

UNIVERSIDADE DE SÃO PAULO
FACULDADE DE FILOSOFIA, CIÊNCIAS E LETRAS DE RIBEIRÃO PRETO
PROGRAMA DE PÓS-GRADUAÇÃO EM ENTOMOLOGIA

“Influência das rainhas na infestação do ácaro parasita *Varroa destructor* em colmeias de abelhas *Apis mellifera*”

Arthur Henrique Puccetti Nascimento

Dissertação apresentada à Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto da Universidade de São Paulo, como parte das exigências para obtenção do título de Mestre em Ciências, obtido no Programa de Pós-Graduação em Entomologia

Ribeirão Preto - SP

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Orientador: Prof. Dr. David de Jong

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RESUMO

A apicultura enfrenta há décadas a Varroatose, patologia apícola causada pelo ácaro *Varroa destructor*, que gerou, e continua gerando, inúmeros prejuízos na criação de abelhas da espécie *Apis mellifera* em várias partes do globo. Muitos manejos, produtos, conhecimento técnico e científico já foram produzidos para conter os danos da Varroatose e permitir maior segurança e sustentabilidade na apicultura, mas ainda existem pontos obscuros na relação parasita/hospedeiro que devem ser desvendados na busca por um controle mais eficaz contra a ação do ácaro. No Brasil, diferentemente de países europeus e norte-americanos, as colmeias sobrevivem sem a utilização de acaricidas e/ou outras técnicas de controle do *Varroa*, isso se deve às características próprias que a abelha africanizada apresenta, como comportamento higiênico mais eficaz e menor volume de alvéolo de cria. O protocolo utilizado pelos pesquisadores brasileiros na década de 80, quando o *Varroa* iniciou sua atividade parasitária no Brasil, de não utilizar em primeira instância acaricidas para controle do ácaro permitiu o desenvolvimento de resistência nas abelhas contra seu novo parasita, esse fator também corroborou para a grande tolerância da abelha africanizada contra a Varroatose. A ausência da abelha-rainha na colmeia também é um fator que gera diminuição nos níveis de infestação dos ácaros no ninho. Este trabalho apresenta dois experimentos de isolamento de rainhas para controle da infestação acarina. No primeiro experimento, a rainha foi isolada em apenas uma colmeia, dentre duas que formam um sobreninho, ao analisar a cria das abelhas, foi constatado que o nível de infestação diminuiu em algumas colmeias. No segundo experimento, a abelha-rainha foi removida totalmente de uma colmeia formada por uma caixa ninho, ao analisar a cria das abelhas, também houve redução nos níveis de infestação, demonstrando que esta técnica pode ser promissora no controle da Varroatose.

PALAVRAS-CHAVE: Varroatose. Abelha africanizada. Colmeias sem rainha. Tolerância ao *Varroa destructor*. *Varroa destructor* no Brasil.

Nascimento, A. H. P. “**Influence of queens on the infestation of the parasitic mite *Varroa destructor* in *Apis mellifera* beehives**” Dissertação - Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto da Universidade de São Paulo, 2023, 69 folhas.

ABSTRACT

Beekeeping has been facing Varroaosis for decades, an apicultural pathology caused by the mite *Varroa destructor*, which has generated, and continues to generate, numerous losses in the breeding of bees of the *Apis mellifera* species in various parts of the globe. Many managements, products, technical and scientific knowledge, have already been produced to contain the damage of Varroaosis, and allow greater safety and sustainability in beekeeping, but there are still obscure points in the parasite/host relationship that must be unraveled in the search for a greater control of the mite action. In Brazil, unlike European and North American countries, hives survive without the use of acaricides and/or other *Varroa* control techniques, this is due to the specific characteristics that the africanized bee presents, such as more effective hygienic behavior and lower volume of brood cell. The protocol used by Brazilian researchers in the 80s, when *Varroa* started its parasitic activity in Brazil, of not using acaricides in the first instance to control the mite, allowed the development of resistance in bees against this new parasite, this factor also corroborated for the great tolerance of the africanized bee against Varroaosis. The absence of the queen bee in the hive is also a factor that generates a decrease in the levels of mite infestation in the nest. This work presents two experiments to isolate queens to control mite infestation. In the first experiment, the queen was isolated in only one hive, among two that form an offspring, when analyzing the brood of the bees it was verified that the level of infestation decreased in some hives. In the second experiment, the queen bee was completely removed from a hive formed by a nest box, when analyzing the bee brood there was also a reduction in infestation levels, demonstrating that this technique can be promising in the control of Varroaosis.

KEYWORDS: Varroaosis. Africanized bee. Queenless hives. Tolerance to *Varroa destructor*. *Varroa destructor* in Brazil.

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1. INTRODUÇÃO GERAL

1.1 Abelha africanizada

Taxonomia da espécie de abelha africanizada – *Apis mellifera* Linnaeus, 1758, segundo Aguiar (2013) e Catálogo Moure:

Domínio – Eukarya

Reino – Animalia

Filo – Arthropoda

Superclasse – Hexapoda

Classe – Insecta

Ordem – Hymenoptera

Subordem – Apocrita

Superfamília – Apoidea

Família – Apidae

Subfamília – Apinae

Tribo – Apini

Gênero – *Apis*

Espécie – *Apis mellifera*

A abelha *Apis mellifera* é uma espécie invasora no Brasil, foi trazida pelos colonizadores no século XIX visando a criação de abelhas para produção de mel e cera, dando continuidade nas tradições europeias, subespécies provenientes da Alemanha (*A. mellifera mellifera*), Itália (*A. mellifera ligustica*) e sudeste da Europa (*A. mellifera carnica*) colonizaram o Brasil e se adaptaram aos biomas sul-americanos (Pereira, 2008). Em 1956, o geneticista Dr. Warwick Kerr trouxe da África a subespécie *A. mellifera scutellata*, com o objetivo de ampliar e melhorar a produção de mel no Brasil (Kerr, 1967). As rainhas da abelha africana mantidas em quarentena escaparam e se espalharam pelo meio natural se reproduzindo com os zangões de raças europeias que já residiam no Brasil, deste cruzamento, entre subespécies, surgiu um tipo de abelha híbrida (Spivak et al., 2019), denominada por Gonçalves (1974) de abelha africanizada, pois nela prevaleciam as características da subespécie

africana, como comportamento mais defensivo, maior formação de produtos da colmeia, maior tendência enxameatória e maior resistência a doenças (De Jong, 1996).

A abelha africanizada, já disseminada pelo território brasileiro, despertou temor da população devido aos ataques proferidos a pessoas e outros animais, gerando um histórico ruim para este híbrido que começava a dominar grande parte da América do Sul (Gonçalves & Stort, 1994; De Jong, 1996). Apicultores temiam trabalhar com este tipo de abelha e pesquisadores buscavam soluções de manejo que beneficiariam a apicultura brasileira. Atualmente, novos instrumentos, vestimentas e manejos proporcionaram certa tranquilidade na atividade de criação de abelhas, inclusive os benefícios se mostraram maiores que os temores, pois este tipo de abelha é mais eficiente na produção de mel e mais resistente a doenças, fatores que tornaram o Brasil um grande produtor de mel que, além de multifloral, possui maior qualidade, pois é livre de contaminantes (Guzmán-Novoa et al., 1999; Gonçalves et al., 2019).

1.2 *Varroa destructor* e seu ciclo de vida

Inicialmente encontrado em Java, na Indonésia, e classificado como *Varroa jacobsoni* por Oudemans (1904), parasita da abelha oriental *Apis cerana* e, posteriormente reclassificado como *Varroa destructor* por Anderson & Trueman (2000), devido a dimorfismos e diferenças no gene da citocromo C oxidase I (CO-I) presente no DNA mitocondrial (Anderson & Trueman, 2000), o ácaro *V. destructor* é um aracnídeo da família Varroidae, apresenta ainda variações na sequência gênica supracitada que confere pelo menos 7 haplótipos diferentes, sendo o haplótipo Coreano/Russo o mais virulento (De Guzman & Rinderer, 1999) e que acarreta enormes prejuízos na apicultura em várias partes do globo.

No início da segunda metade do século XX, através de tráfegos na ferrovia Transiberiana, apicultores transportando colmeias de *A. mellifera* vindas de países europeus para o oriente, bem como camponeses migrando do extremo oriente russo para Rússia Europeia transportando ninhos de *A. cerana*, proporcionaram o contato de colmeias de subespécies europeias com colmeias de *A. cerana*, ocasião que pode ter possibilitado os primeiros acessos ao ácaro *V. destructor* ao seu novo hospedeiro, a abelha *A. mellifera* (Crane, 1978; Oldroyd, 1999). Em 1975, é publicado uma nota de aviso sobre a disseminação do ácaro *V. destructor* (denominado na época de *V.*

jacobsoni) em países europeus, vindos da antiga URSS (Akkratanakul & Burgett, 1975), fato que já era de conhecimento de apicultores e motivo de preocupações devido às grandes perdas de colônias em países orientais causadas pela Varroatose.

Os danos causados pelo *V. destructor* em seu hospedeiro natural não são muito evidentes, pois *A. cerana* possui um eficiente comportamento defensivo (grooming) contra o ácaro e, além disso, a reprodução do *Varroa* fica restrita às células de zangões (Peng *et al.*, 1987; De Jong, 1988), fator que não interfere de maneira acentuada na manutenção da colmeia; já na espécie *A. mellifera*, o ácaro é capaz de gerar mais danos (De Jong, 1990), pois além de prejudicar a saúde da abelha adulta ao sugar sua hemolinfa e transmitindo patógenos, parasita também, tanto larvas e pupas de zangão, quanto de operárias (De Jong, 1984; Fries *et al.*, 1994). De Jong *et al.* (1982a) encontrou relação entre a infestação de ácaros em crias de *A. mellifera* com a perda de peso em operárias recém-emergidas, com perdas de massa entre 6% - 25% em comparação com operárias saudáveis (que não foram parasitadas na fase da cria); Duay *et al.* (2003) fez uma correlação semelhante, mas analisando perda de massa em pupas de zangão parasitadas por *Varroa*, onde, além de terem redução de peso de pelo menos 10% em comparação com zangões não parasitados, mostrou também que a redução de peso é diretamente proporcional à quantidade de ácaros fêmea na célula de cria de zangão. Após o contato e início da relação de parasitismo do ácaro com *A. mellifera*, devido à grande circulação de colmeias pela Europa, África e Ásia, esse parasita se espalha pelo Velho Mundo (Ritter, 1981) e começa a gerar prejuízos na apicultura em geral.

