect. Immune system suppression can occur due to several factors, such as pesticides (Mason et al., 2013; Sánchez-Bayo et al., 2016), pests (Navajas et al., 2008; Chaimanee et al., 2012), and mechanical injuries (Koleoglu et al., 2017). In turn, immunosuppression can promote several effects, such as greater susceptibility to diseases and pests (Nazzi & Pennacchio, 2014); down-regulation of vitellogenin and juvenile hormone genes, both related to labor differentiation of worker bees (Bordier et al., 2017); and also, impairment of foraging activity, affecting pollen collection and consequently the colony's nutritional balance (Bordier et al., 2018).

For the HSP70 gene, up-regulation was observed 24 and 72 hours after transport when performed for one and two hours, respectively. Hexamerins are considered important storage proteins, acting as molecular chaperones in thermal stress situations, assisting in refolding proteins that denature with temperature increases and preventing their erroneous binding to other compounds (Sahebzadeh & Lau, 2017). Thus, transport may have caused the expression of this gene related to the physiological regulation of body temperature variations. Also, the period considered in the experiment was not enough to verify the necessary time to restore normal levels of HSP70 for the transported treatments.

Bee mortality and population growth analysis did not change in transported colonies compared to control colonies. For bee mortality, this result indicates that the method used to prepare the colonies for transportation minimizes a stressful effect, which is the death of bees and the consequent population decrease. Finally, for population growth, despite a decrease in the control and T50 colonies for closed brood areas, there was no change in the colonies of the T100 treatment. This may also indicate a positive effect of the colony preparation method. However, as migratory beekeeping aims to move colonies to areas with a high abundance of floral resources, the stressful effect of this management can be masked by the increase in food availability (Zhu et al., 2014). This can be seen by the increase in the area of stored food for both control and T50 treatments since the colonies were transported to the same apiary where the control colonies were placed.

Colony transportation for both pollination and honey production is a common practice in beekeeping worldwide. Nevertheless, different transportation protocols are used by beekeepers to make it a fast and practical operation, only sometimes prioritizing the well-being of bees, which could promote stress in the colonies. For instance, transporting colonies during the day, when temperatures are higher, can promote an increase in the colony's internal temperature, in addition to the loss of worker bees in the foraging stage if the colonies are not closed at night. Likewise, transporting colonies with closed entrances helps to reduce the colonies' population loss and, consequently, their population growth. In this work, colony transportation was carried out to minimize possible stressful effects on the colonies, using a screened lid for ventilation, closed entrance, and transport at night. Thus, it can be inferred that when colonies are transported in an attempt to maintain the minimum conditions of well-being for the bees, the losses of this management can be reduced. However, some physiological changes can be observed.

Conclusion

Colony transportation for up to two hours promotes modulation of immune system gene expression, although there are no significant changes in bee mortality and population growth in the colonies.

Authors' Contribution

MFS: Conceptualization, methodology, investigation and formal analysis.
SMK: Conceptualization, methodology, investigation, formal analysis and writing & editing.
DDM: Conceptualization, methodology and formal analysis.
PEMR: Investigation and writing & editing.
ROO: Conceptualization, methodology, investigation, formal analysis and writing & editing.

Funding

This work was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES), Financial Code 001.

References

Accorti, M., Luti, F. & Tarducci, F. (1991). Methods for collecting data on natural mortality in bees. Ethology Ecology and Evolution, 3: 123-126. doi: 10.1080/03949370.1991.10721924

Ahn, K., Xie, X., Riddle, J., Pettis, J. & Huang, Z.Y. (2012). Effects of long distance transportation on honey bee physiology. Psyche: A Journal of Entomology. Special Issue. 1-10. doi: 10.1155/2012/193029

Alger, S.A., Burnham, P.A., Lamas, Z.S., Brody, A.K. & Richardson, L.L. (2018). Homesick: impacts of migratory beekeeping on honey bee (*Apis mellifera*) pests, pathogens, and colony size. PeerJ, 6: e5812. doi: 10.7717/peerj.5812

Al-Tikrity W.S., Hillmann R.C., Benton A.W. & Clarke W.W. (1971). A new instrument for brood measurement in a honeybee colony. American Bee Journal, 111: 20-26.