O ácaro necessita de abelhas do gênero *Apis* como hospedeiras para realizar seu ciclo reprodutivo, que ocorre no interior do alvéolo de cria, onde o ácaro se alimenta da hemolinfa e do tecido adiposo das larvas e pupas das abelhas, e para seu ciclo dispersivo utiliza abelhas adultas, das quais se alimenta da hemolinfa e se dispersa pelo ninho em busca de outros alvéolos com larvas em estágio L5 (último estágio larval) para invadir, recomeçando seu ciclo de vida (Rosenkranz, P. *et al.* 2010; Nazzi & Le Conte, 2016).

Os ácaros se locomovem, no interior do ninho, aderidos ao corpo das abelhas nutrizas (ciclo dispersivo), ao localizarem um alvéolo adequado para invasão, se desalojam da abelha nutriz e rapidamente seguem direto ao alvéolo (Ritter, W. 1981; Beetsma, *et al.* 1999). Ao adentrar, se direcionam ao fundo da célula de cria e “mergulham” no alimento larval (Ifantidis, M. 1988; Boot, *et al.* 1992), deixando apenas

seu peritrema sobremeroso para a respiração (De Jong, 1984). Assim que a larva consome todo o alimento, o ácaro é liberto para transitar no interior do alvéolo (De Jong et al., 1982b). A oviposição ocorre, aproximadamente, após 60h da operculação do alvéolo, deste primeiro ovo eclode um ácaro macho, posteriormente, em um intervalo de aprox. 30h, são ovipostos mais ovos (cerca de seis) (Martin, S. 1994). Após a conclusão do desenvolvimento da cria do ácaro até a fase adulta ocorrer (aprox. seis dias), o *Varroa* macho copula com suas irmãs e as insemina (Grobov, 1977; Accorti, M. et al. 1990). Com a emergência da abelha adulta, saem também, acoplados a ela, a fêmea matriz e as fêmeas filhas inseminadas, iniciando o ciclo dispersivo com a busca por abelhas nutrizas (Le Conte & Arnold, 1987), o *Varroa* macho permanece no alvéolo e morre (Fries, et al. 1994).

Os imaturos parasitados pelos ácaros sofrem inúmeros danos histofisiológicos, perdem peso e podem se contaminar com microorganismos patogênicos transmitidos pelos ácaros (Anshakova et al., 1978; De Jong et al., 1982a; Dainat, B. 2012), como o DWV (Deformed wing virus), que impossibilita o voo das abelhas devido à deformidade que causa nas asas (Kielmanowicz, M. et al., 2015).

1.3 A Varroatose na apicultura mundial

A Varroatose é uma parasitose que acomete abelhas do gênero *Apis*, seu agente causador é o ácaro *V. destructor*. Essa patologia apícola prejudica o setor de apicultura há muitas décadas, sendo considerada uma das pragas mais desafiadoras que atinge a espécie *Apis mellifera* e cujas consequências negativas são sentidas em muitos países do mundo (Le Conte et al. 2010; Rosenkranz et al. 2010; Dietemann, V. et al. 2012; Traynor et al. 2020; Jack & Ellis, 2021).

É difícil mensurar o impacto negativo que a Varroatose causa no setor apícola em termos financeiros, para encontrar possíveis cifras que quantifiquem esse problema é essencial analisar os valores médios que os serviços de polinização geram na economia mundial. No Brasil, por exemplo, os serviços de polinização em 2018 foram estimados em R\$ 43 bilhões (Wolowski et al. 2019), Gallai et al. (2009) também investigando a importância da polinização na produção de alimentos, apresenta o valor de £ 153 bilhões ao ano, o que equivaleu a 9,5% do valor da produção agrícola mundial; sendo as abelhas os principais animais polinizadores que existem (Shepherd et al. 2003), fica patente o prejuízo que as doenças e as perdas de colmeias geram

na produção de alimentos e na economia mundial. Com a crescente demanda na agricultura, o uso dos insetos polinizadores está cada vez mais em voga, a importância da preservação e sanidade destes seres é essencial para o crescente desenvolvimento desse serviço produtivo.

Deve-se, ainda, considerar na somatória total dos valores econômicos do serviço de polinização prestado pelas abelhas *Apis mellifera* os custos com os tratamentos químicos usados no controle do ácaro *V. destructor*. Diferentemente do Brasil, onde o tipo de abelha *A. mellifera*, conhecida como abelha africanizada, possui tolerância contra o *Varroa* e não necessita de uso de acaricidas, os países europeus e norte-americanos necessitam obrigatoriamente de tais produtos a nível de que, sem eles, as colmeias padecem em poucos anos (De Jong, 1984; Goulson et al., 2015). Assim, além de altos custos gastos com acaricidas e outros produtos químicos específicos, a contaminação dos produtos da colmeia ocorre de maneira recorrente e interfere negativamente na qualidade de tais produtos.

Ainda considerando a sanidade e o controle de pragas das abelhas *A. mellifera*, obviamente não se deve apenas considerar o papel de polinizadores nas culturas agrícolas para uso humano, mas também (e principalmente) o papel que esses insetos possuem como polinizadores naturais dos ecossistemas em geral. O nicho ecológico das abelhas é imprescindível para a reprodução dos vegetais, com consequente produção de frutos, dispersão de sementes e alimentação dos animais. Portanto, as abelhas asseguram a perpetuação dos meios naturais, fauna e flora, com sua atividade polinizadora (Costanza, R. et al. 1997; Buchmann, S. L., & Nabhan, G. P. 2012).

1.4 Fatores que promovem a invasão dos ácaros às células de cria

Inúmeros fatores físico-químicos estimulam e sinalizam aos ácaros o momento e o local adequados para realizarem a invasão nas células de cria. A fase de desenvolvimento da larva da abelha adequada para a invasão acarina é a fase L5 (Aumeier, P. et al. 2002), último instar larval, anterior à fase de pupa. O imaturo libera sinais químicos de sua cutícula que estimulam as abelhas operárias a opercularem o alvéolo, estes sinais, no entanto, funcionam também como cairomônios, sinalizando aos ácaros o momento ideal para a entrada no alvéolo (Le Conte, et al. 1990;

Calderoni, N.W. & Lin, S. 2001), que ocorre, nas operárias, 20h antes da operculação (Beetsma J., Boot W.J., Calis J. 1999).

A posição da larva no alvéolo também é um fator que indica ao ácaro possibilidades de invasão. Quanto mais próxima a larva está da borda do alvéolo, mais atrativa ela será para o ácaro (Goetz, B., & Koeniger, N. 1993; Boot, W.J. et al. 1995), bem como larvas em alvéolos menos profundos que a medida padrão são também mais atrativas (De Ruijter, A. & Calis, J. 1988). Com menor espaço entre as bordas e mais expostas devido à profundidade menor do alvéolo, a liberação dos feromônios da larva, que funcionam como caimônios, é mais intensa e mais receptiva para o *Varroa*, estimulando maiores invasões nas células com essas características.

Os ácaros preferem invadir alvéolos em favos já usados, com maior tempo de vida, do que alvéolos em favos recém-construídos. Nos favos velhos, a impregnação de substâncias cuticulares, resquícios de compostos do alimento larval e outros elementos liberados pelas abelhas é maior, portanto, a atratividade ao ácaro é diretamente proporcional, acionando sua preferência nessas condições específicas (Piccirillo, G.A. & De Jong, D. 2004). Segundo De Jong (1981), a construção de células de cria de rainha também funciona como atrativo para o ácaro, o autor observou um grande aumento na invasão dos ácaros após a construção de tais células, principalmente no lado do favo onde a célula real se encontrava.

1.5 Tolerância da abelha africanizada à Varroatose

A abelha africanizada apresenta maior tolerância ao ácaro *V. destructor* em comparação com outras subespécies da abelha *A. mellifera*. Vários estudos foram publicados comprovando tal tolerância, desde que os apicultores e pesquisadores iniciaram os manejos e estudos com este híbrido de raça africana com raças europeias, as análises dos resultados de campo e laboratório revelaram características muito interessantes e singulares da relação parasita/hospedeiro entre o *Varroa* e a abelha.

A abelha africanizada apresenta maior rapidez e agilidade na remoção dos ácaros alojados em seus corpos em comparação com a raça italiana. Em um experimento realizado por Moretto et al. (1991), cinquenta abelhas africanizadas e cinquenta abelhas italianas foram inoculadas com um *Varroa* fêmea cada e, durante

30 minutos, foram observadas quanto à detecção e remoção do ácaro. As abelhas africanizadas obtiveram uma proporção de remoção de 38,5%, já as abelhas italianas, apenas 5,75%.

O tamanho da célula de cria da abelha africanizada é menor do que a célula de cria das raças europeias, fator que também promove uma maior tolerância da abelha ao ácaro. Gonçalves & Message (1995) e Picirillo & De Jong (2003) observaram uma maior taxa de infestação do ácaro nas abelhas de raças europeias em comparação com a africanizada, devido à diferença do tamanho dos alvéolos.

O comportamento higiênico se configura como uma capacidade de detecção e remoção de parasitas e/ou imaturos contaminados do ninho, essa característica confere às abelhas maior resistência às doenças e parasitas em geral (Spivak & Downey, 1998). O comportamento higiênico, expressado pela abelha africanizada, é mais eficaz contra o *Varroa* do que outras raças de *A. mellifera*. Guerra et al. (2000) e Moraes et al. (2009) observaram em seus experimentos praticamente o dobro de remoção de cria infestada pelo ácaro nas abelhas africanizadas, quando comparadas com raças europeias, comprovando maior eficiência deste tipo de abelha na defesa contra o ácaro.