Antúnez, K., Martín-Hernández, R., Prieto, L., Meana, A., Zunino, P. & Higes, M. (2009). Immune suppression in the honey bee (*Apis mellifera*) following infection by *Nosema ceranae* (Microsporidia). Environmental Microbiology, 11: 2284-2290. doi: 10.1111/j.1462-2920.2009.01953.x

Aronstein, K. & Saldivar, E. (2005). Characterization of a honey bee Toll related receptor gene Am18w and its potential involvement in antimicrobial immune defense. Apidologie, 36:3-14. doi: 10.1051/apido:2004062

Bordier, C., Klein, S., Le Conte, Y., Barron, A.B. & Alaux, C. (2018). Stress decreases pollen foraging performance in honeybees. Journal of Experimental Biology, 221: 1-5. doi: 10.1242/jeb.171470

Bordier, C., Suchail, S., Pioz, M., Devaud, J.M., Collet, C., Charreton, M., Conte, Y.L. & Alaux, C. (2017). Stress response in honeybees is associated with changes in task-related physiology and energetic metabolism. Journal of Insect Physiology, 98: 47-54. doi: 10.1016/j.jinsphys.2016.11.013

Casteels, P., Ampe, C., Rivière, L., Van Damme, J., Elicone, C., Fleming, M., Jacobs, F. & Tempst, P. (1990). Isolation and characterization of abaecin, a major antibacterial response peptide in the honeybee (*Apis mellifera*). European Journal of Biochemistry, 187: 381-386. doi: 10.1111/j.1432-1033.1990.tb15315.x

Chaimanee, V., Chantawannakul, P., Chen, Y., Evans, J.D. & Pettis, J.S. (2012). Differential expression of immune genes of adult honey bee (*Apis mellifera*) after inoculated by *Nosema ceranae*. Journal of Insect Physiology, 58: 1090-1095. doi: 10.1016/j.jinsphys.2012.04.016

Crane, E. (1999). The world history of beekeeping and honey hunting. Routledge. 682p.

Cunha, A.R. & Martins, D. (2009). Classificação climática para os municípios de Botucatu e São Manuel, SP. Irriga, 14: 1-11. doi: 10.15809/irriga.2009v14n1p1-11

Elsik, C.G., Worley, K.C., Bennett, A.K., Beye, M., Camara, F., Childers, C.P. & Elhaik, E. (2014). Finding the missing honey bee genes: lessons learned from a genome upgrade. BMC Genomics, 15: 86. doi: 10.1186/1471-2164-15-86

Even, N., Devaud, J.M., & Barron, A.B. (2012). General stress responses in the honey bee. Insects, 3: 1271-1298. doi: 10.3390/insects3041271

Evans, J.D. (2004). Transcriptional immune responses by honey bee larvae during the invasion by the bacterial pathogen, *Paenibacillus larvae*. Journal of Invertebrate Pathology, 85: 105-111. doi: 10.1016/j.jip.2004.02.004

Glenny, W., Cavigli, I., Daughenbaugh, K.F., Radford, R., Kegley, S.E. & Flenniken, M.L. (2017). Honey bee (*Apis mellifera*) colony health and pathogen composition in migratory beekeeping operations involved in California almond pollination. PloS one, 12: e0182814. doi: 10.1371/journal.pone.0182814

Goulson, D., Nicholls, E., Botías, C. & Rotheray, E.L. (2015). Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. Science, 347: 1255957. doi: 10.1126/science.1255957

Honeybee Genome Sequencing Consortium. (2006). Insights into social insects from the genome of the honeybee *Apis mellifera*. Nature, 443: 931-949. doi: 10.1038/nature05260

Iwasaki, A. & Medzhitov, R. (2015). Control of adaptive immunity by the innate immune system. Nature Immunology, 16: 343-353. doi: 10.1038/ni.3123

Lourenço, A.P., Florecki, M.M., Simões, Z.L.P. & Evans, J.D. (2018). Silencing of *Apis mellifera* dorsal genes reveals their role in expression of the antimicrobial peptide defensin-1. Insect Molecular Biology, 27: 577-589. doi: 10.1111/imb.12498

Maderson, S. & Wynne-Jones, S. (2016). Beekeepers' knowledges and participation in pollinator conservation policy. Journal of Rural Studies, 45: 88-98. doi: 10.1016/j.jrurstud.2016.02.015

Mason, R., Tennekes, H., Sánchez-Bayo, F. & Jepsen, P.U. (2013). Immune suppression by neonicotinoid insecticides at the root of global wildlife declines. Journal of Environmental Immunology and Toxicology, 1: 3-12. doi: 10.7178/jeit.1

Nation, J.L. (2015). Insect Physiology and Biochemistry. Boca Raton: CRC Press. 690p.