O clima também é um fator que interfere na relação de parasitismo entre o ácaro e a abelha *A. mellifera*, influenciando negativamente a reprodução do parasita (Ritter & De Jong, 1984; Camazine, 1986; Anguiano-baez et al. 2016). De Jong et al. (1984), ao investigar a Varroatose em países de clima temperado (Argentina e Uruguai) e países de clima tropical (Brasil e Paraguai), encontrou níveis de infestação maiores nos países de clima temperado (1.07 ácaros por abelha vs. 0.1 ácaros por abelha).

1.6 Controle da Varroatose

Países europeus e norte-americanos, principalmente, não podem deixar de utilizar controle químico com acaricidas para manter as colmeias com boa sanidade e produzindo, sem tais manejos as colmeias perecem em poucos anos (De Jong, 1997; Haber et al. 2019), em contrapartida, o uso de tais produtos contamina os produtos da colmeia (Wallner, K. 1999) e pode, ainda, possibilitar o desenvolvimento de linhagens de ácaros resistentes aos acaricidas (Milani, N. 1999).

Além do uso de acaricidas, a seleção artificial de linhagens resistentes, ou ao menos mais tolerantes ao ácaro, é uma técnica bastante explorada pelos pesquisadores e apicultores, especialmente buscando gerações com comportamento higiênico mais eficaz, característica importante na defesa da abelha contra o ácaro (Büchler et al., 2010). O monitoramento da população do ácaro também auxilia no controle da infestação e pode fornecer uma previsão de possíveis manejos a serem utilizados, a observação dos ácaros mortos pelas abelhas no fundo da colmeia é uma forma de monitoramento acessível e significativa (Imdorf, A. et al. 1996).

Outra técnica que pode beneficiar o controle do *Varroa* é a interrupção da ovipostura e conseqüente desenvolvimento de imaturos durante o verão, estação anterior ao período crítico para as colmeias, pois a Varroatose gera mais danos nas estações frias. Procedimentos como separação da abelha-rainha em gaiolas, uso de ácido oxálico e remoção total das larvas são utilizados neste manejo (Büchler et al. 2020).

Os países da América do Sul, onde a abelha africanizada se adaptou e reside, sofrem bem menos com a Varroatose, já que por inúmeros fatores este tipo de abelha não necessita de ajuda externa para se defender contra o parasitismo do ácaro *V. destructor* (Calderón, et al. 2010). O ácaro foi detectado no Brasil em 1978 (Morse & Gonçalves, 1979) e durante os cinco primeiros anos parasitando *A. mellifera* no território brasileiro, os níveis de infestação foram muito elevados, gerando sérias preocupações aos apicultores, mas após esse período os níveis baixaram bruscamente, atestando uma possível adaptação da abelha ao seu parasita (De Jong & Gonçalves, 1998). Essa situação natural dispensou o controle químico nas colmeias no Brasil, manejo que estava em uso em outras partes do mundo, onde o *Varroa* já se relacionava com a abelha *A. mellifera*.

O controle da Varroatose é imprescindível para que a apicultura se desenvolva plenamente e produza itens da colmeia com qualidade e segurança alimentar para a sociedade humana. Inúmeros procedimentos e produtos são explorados pelos pesquisadores e apicultores ao redor do mundo, mas ainda há indizíveis perdas de colmeias devido a essa patologia, por isso a investigação de novas formas de controle é importantes e deve ser testada e divulgada para o enriquecimento desta atividade e do conhecimento científico.

2. OBJETIVO GERAL

Determinar a influência da rainha na infestação do ácaro parasita *Varroa destructor* em abelhas adultas e imaturas da espécie *Apis mellifera*.

3. CAPÍTULO 1

Title

“Relevant aspects of the tolerance of the africanized honey bees in Brazil to the parasitic mite *Varroa destructor*”

Abstract

Varroatosis, a parasitosis caused by the mite *Varroa destructor*, causes numerous losses in beekeeping worldwide, since its identification in the mid-twentieth century, to the present day. In Brazil, the hybrid bee of the species *Apis mellifera*, called the africanized bee, when it came into contact for the first time with the mite in the 70s, suffered severe losses in its population, generating fear in beekeepers who were faced with a new bee pathology. Upon recognizing the threat, Brazilian researchers began research to understand the ecology and physiology of *Varroa*, seeking to develop techniques to control the parasite and, unlike European countries, which used acaricides and other chemical substances to control the mites, they opted first to understand the dynamics of the parasite relationship to later determine which protocol would be followed by beekeepers. After a period of contact between the africanized bee and the mite, infestation levels dropped to a great extent, revealing the bee's development of a tolerance for *Varroa*, a situation that greatly benefited beekeeping and aroused curiosity among scientists. Although it does not generate major losses in Brazilian beekeeping, the development of Varroatosis control managements that avoid, or reduce, the use of chemical substances is paramount, for this, it is clear to know the factors that stimulate the invasion of mites in the brood cells, as well as to know the aspects that generate tolerance of the africanized bee by the mite, as this knowledge can help in the development of control protocols in other countries.

Key words: Africanized honey bee. *Varroa destructor*. Varroatosis.

Introduction

***Varroa destructor* mite and the africanized honeybee**

The mite *Varroa destructor* (Acari: Varroidae) is considered one of the greatest threats to beekeeping in various parts of the world, parasitizing immature and adult bees *Apis mellifera* which, with the exception of some countries, such as Brazil, where there are no records of colony losses and not much interference in the production of bee products, generates high mortality rates in hives (De Jong et al., 1982; Rosenkranz et al., 2010; Traynor et al. 2020).

In Brazil, the mite *V. destructor* was probably introduced in 1972, coming from infested hives from Japan via Paraguay (Crane, 1978; De Jong & Gonçalves, 1981; de Guzman et al., 1997) and in the United States of America, was detected in 1987 (de Guzman & Rinderer, 1999).

In 1978, the mite *V. destructor* was officially detected in honey bee colonies in Brazil (Morse & Gonçalves, 1979), raising awareness and concern among Brazilian researchers and beekeepers, as the damage caused by the parasite was already known. In 1979 and 1980, researchers from the Department of Genetics, Faculty of Medicine, University of São Paulo in Ribeirão Preto, surveyed hives in the region, detecting high levels of infestation of 50 mites per 100 bees. Thus, determined to prevent a massive infestation of mites in hives, Brazilian researchers went to APIMONDIA in Greece, in 1979, in search of more information about this parasite and possible controls for it. In this congress, in addition to information about the mite, numerous acaricidal products were offered and companies saw an opportunity for trade in Brazilian beekeeping, which was now beginning to face another challenge, Varroaosis. The Brazilian researchers initially opted to understand the parasitic relationship between the mite and the Africanized honeybee, that is, to deepen their knowledge of Varroaosis before encouraging and guiding treatment with acaricides, a situation that, even if they could control infestations at certain levels, could also contaminate the hive products. In this way, observations were conducted in hives infested with *Varroa* without the use of chemical treatment and hives with the use of chemical treatment, each apiary located in a different city. Products such as SINEACAR (Stort et al., 1981), tobacco and formic acid were tested, but without effective success in controlling the mite, on the other hand, in the monitoring of hives without chemical treatment, a decrease in infestation rates was observed, so in 1983

there was an average of only 5 mites per 100 bees and no hive loss had been recorded, possibly revealing a parasite/host adaptation, which is observed up to the present day, with low rates of infestation and rare hive losses by Varroaosis in Brazil (De Jong & Gonçalves, 1998).

Since the 1960s, in Brazil, the most abundant bee of the genus *Apis*, and possibly currently the only race present, is a polyhybrid resulting from the crossing of European subspecies with an African subspecies (Francoy, 2009), a miscegenation that generated the africanized honeybee. (Gonçalves, 1974). This bee expresses characteristics mostly of the African race, such as aggressiveness, greater tendency to swarm, great resistance to diseases and greater efficiency and speed in the production of nests and food reserves (De Jong, 1996). Shortly after the detection of this new hybrid breed of bee, reports and media reports of accidents involving animals and people who were stung began to appear, soon the name “killer bee” (Winston, 1992; Gonçalves & Stort, 1994) began to be used and bring insecurity to those who worked with beekeeping. Despite the bad repercussion that the hybridization and domination of the Brazilian territory by the africanized honeybee generated, after a few years Brazilian researchers developed management and instruments to deal with this type of bee, a situation that provided safe control for beekeepers in dealing with the bee, since then beekeeping and the consequent production of Brazilian bee products has grown enormously, and today the africanized honeybee is managed successfully and its qualities are well recognized (De Jong, 1996; Gonçalves, 2004; Gonçalves et al., 2019).

Factors that stimulate the invasion of mites in brood cells:

1. Chemical stimuli – pheromones

Communication in the nest is mainly through chemical signals. The queen, for example, communicates with the workers by releasing chemical substances from her mandibular glands, the immatures signal their stages of development to the workers through pheromones, just as the workers communicate through such substances, maintaining an adequate social homeostasis for the proper functioning of the nest (Slessor et al. 2005).

There is a select variety of substances that act as chemical signals, the synergy between these substances is something complex and, to a certain extent, unexplored by researchers, to the point of being an obscure point for the full understanding of communication between these insects. Some chemical signals released by the larvae in the last instars of development, whose function is to indicate to the workers the release for the operculation of the alveoli, can also function as kairomones, attracting the mites into the alveoli in the execution of the reproductive cycle (Boot et al., 1994).

Components present in the larval food also act as semiochemicals for the mites, as well as volatized extracts from the cuticle of the larvae, the larval food attracts the mites to the alveoli (Nazzi, 2001).

2. Physical structure of the comb and position of the larva in the alveolus

The physical structure of the comb influences the mite invasion rate. Older combs, even with smaller cell diameter, had a greater number of alveoli invaded by *Varroa*, as described by Piccirillo & De Jong (2004). Mites prefer cells with a larger diameter, but between old combs and newly constructed combs, there was a preference for old combs, an observation that may prove that substances present in already used combs (e.g. brood pheromones; cuticular extracts) may have generated greater stimuli, than the cell diameter.

The depth of the brood cell can also interfere with mite invasion. By inserting in hives infested by *Varroa*, combs with cells with a depth smaller than the standard (3 – 4mm smaller), De Ruijter, A. & Calis, J. (1988) observed that there was a greater invasion of mites in the cells less deep, in these alveoli, the larva is more exposed to the outside, a fact that can be combined with the observation made by Boot et al. (1995) who proved that the distance between the larva and the edge of the cell influences the attractiveness of the mite, the attraction increases as the distance from the larva to the edge decreases.

Factors that may explain the greater resistance of the africanized honeybee to the mite *V. destructor*:

1. Climate

Most of Brazil is located in the Intertropical Zone, with hot and humid climates, with a low temperature range and high temperatures throughout the year, with the exception of the southern region of Brazil, with more evident seasons and a subtropical climate. This Brazilian climate differs from much of the European and North American continent, which has a temperate climate.

When comparing levels of *V. destructor* mite infestation in *A. mellifera* bee hives in European and North American countries with infestations registered in Brazil, great differences are found, with higher infestation rates in temperate regions, showing that the climate factor is deterministic in the relationship of parasitism between the mite and the bee.

Moretto et al. (1991a) found significant differences ($P < 0.001$) in infestation in three Brazilian cities that had different mean temperatures. In the first city, with an average temperature of 21°C (southeast), the infestation level was 3.5 mites per 100 bees; in the second city, which had an average temperature of 18°C (south), the infestation level was 5.11 mites per 100 bees; in the third city, with an average temperature of 13°C (south), the result was 11.37 mites per 100 bees. It is possible to see through these data how the climate interferes with the levels of infestation of the mite *V. destructor*.

De Jong et al., (1984b) also compare temperate climate with tropical climate in relation to different levels of infestation in South America. High hive mortality rates are described in Argentina and Uruguay (temperate climate), with average infestation levels of 1.07 mites per bee, while in Brazil and Paraguay (tropical climate), average levels are around 0.1 mites per bee, and the losses of hives by Varroaosis are rare. Proving the direct relationship of climate to infestation.

Ritter & De Jong (1984) and Camazine (1986) demonstrate how the climate is a factor that interferes with the reproduction of *V. destructor*, and consequently with the levels of infestation in hives of *A. mellifera*, since the virulence of the mite is linked also to its reproduction (Corrêa-Marques et al., 2003). In their investigations, the reproduction rates of the mite were higher in temperate climate regions than in tropical climate regions. Anguiano-BAEZ et al. (2016), also found significant differences between colonies from temperate and tropical climates in relation to levels of *V. destructor* infestation and DWV infection, colonies from temperate climates had higher rates of both parasites.

2. The africanized honeybee

The type of bee *A. mellifera* influences the bee's tolerance for the mite *V. destructor*. During the period of Brazilian colonization, in the middle of the 19th century, European immigrants introduced hives of *A. mellifera* in order to practice beekeeping, continuing the European traditions of raising honey bees (Pereira, 2008). Subspecies from Germany (*A. mellifera mellifera*), of Italian origin (*A. m. ligustica*) and from southeastern Europe (*A. m. carnica*) (Jensen et al., 2005) were introduced in Brazil, mainly in the region South of the country. In 1956, geneticist Dr. Warwick Kerr, introduces the African subspecies *A. m. scutellata*, with the aim of using it in genetic improvement research to increase the production of Brazilian honey, but due to an oversight by a technician in the quarantine sector where the African hives were, the queens escaped into the wild, and reproduced with drones of European breeds that already resided in Brazil, thus creating the poly hybrid, called africanized honeybee (Spivak et al., 2019). Currently, almost 65 years after the hybridization event, it is found in Brazilian territory, including from Argentina to the North American states of Texas and California (Kerr et al., 1982; Sheppard et al., 1991; Lin et al., 2018), mostly the africanized honeybee.

Moretto et al. (1991b) compared mite extraction behavior in adult, africanized and Italian bees. In this experiment, adult bees (n = 50) were inoculated with a female *Varroa* on their body and placed under observation for 30 minutes in order to analyze the behavior of recognition and extraction of the parasitic mite. The africanized bees were more agitated and more agile in removing the mite from their bodies, while such behavior was milder and less efficient in the Italian bees, with a removal rate during the 30 minutes of 5.75% in the Italian bees and 38, 5% in africanized bees.

Guerra et al. (2000) compared the efficiency in detecting and removing brood infested by the mite *V. destructor* in africanized bees, Italian bees (*A. m. ligustica*) and hybrids of Italian and africanized bees. In his investigations, he found a higher proportion of removal and detection of broods infested by the mite in hives of africanized bees than in hybrids (51% vs. 25%, respectively), as well as in the comparison between africanized and Italian (59% vs. 31%, respectively), revealing that the africanized bee type is more efficient against mite parasitism.

Gonçalves & Message (1995) also confirmed the greater tolerance of the africanized bee to the mite *V. destructor* in comparison with the Italian race, analyzing

the effect of the brood cell size of the two aforementioned races, where the africanized builds smaller brood cells. When analyzing the mite infestation rate in the two types of cell, the africanized bee, with its smallest cell, presented 7.3% while the Italian bee 15.7%, with its largest cell. The amount of cells containing immature stages of the mite was also higher in the Italian bee (11.9% vs. 4.7%), a factor that proves the effectiveness of the reproduction of the mite. Picirillo & De Jong (2003), also proved that the smaller size of the brood cell of the africanized bee positively interferes in its tolerance against the mite *V. destructor*. In their investigations, the Carniolan bee (European race) had a higher level of infestation (19.3%) than the Italian bee (13.9%) and the africanized honeybee (10.3%).

The comparison between the africanized bee and European races regarding the reproductive success of the mite *V. destructor* was investigated by Corrêa-Marques et al. (2003). In the comparative study, the reproductive success of the mite in africanized bees in Brazil, Mexico and European races in England was investigated, using the *Varroa* family reconstruction parameter, an effective tool in confirming the mite virulence. Africanized bees, both in Brazil and Mexico, had 40% of viable brood of the mite in their hives, while the European races had 75% of viable brood. Non-viable broods were also verified, when there are no mites of opposite sexes to carry out reproduction (Medina & Martin, 1999), where in africanized bees the percentage was 38% in Brazil and 48% in Mexico, while in European races in England was 16%, also revealing greater reproductive success of the mite in European breeds.

3. Hygienic behavior

Hygienic behavior in *A. mellifera* bees is configured as a defense against parasites, as well as a mechanism of resistance to various diseases, where the adult bee detects and removes the brood infected by the foreign agent, avoiding or mitigating greater damage to the nest (Spivak & Downey, 1998).

Moraes et al. (2009) conducted an experiment to analyze the hygienic behavior of africanized bee hives in Brazil. The rate of detection and removal of perforated pupae by the pin-killed method and the duration of this process in hygienic and non-hygienic hives were observed. Hygienic hives, 1h after the start of observation, detected 43% of damaged pupae, while non-hygienic hives detected 25%. After 2 hours of perforation of the pupae, the hygienic hives had already uncapped 67% of the

detected cells against 25% of uncapping in the non-hygienic hives. The percentage of removal of dead pupae is also more effective in hygienic hives, where after 8h of perforation, 87% of the pupae were removed from the cells, while in non-hygienic hives, only 32% were removed. The authors also compare their results with data from Gramacho (1999), who carried out observations of hygienic behavior in European bees (*A. m. carnica*), and found removal rates of dead pupae of 31.6% after 6h of perforation, while Moraes et al. found a removal rate of 58% in hygienic hives, revealing greater efficiency in this behavior in africanized honeybees.

Conclusion

Present in Brazil for several decades, parasitizing bees of the genus *Apis*, the *Varroa destructor* mite does not carry serious concerns for beekeepers, as it does not generate mortality rates or irreversible damage to hives to the point of requiring the use of acaricides, as is the case in other countries where Varroaosis is one of the biggest threats to beekeeping (Locke, B. 2016).

Through the analysis of numerous comparative works that investigate Varroaosis in *A. mellifera* honeybees, some factors are found that provide different infestation rates in European bees and the polyhybrid, the africanized honeybee. Such factors, cited in the body of the text, reveal relevant aspects and concrete data that can help in understanding the tolerance capacity of the africanized bee to the mite *V. destructor*.

Infestation levels are higher in hives located in temperate climates and/or in seasons with lower temperatures. The climate also interferes with the reproductive success of the mite, an essential factor in its virulence. The type of bee, within the *A. mellifera* species, is also a factor that corroborates the bee's tolerance to *Varroa*, when comparing levels of infestation of africanized bees with bees of European subspecies, higher rates are observed in European races. Some characteristics present in the africanized honeybee that allow greater tolerance to Varroaosis are: more effective hygienic behavior, both in grooming and in the detection and removal of parasitized brood; construction of smaller brood cells, a factor that reduces the invasion of female mites, possibly due to *Varroa*'s preference for larger brood cells, such as those of drones, for example.

After 5 years of official registration of *Varroa* parasitizing bees in Brazil (1978 – 1983), infestation rates in hives went from an average of 50 mites per 100 bees to an average of 5 mites per 100 bees, a scenario that indicated a possible Africanized honeybee adaptation to *V. destructor*. The researchers responsible for monitoring Varroaosis in Brazil, when observing this large reduction in infestation levels, and still without finding losses of hives due to the parasitosis, realized that it would not be necessary to use chemical treatments to control the mite, as is done in some European countries and the USA (De Jong & Gonçalves, 1998). Chemical treatment with the use of acaricides applied to the hives interferes with the natural selection that acts on the bees for a possible adaptation and resistance to the mites and can even generate tolerance of the mite to the acaricide, enabling more resistant strains (Milani, N. 1999). In Brazil, due to the non-use of chemical substances against mites, Brazilian hives of *A. mellifera* had initial contact with the mite, a situation that generated high infestations between 1978 and 1983, but which also allowed natural selection to act freely in the interaction parasitic to the point of selecting lineages that are increasingly tolerant to the mite, after all the presence of the mite in the hives potentiated and maintained an essential selective pressure for the survival of the bees (Fries et al., 2006), a factor that probably interfered positively in the drop in infestation rates.