Navajas, M., Migeon, A., Alaux, C., Martin-Magniette, M.L., Robinson, G.E., Evans, J.D. & Le Conte, Y. (2008). Differential gene expression of the honey bee *Apis mellifera* associated with *Varroa destructor* infection. BMC Genomics, 9: 1-11. doi: 10.1186/1471-2164-9-301

Nazzi, F. & Pennacchio, F. (2014). Disentangling multiple interactions in the hive ecosystem. Trends in Parasitology, 30: 556-561. doi: 10.1016/j.pt.2014.09.006

Pfaffl, M.W. (2001). A new mathematical model for relative quantification in real-time RT–PCR. Nucleic Acids Research, 29: e45. doi: 10.1093/nar/29.9.e45

Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O. & Kunin, W.E. (2010). Global pollinator declines: trends, impacts and drivers. Trends in Ecology and Evolution, 25: 345-353. doi: 10.1016/j.tree.2010.01.007

Randolt, K., Gimple, O., Geissendörfer, J., Reinders, J., Prusko, C., Mueller, M.J. & Beier, H. (2008). Immune-related proteins induced in the haemolymph after aseptic and septic injury differ in honey bee worker larvae and adults. Archives of Insect Biochemistry and Physiology, 69: 155-167. doi: 10.1002/arch.20269

Richard, F.J., Holt, H.L. & Grozinger, C.M. (2012). Effects of immunostimulation on social behavior, chemical communication and genome-wide gene expression in honey bee workers (*Apis mellifera*). BMC Genomics, 13: 1-18. doi: 10.1186/1471-2164-13-558

Sahebzadeh, N. & Lau, W.H. (2017). Expression of heat-shock protein genes in *Apis mellifera* meda (Hymenoptera: Apidae) after exposure to monoterpenoids and infestation by Varroa destructor mites (Acari: Varroidae). European Journal of Entomology, 114: 195-202. doi: 10.14411/eje.2017.024

Scharlaken, B., de Graaf, D.C., Goossens, K., Peelman, L.J., & Jacobs, F.J. (2008). Differential gene expression in the honeybee head after a bacterial challenge. Developmental and Comparative Immunology, 32: 883-889. doi: 10.1016/j.dci.2008.01.010

Schlüns, H. & Crozier, R.H. (2007). Relish regulates expression of antimicrobial peptide genes in the honeybee, *Apis mellifera*, shown by RNA interference. Insect Molecular Biology, 16: 753-759. doi: 10.1111/j.1365-2583.2007.00768.x

Siede, R., Meixner, M.D. & Büchler, R. (2012). Comparison of transcriptional changes of immune genes to experimental challenge in the honey bee (*Apis mellifera*). Journal of Apicultural Research, 51: 320-328. doi: 10.3896/IBRA.1.51.4.05

Simone-Finstrom, M., Li-Byarlay, H., Huang, M.H., Strand, M.K., Rueppell, O. & Tarpy, D.R. (2016). Migratory management and environmental conditions affect lifespan and oxidative stress in honey bees. Scientific Reports, 6: 1-10. doi: 10.1038/srep32023

Whynott, D. (1991). Following the bloom: across America with the migratory beekeepers. Stackpole Books. 214p.

Yang, X. & Cox-Foster, D.L. (2005). Impact of an ectoparasite on the immunity and pathology of an invertebrate: evidence for host immunosuppression and viral amplification. Proceedings of the National Academy of Sciences USA, 102: 7470-7475. doi: 10.1073/pnas.0501860102

Zar, J.H. (1996). Bioestatistical Analysis. New Jersey: Pretince Hall.

Zhu, X., Zhou, S. & Huang, Z.Y. (2014). Transportation and pollination service increase abundance and prevalence of *Nosema ceranae* in honey bees (*Apis mellifera*). Journal of Apicultural Research, 53: 469-471. doi: 10.3896/IBRA. 1.53.4.06