Parallel to the aforementioned factors, which configure a certain tolerance of the Africanized honeybee to the *V. destructor* mite, another aspect is recognized that can amplify the reasons for such resistance. Initially, when the mite was introduced in Brazil, the invasive haplotype was of the Thai/Japanese type (de Guzman et al., 1997), considered less virulent than the Korean/Russian haplotype, found in countries in Europe and North America, therefore, it is possible that Brazilian bees first developed tolerance to a less virulent type of mite, and only at the beginning of the 21st century did they come into contact with the Korean/Russian haplotype, but at that time already with resistance to the mite species, which probably contributed to more easily develop resistance to the Korean/Russian type (De Jong, 2006).

Regardless of the factors that generate tolerance of the bee to the mite, the fact that the Africanized bee resists parasitism, presenting very low infestation levels, in comparison with European and North American countries, demonstrates the possibilities of developing mite-tolerant lineages without the need for use of acaricides. Brazil is not the only country that obtained selection of populations that are not affected to a great extent by Varroaosis, Le Conte et al., (2007) reveals populations of bees

that survived years to Varroaosis without control by chemical treatments in France, the author cites a probable development of tolerance due to natural selection, because in the absence of external control (acaricides, for example) bees and mites achieved a balance in the parasite/host relationship. Fries et al., (2006) also present colonies that not only survived, but decreased infestation levels and increased swarming without the use of chemical treatments, on an island in the Baltic Sea. There are many other examples of bee populations that have developed tolerance, or at least resist more to the damage caused by Varroaosis in countries that have historically suffered from this pathology for decades, the knowledge of these examples can help to build more sustainable and effective control models.

Despite several studies presenting data and methodologies that provide better management in beekeeping to control Varroaosis, there are still numerous cases of hive losses and serious damage in the production of bee products in several countries around the world. There are still obscure points in the parasitic relationship of the mite in the bee *A. mellifera* that must be investigated to benefit the beekeeping, processes such as attraction of the mite to the brood cell, interference of the queen bee in the levels of infestation and relationship between food stock of the hive with the *Varroa* invasion need to be better understood.

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4. CAPÍTULO 2

Title

“*Apis mellifera* hives with a queen excluder showed lower rates of *Varroa destructor* mite infestation”

Abstract

The mite *Varroa destructor*, which causes the bee pathology Varroosis, causes countless losses in beekeeping in a large part of the globe. The use of control chemicals, such as acaricides for example, contaminates the hive products and can select more resistant mite strains, which is why the development of management techniques for control and full knowledge of the parasite/host relationship can help in breeding of bees in a more sustainable and safe way. Creating strains of bees that are more resistant to the mite is a way to control Varroosis and also reduce the use of chemicals in hives, as well as population control management, by isolating the queen before the colder seasons, can have positive results in reducing of the mites. The absence of the queen bee in the nest exerts a positive influence on the control of the mite. The level of infestation in the offspring of the bee *Apis mellifera* (africanized) was evaluated in ten starter finisher colonies composed of two Langstroth deep boxes, separated by a queen excluder. In the hives where the queen was present, 155 mites were counted in total, while in the hives where the queen was absent, 79 mites were counted in total. The difference between the amounts of mites points to an interfering factor in the levels of mite infestation, where the management of isolating the queen bee can reduce the number of mites in the nest.

Key words: *Apis mellifera*. *Varroa destructor*. Queen excluder. Africanized honeybee. Queenless hives.

Introduction

Varroaosis is a parasitosis that affects bees of the genus *Apis*, whose etiologic agent is the ectoparasite mite *Varroa destructor* (De Jong et al., 1982a). A bee pathology discovered more than 50 years ago was and continues to be one of the biggest challenges faced by beekeepers in various parts of the world, bringing high death rates in hives and serious damage to the bee products sector (Rosenkranz et al., 2010; Jack & Ellis, 2021).

The mite *V. destructor*, previously called *V. jacobsoni*, but reclassified by Anderson & Trueman (2000), feeds on the hemolymph of adult bees when it performs its dispersive cycle, and in its reproductive cycle, inside the brood alveolus whose larvae and pupae develop in the combs, feed on the adipose tissue and hemolymph of immature bees (Oldroyd, 1999; Rosenkranz et al., 2010; Ramsey et al. 2019). With this parasitic action, the bees suffer histophysiological damage, developmental deficiency, weight loss and can become contaminated with pathogenic microorganisms; the nest, in general, suffers from this pathology (De Jong et al., 1982b).

When looking for solutions to control the mite *V. destructor*, it is necessary to understand the dynamics of the parasite/host relationship, therefore, mechanisms and substances that act as attractants for the mite need to be widely understood.

Mites are attracted both by larval food and by hydrocarbons present in the cuticle of the larvae (Nazzi, F. et al. 2001; Nazzi, F. & Pennacchio, F. 2014), numerous volatile compounds have been studied, seeking to describe the attractive substances that stimulate the invasion of the mites in the brood cells. Methylated esters, released from the cuticle of the larvae moments before the operculation, function as a chemical signal for the worker bees to operculate the alveolus, but also act as kairomones attracting the mites, which are dislodged from the adult bees and go to the alveolus, where the L5 larva is lodged (Le Conte, et al. 1990; Trouiller, J. et al. 1992; Calderoni, N.W. & Lin, S. 2001), invasion occurs within a 20h interval before operculation (Beetsma J., Boot W.J., Calis J. 1999). Boot, W.J. (1994a), when investigating methyl palmitate, described as the only chemical signal released by the larva that acts as an attractant for *Varroa*, discovered that there are several cuticular chemical components that act as attractants, and not just one.

Thus, numerous chemical signals triggered both by the larva and by its food, stimulate the invasion of mites to the brood cells, on the other hand, there are substances present in the nest that act as repellents to the mites, such as octanoic acid, present in large amounts in queen brood cells (Calderone et al., 2002; Nazzi et al., 2009) and (Z)-8-heptadecene, present in the cuticle of foragers that carry pollen, which interferes with the orientation of the mite to find nursing workers, the adult bee stage which mites use to carry out their dispersive cycle (Del Piccolo et al. 2010).

The physical structure of the alveolus, the position of the larva in the brood cell and the lifespan of the comb have also been shown to be influencing factors in the rates of mite infestation.

De Ruijter, A. & Calis, J. (1988), by developing brood cells with a smaller depth than the standard cells (3 – 4mm smaller), demonstrated that the mites invade more the shallower cells, than cells with standard size, because in cells with less depth, the larvae are more exposed to the external environment, so their volatile chemical signals reach the outside of the cell with more intensity, facilitating the location of the mite. Goetz, B., & Koeniger, N. (1993) and Boot, W.J. et al. (1995a) points out that the distance between the larva and the edge of the cell (before operculation) also interferes with the attractiveness of the mite to the larva, the smaller the distance, the greater the attraction, and vice versa, an observation that corroborates with De Ruijter, A. & Calis, J. (1988), because with a smaller distance between the larva and the edge, the release of semiochemicals becomes more evident for capturing the mite. Piccirillo, G.A. & De Jong, D. (2004) when investigating the preference of mites for old or new combs, found approximately twice the infestation (22.6% vs. 9.75%) in old combs, even these having a smaller diameter in their alveoli, in this matter the greatest attractiveness may be in the accumulation and impregnation in the wax of attractive compounds released by the immature and its food, the most striking characteristic in already used combs.

The amount of larvae at the appropriate stage (L5) for mite invasion also influences the amount of mites invading the cells, the more larvae available in L5, the more mites entering the alveoli for the reproductive cycle (Boot et al. 1994b).

De Jong (1981) observed that the construction of brood cells for queens influenced the infestation of mites in the alveoli. In his experiment, the newly capped worker brood cells were analyzed for the entry of mites, after three days of investigation, the queen was experimentally removed from the nest and during three subsequent days the newly capped brood cells were again analyzed, looking for the

invading mites. It was then observed that, after the construction of the cell, the infestation rate in the frame whose cell was constructed, increased to a great extent (64 mites in 79 cells before construction of the cell as opposed to 721 mites in 374 cells after construction of the cell), showing a significant difference and which may reveal that there is some relationship between the presence of the queen and the rate of infestation of the mite *V. destructor*. This fact, added to the essential role played by the queen bee in the nest, which demonstrates control of social homeostasis through the release of pheromones (Winston, M.L. 1991; Brockmann, A. et al., 2006) and the analysis of the processes responsible for the attraction of mites to the brood cells, was a trigger to investigate whether the presence and/or absence of the queen bee in the hive influences the levels of infestation of the mite *V. destructor* in the brood of *A. mellifera*, no previously published study related the presence of the queen bee with levels of *V. destructor* infestation in Africanized bees.

Materials and methods

The analyzes were carried out in the experimental apiary of the Laboratory of Biology, Behavior and Genetics of Bees (Apilab), integrated to the Department of Genetics of the Faculty of Medicine of Ribeirão Preto - SP (FMRP - USP), between the months of March to August, 2021. Ten starter finisher colonys composed of two Langstroth deep boxes of the *Apis mellifera* species (africanized bee) were used, such hives contains 9 frames for the production of combs and 1 feeder frame in each hive.

For the analysis of *Varroa destructor* mite infestation in hives, they were divided by a queen excluder, an instrument that only allows the workers to move from one hive to another, due to the specific diameter of the spaces between the grids.

We tried to compare the number of mites that would infest the brood cells in the box with and without the queen, for that, 200 brood cells from 10 hives were analyzed, 100 brood cells in the nest with the presence of the queen and 100 brood cells in the nest without the presence of the queen. The introduction of the queen excluder in the hives occurred on average 7 days before the beginning of the infestation analysis, so combs with brood uncapped at the beginning of larval development were allocated in both hives and left to continue their development and subsequent capping by the

worker bees. Frames with brood and food stock were divided into both hives so that, after placing the queen excluder, the two hives would obtain a similar configuration.

After operculating the alveoli, 1 frame with brood was removed from each hive (with and without queen) of the same nest and, using entomological forceps, the brood cell was uncapped, the larva in stage L5, the pre-pupa and /or the pupa, removed and checked if there were mites residing in the alveolus and/or adhered to the body of the immature. After removing 100 young bees from each frame and recording the mites found, the ratio between the number of mites infesting the alveoli with the number of uncapped broods was calculated, multiplying the result by 100 to obtain the percentage of infestation rate.

In addition to this activity, the following were also described: presence/absence of drone offspring in the frame and adult drones in the hive; presence of *Galleria mellonella* (or other parasite in the hive) feces; the stage of development that the uncapped brood was in; if there was more than one female mite in the alveolus; whether there were offspring of the mite in the alveolus.

Two months after the last hive analyzed, between the months of October and December, the infestation evaluations were repeated, following the same methodology with the exception of the sample number of hives and uncapped cells. Six hives were analyzed and 50 cells were uncapped from each frame, in both hives (with and without queen bee).

The software used for the statistical test of the two infestation evaluations was the Wilcoxon Signed-Rank Test.

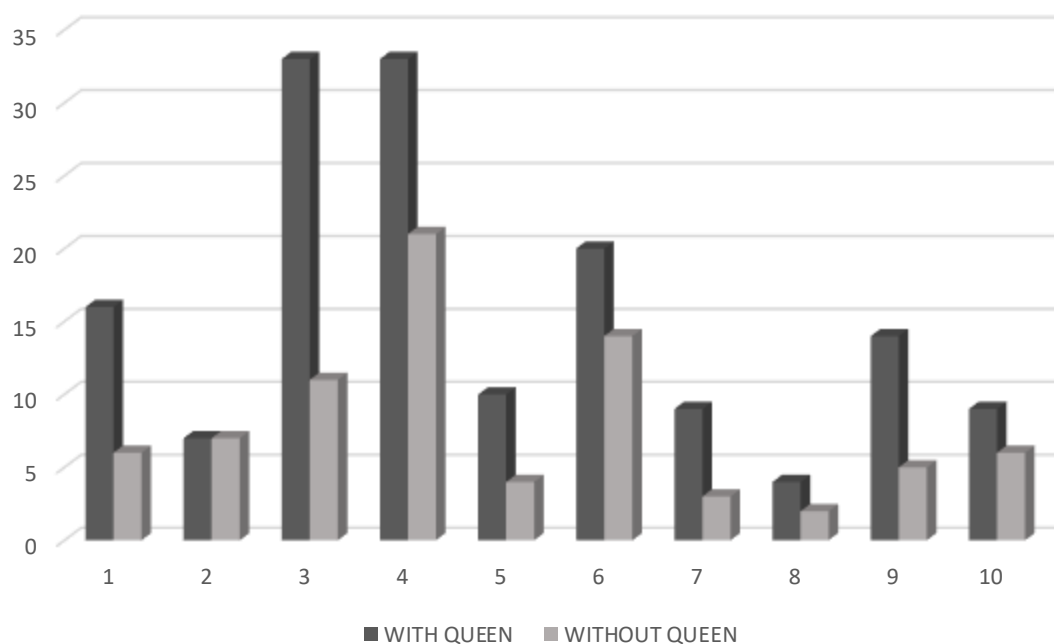
Results

The infestation analyzes revealed that the nest boxes without the presence of the queen bee had lower rates of *V. destructor* mite infestation. In the ten boxes without the presence of the queen, a total of 79 mites were found, while in the ten boxes with the presence of the queen, a total of 155 mites were found (Table 1 and Figure 1). The results demonstrate significant differences between the treatment with the presence of the queen and without the presence of the queen, when analyzing the total sum of mites found, the statistical test revealed a value of $p < 0.05$.

Table 2. 1 - Analysis of infestation in hives, with the queen bee isolated in a box by an queen excluder. N uncapped cells = 100.

Hive with queen	Varroas	Cells	Infestation rate	Hive without queen	Varroas	Cells	Infestation rate
1	16	100	16%	1	06	100	6%
2	07	100	7%	2	07	100	7%
3	33	100	33%	3	11	100	11%
4	33	100	33%	4	21	100	21%
5	10	100	10%	5	04	100	4%
6	20	100	20%	6	14	100	14%
7	09	100	9%	7	03	100	3%
8	04	100	4%	8	02	100	2%
9	14	100	14%	9	05	100	5%
10	09	100	9%	10	06	100	6%
TOTAL	155	1000	15,5%	TOTAL	79	1000	7,9%

Figure 1. Comparison between the amounts of mites found in brood cells in hives with and without the queen bee. Uncapped cells = 100



Hives 1, 3, 4 and 9 showed significant differences ($p < 0.05$) in relation to the amounts of mites found in the broods in the box with the queen and in the box where the queen was absent. Hives 6 and 10 did not show significant differences ($p > 0.05$) for the amounts of mites found in the box with and without a queen. In hives 5, 7 and 8, due to the small amount of mites found, it was not possible to analyze the difference between the box with and without a queen using a statistical test. Hive 2 had the same amounts of mites found in the treatment with and without a queen. In all analyzed

hives, with the exception of hive 2, more mites were found in the boxes where the queen was present.

Hives number 5, 7, 8 and 10 contained drone brood cells and adult drones in the nest.

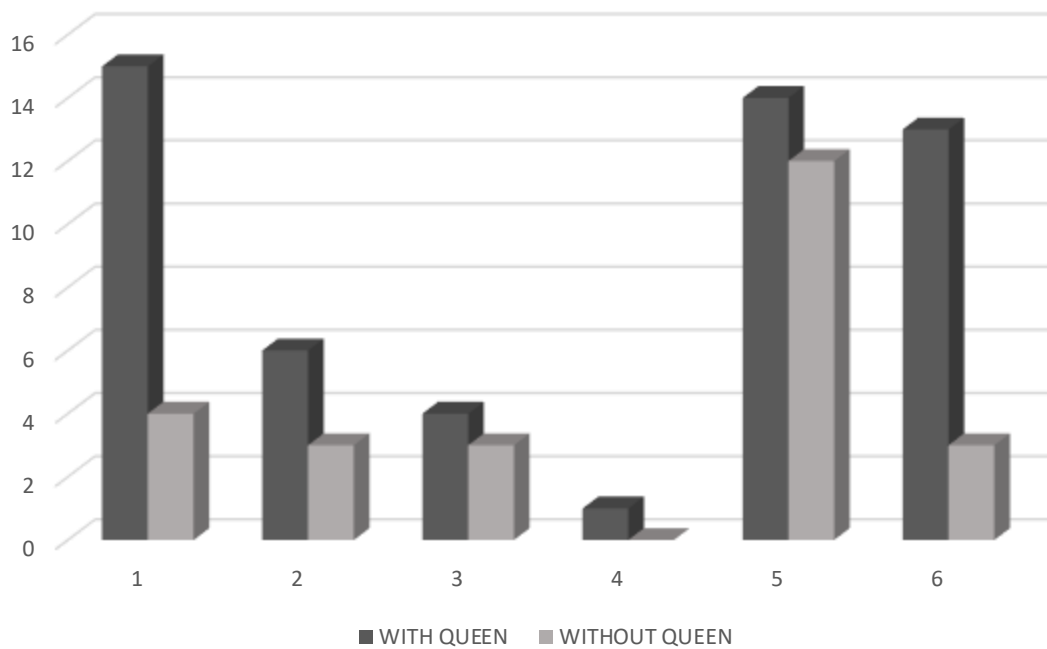
Hives 3, 4, 5, 6, 8 and 10 contained moth (*Galleria mellonella*) feces inside some brood cells; in hives 6 and 7 some hive beetles (*Aethina tumida*) were found.

The results of the second analysis of infestation, whose methodology was changed in terms of the number of hives and uncapped cells, revealed the same pattern as the first analysis, where the boxes without the presence of the queen had lower rates of infestation. In the boxes without the presence of the queen, a total of 25 mites were found, in the hives with the presence of the queen, a total of 53 mites were found (Table 2 and Figure 2), the statistical test revealed a value of $p < 0.05$, as in the first analysis, with significant differences between the two treatments when considering the total sum of mites found.

Table 2. 2 - Second analysis of infestation in hives with the queen bee isolated in a box by an queen excluder. N uncapped cells = 50.

Hive with queen	Varroas	Cells	Infestation rate	Hive without queen	Varroas	Cells	Infestation rate
1	15	50	30%	1	04	50	8%
2	06	50	12%	2	03	50	6%
3	04	50	8%	3	03	50	6%
4	01	50	2%	4	00	50	0%
5	14	50	28%	5	12	50	24%
6	13	50	26%	6	03	50	6%
TOTAL	53	300	17,6%	TOTAL	25	300	8,3%

Figure 2. Comparison between the amounts of mites found in brood cells in hives with and without the queen bee. Uncapped cells = 50



Hives 1 and 6 showed significant differences ($p < 0.05$) in relation to the number of mites found in hives with and without a queen. Hive 5 did not show a significant difference ($p > 0.05$) regarding the amounts of mites found in the two treatments. In hives 2, 3 and 4 the amounts of mites found were low, not allowing statistical tests. All hives had more mites in the treatment with the queen.

Discussion

The sixteen starter finisher colonies composed of two Langstroth deep boxes used in this experiment were divided by a queen excluder, which allowed access to both boxes only to worker bees, which freely moved around the entire nest when performing their hive maintenance services. In this passage through the two boxes, the workers possibly (and naturally) had contact with the queen bee pheromones, and as the queens that were in the sixteen hives analyzed were laying eggs, it is believed that their reproductive ability was acceptable for the nest, therefore the worker population perceived and felt the influence of the queen in the nest, in addition there was no production of real cells with larvae in the boxes where the queen did not have access, which may indicate, in theory, that the chemical signals released by the queen were being captured by the workers, thus inhibiting the production of new queens.

Despite this observation, the total sum of mites found was higher in the boxes where the queens were allocated, in comparison with the boxes without the presence of the queens, a situation observed in fifteen hives, out of the sixteen used.

De Jong (1981) analyzed the influence that the construction of brood cells for the queen bee exerts on infestation levels; by analyzing the observations made, it is possible to conclude that there is a certain influence of the production of such brood cells on the increase in the number of alveoli invaded by the mites. However, the work of De Jong (1981) contradicts the observations made by Calderone et al. (2002) and Nazzi et al. (2009), where they describe the repellency of octanoic acid, present in abundance in real cells, to mites, which indicates that *Varroa* would not be attracted to brood cells close to real cells.

Still on the work of De Jong (1981), the increase of mites invading the cells after the removal of the queen observed by the author, represents an antagonistic result in comparison with the results of this work, and can be explained, in part, by the difference in the *Varroa* haplotype present in the hives in both works. In 1981, the parasitic haplotype of the mite *V. destructor* was the Japanese (Thai) type of mite less virulent than the parasitic haplotype present in Brazil since the beginning of the 21st century, the Korean (Russian) (Guerra et al. 2010), which parasitized the hives in the experiment of the work in question. Therefore, the difference in the types of mites in the two experiments may have generated different results.

Another observation that may explain the discrepancy in the number of mites parasitizing the brood between boxes with and without a queen is the transmission of pheromones from the queen bee, which ends up being diluted due to the large number of worker bees in the two boxes (approx. 100,000 bees) in addition to the queen's restriction situation in only one of the boxes, due to the isolation by the queen excluder, which hinders the transmission of pheromones between the two boxes. Queen pheromones control the inhibition of worker fertility, the production of new queens, swarming and, in general, nest homeostasis (Slessor et al. 2005), are released by the queen's mandibular glands (QMP) and transmitted to the queen's workers keeping an retinue around them, from the retinue are transmitted to the other workers by contact and grooming behavior thus, the greater the number of bees in the nest, the greater the demand for transmission of chemical signals and the more difficult such transmission will be (Trhlin et al. 2011), even more so with the queen isolated in one of the boxes. The QMP also delays the age development of the workers, controlling

the establishment of the functions of the bees in the nest, in the absence of this pheromone, the workers accelerate their development until the foraging stage (Hoover et al. 2003; Slessor et al. 2019), a situation which is not favorable for the mites, because with the acceleration of the development of the workers there will be less nursing mothers in the nest, necessary phase for the dispersive cycle of the mite (Del Piccolo et al., 2010) and that can impair its parasitic action in addition to the repellency of (Z)-8-heptadecene, present in the cuticle of foragers that carry pollen (Nazzi & Le Conte, 2016).

When analyzing each hive independently, not all of them showed significant differences in relation to the number of mites found, taking into account the fact that the high density of bees in the two boxes generates negative interference in the transmission of chemical signals from the queen, it can be concluded that, in the boxes where there were no significant differences between the two treatments, the queen bee could be closer to the box that did not have access, transiting on the excluder screen itself, for example, benefiting the transmission of her pheromones to the workers in the other nest box.

Hives 5, 7, 8 and 10 contained brood cells of drones and adult drones in the nest, these hives showed small differences in the amount of mites between the box with and without queen bee, even making statistical analysis impossible in hives 5, 7 and 8. The fact that Varroas prefer drone larvae to parasitize (Fuch, 1990; Boot et al. 1995b; Nazzi & Le Conte, 2016) may be an important factor in this small discrepancy in numbers, so it is expected that the drone cells present these hives were infested with mites, which may decrease the amount of infested worker cells.

In addition to numerous interfering factors in the levels of infestation already widely studied, such as: the type of climate, type of bee, the hygienic behavior of the investigated population and chemical and physical factors of the biotic and abiotic components of the nest, the comparison between the presence and absence of the queen bee in the hive showed discrepant results regarding infestation rates, with higher rates in boxes with the presence of the queen bee, indicating that there is some influence of the queen bee on the infestation of the mite *V. destructor*.

New studies linking the presence of the queen with Varroaosis must be carried out, seeking to understand which mechanisms interfere and influence the fall of mites invading the alveoli in hives without the queen bee, so that new managements can

emerge in beekeeping aimed at reducing the use of chemical treatments for improvement in the bee products sector.

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5. CAPÍTULO 3

Title

“*Apis mellifera* bee hives without a queen bee showed lower rates of *Varroa destructor* mite infestation”

Abstract

Varroatosis is one of the main apicultural pathologies in the world, it negatively interferes with beekeeping and, consequently, impairs the production of bee products. This research investigated the role of the queen bee in the infestation rate of the *Varroa destructor* mite, for this, the levels of infestation in *Apis mellifera* (africanized) hives, with and without queen bee, in adult bees and in the offspring were evaluated. Six hives in Langstroth-type boxes were evaluated, in the control experiment, in the population with the queen bee, brood cells were uncapped for three subsequent days and the mites present in the cells were counted, later the queen bee was removed and a new infestation assessment was made in the young, now with the absence of the queen. The hives where the queen bee was removed showed lower levels of brood infestation, both in the total amount of mites and in the number of infested brood cells. For the evaluation of parasitic mites in adult bees, a total of bees equivalent to 150 ml of a beaker were collected in a container, treated with alcohol and shaken for uncapping and counting of mites. In adult bees, the difference between hives with and without a queen bee was not significant. The analysis of these results can infer that the presence/absence of the queen bee in the hive interferes with the rates of varroatosis in bee offspring.

Key words: Varroatosis. *Apis mellifera*. Queenless hive. Africanized honeybee. Tolerance against *Varroa*

Introduction

Varroa and associated viruses is the most important disease problem for honey bees worldwide (De Jong & De Jong, 1983; Rosenkranz et al., 2010).

Despite research and field experience of over 60 years, in most regions of the world the Western honey bee, *Apis mellifera*, requires treatments in order to survive (De Jong et al., 1982; De Jong, 1997; Le Conte et al., 2010; van Engelsdorp et al., 2012; Goulson et al., 2015; Haber et al. 2019).

Chemical treatments have allowed beekeeping to continue, but there are various associated problems, including costs of treatments, side effects for the bees, resistance development by the mites, and contamination of honey and other bee products. Natural chemical controls, such as formic and oxalic acids, often can substitute hard chemicals, but they also have costs involved and are normally not as effective (De Jong, 1984; Behrens et al., 2011; Johnson, 2013).

Some local populations of bees have been documented as tolerant of *Varroa* infestation and can survive without treatment (De Jong & Soares, 1997; De Mattos et al., 2016); however, this has not resulted in the availability of bee varieties that can be maintained treatment free in commercial and sideline beekeeper apiaries.

Africanized bees in Brazil and in various other regions of Latin America are an exception, allowing beekeeping without a need for treatment (De Jong, 1996; Ratnieks & Carreck, 2010; Medina et al., 2014; De Jong et al., 2016). Similarly, the introduction of *Varroa* to african countries has generally demonstrated that african bees are able to survive and remain productive without chemical controls (Allsopp et al., 2007; Frazier et al., 2010).

Considerable effort has been made to find and breed for resistance to *Varroa* infestation in European bees, which are the basis of apiculture in North America, Europe, and various other regions of the world. Selection has often focused on hygienic behavior and on *Varroa* specific hygienic behavior (Harbo & Harris, 1999; Harbo & Harris, 2005; Büchler et al., 2010; Büchler et al., 2014). Various of these selected bee varieties are available commercially. However, though they are often demonstrably more tolerant of *Varroa* infestations, they normally also require chemical controls in order to thrive and remain productive.

Understanding what factors allow bees to keep *Varroa* infestations from increasing to dangerous levels could help in determining selection parameters and management options that would be useful to deal with this mite parasite (Moritz et al., 2010; Locke & Fries, 2011)

It is well known that *Varroa* mites preferentially select drone brood over worker brood for reproduction in both the original host, *Apis cerana*, and in its new host *Apis mellifera* (De Jong, 1984; Rosenkranz et al., 2010). However, the selection of worker brood cells is not completely random. When both large and somewhat narrower width worker brood cells are available, the mites tend to infest more heavily the wider cells. Also if some brood cells protrude from the comb surface, due to irregularities in the comb, which can be natural or artificially induced by a poorly embedded reinforcing wire in comb foundation, such tall cells are preferred by the mites (Kuenen & Calderone, 2000).

The distance from the edge of the cell wall and the larva has been found to influence the brood cell invasion behavior of the *Varroa* mites, which could explain why protruding cells are more heavily infested. As the distance from the larva to the cell opening edge decreases, the probability of a mite invading the brood increases (Boot, et al., 1995).

Another factor that has been found to influence the preference of the mites for specific brood cells is the age of the comb. Old comb cells tend to be more heavily infested than new comb cells (Piccirillo & De Jong, 2003).

It has been suggested that queen age and condition could affect *Varroa* populations (Akyol et al., 2007; Nazzi & Le Conte, 2016). In an early experiment originally designed to determine the flow of mites from the phoretic phase on adult bees to the reproductive phase in worker brood cells, it was found that the brood infestation increased considerably after the queen was removed and queen cells were constructed. This behavior was consistent in various repetitions. As the number of brood cells decreased, due to a lack of new brood in the queenless colonies, the number of invading mites per brood cell increased dramatically, resulting in infestations of up to 24 original mother mites in a single worker brood cell (De Jong, 1981).

Attempts to repeat this in later years showed an opposite tendency.

Materials and methods

The analyzes were carried out in the experimental apiary of the Laboratory of Biology, Behavior and Genetics of Bees, integrated to the Department of Genetics of the Faculty of Medicine of Ribeirão Preto - SP, Brazil, between the months of July 2022 to January 2023.

Six bee hives of the *Apis mellifera* species (africanized bee) were used. Each hive had ten frames of brood and food (pollen and honey) in a Langstroth hive.

All frames, on each side of the combs, were marked with a pen to arrange them in the same configuration during return after analysis, so that possible changes in the combs could be checked and recorded.

With the aim of analyzing the existence and accounting for the amount of mites of the *Varroa destructor* species parasitizing the bee population, it was determined to remove brood frames for three subsequent days and, using forceps, the newly capped larvae were removed from the combs, in search of parasitic mites. This first activity represents the control experiment, as the analyzes were carried out in standard hives, that is, with a typical population with the natural castes of the species, food reserve and no other interfering pathology.

After counting the mites present in a given hive, the queen bee was removed and stored in a specific cage, with food and nursing bees, on the same day, frames were marked with still uncapped immatures in order to monitor, over the days, the operculation of the brood cells by the workers, since the objective was to analyze only newly operculated brood cells. After 48 hours, the hive without a queen bee had its marked frames removed and, in the laboratory, using the same extraction technique, the larvae were removed to look for the mites. Unlike the analysis of the control experiment, where it was carried out in 3 subsequent days, the investigation of the hive without the queen bee took 3 days as well, but with an interval of 24h to 48h between the previous days.

A large part of the newly sealed brood cells were examined in the search for mites. When the combs contained few closed cells, all were uncapped, when the entire comb was full of closed cells, about 200 cells were randomly uncapped on each side of the frame.

At the end of the analysis of a given hive, first with the presence of the queen bee and later without the queen bee, the amounts of mites found were compared.

To calculate the mite infestation rate, the mites and larvae removed from the brood cells are counted, the number of mites is divided by the number of larvae and multiplied by 100, resulting in the percentage of parasitic mites in that sample.

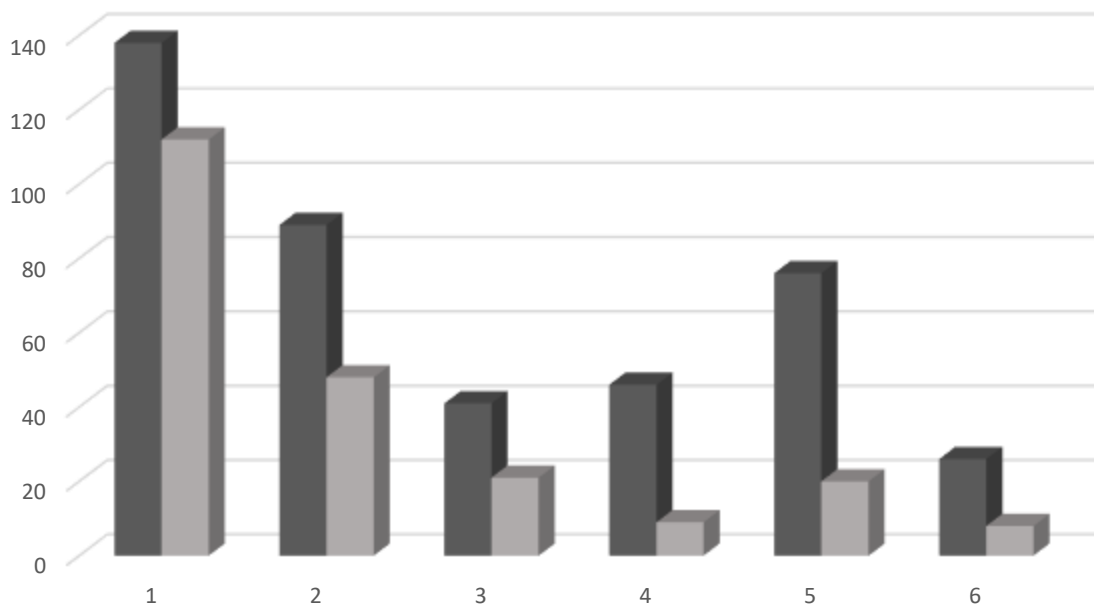
In each of the six hives used, the presence of mites in adult bees was also investigated. An amount of worker bees equivalent to 150ml in the beaker was collected in a 600ml beaker with 200ml of 90% ethyl alcohol. The contents of the beaker were poured into a specific container (Varroa easyCheck™) to remove mites from adult bees and shaken by hand for 1 minute. The dislodged mites are counted at the bottom of the container and subsequently the bees are also counted.

Results

The difference found between the two treatments of the six hives used was significant, both in relation to the number of infested worker brood cells and the total number of mites infesting such cells.

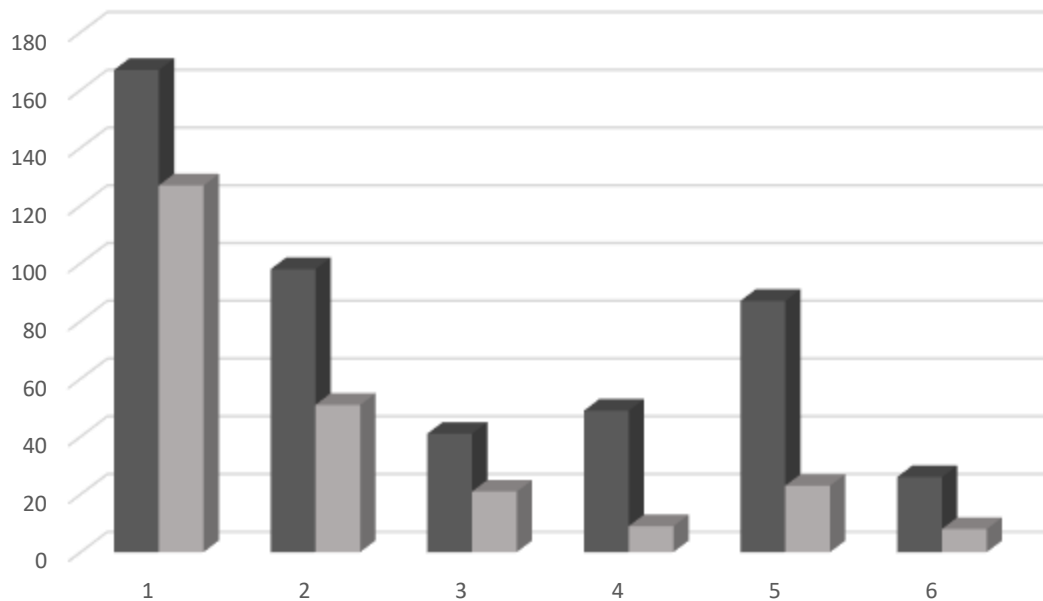
The amount of brood cells infested with mites was lower in the treatment of hives without the queen bee, when compared to the treatment of control hives, with the presence of the queen bee ($P = 0.0350$ Paired t-test $T = 2.87 - 5$ df) (Figure 1).

Figure 1 – Graph representing the number of varroa-infested brood cells in the six analyzed hives: black bar = control hives (with queen); gray bar = queenless hives.



Regarding the total amount of mites found in brood cells, there was also a greater amount in hives with a queen ($P = 0.0367$ Paired t-test $T = 2.83 - 5$ df) (Figure 2).

Figure 2 – Graph representing the total amount of mites infesting the brood cells; black bar = control hives (with queen); gray bar = queenless hives.



The largest number of uncapped alveoli occurred in hive number 1, in the “queenless” treatment, with a total of 894 open cells. The lowest amount of uncapped alveoli occurred in hive number 5, in the control treatment, with a total of 267 open cells. This discrepancy in quantities is due to the availability of newly capped brood cells to be used in the analyses.

In hive number 1, 117 more cells were opened in the “without queen” treatment, and 26 less infested cells were recorded than in the control (with queen).

In hive number 2, 63 more cells were opened in the control treatment, and 41 more infested cells were registered than in the “queenless” treatment.

In hive number 3, 68 more cells were opened in the “queenless” treatment, and 20 less infested cells were registered than in the control.

In hive number 4, 26 more cells were opened in the “queenless” treatment, and 37 less infested cells were recorded than in the control.

In hive number 5, 98 more cells were opened in the “queenless” treatment, and 56 less infested cells were registered than in the control.

In hive number 6, 24 more cells were opened in the “queenless” treatment, and 18 less infested cells were recorded than in the control.

Of the six hives analyzed, four presented more than twice the difference between the control and “queenless” treatments, both in the total number of infested cells and in the total number of mites found in the brood cells (Table 1).

Analyzing each hive individually, all showed significant differences between the amounts of mites found in the two treatments ($p < 0.05$).

Table 3. 1 - Number of brood cells analyzed, number of infested cells and respective number of mites found in each hive. Column 1 indicates the identification of the hive and which treatment the hive underwent (Q = with queen; no Q = without queen).

colony	brood cells	infested	% infested	n Varroa	mean V/100 cells	V/infested cell
1 Q	777	138	17,8	167	21,5	1,21
1 no Q	894	112	12,5	127	14,2	1,13
2 Q	774	89	11,5	98	12,7	1,10
2 no Q	711	48	6,8	51	7,2	1,06
3 Q	507	41	8,1	41	8,1	1,00
3 no Q	575	21	3,7	21	3,7	1,00
4 Q	522	46	8,8	49	9,4	1,07
4 no Q	548	9	1,6	9	1,6	1,00
5 Q	267	76	28,5	87	32,6	1,14
5 no Q	365	20	5,5	23	6,3	1,15
6 Q	306	26	8,5	26	8,5	1,00
6 no Q	330	8	2,4	8	2,4	1,00

The hives analyzed did not have drone brood cells during the experiments and observations. The existence of such drone cells in the analyzed hive may interfere with the levels of infestation of worker brood cells, since the mites prefer drone brood to complete their reproductive cycle.

No other parasites were detected either at high levels of contamination in the investigated hives, at least not to the point of harming the development of the nest. In some combs, sporadically, the following individuals were found: *Aethina tumida* (hive beetle) and *Galleria mellonella* (wax moth); no bee larvae with signs of apparent bacteriosis or mycoses were observed.

After, on average, 48 hours after the queen bee was removed, there were already queen brood cells, with the larva inside, under construction in the combs. The maintenance and development of the real brood cells was monitored and the operculations were observed, revealing the conventional behavior of the workers when producing new queens because they noticed the absence of the original queen.

The mites found inside the brood cells were mother females, other mites in other stages of development were not found (e.g. protonymph, deutonymph), since uncapping occurred a maximum of 48 hours after operculation by the workers, and as the female mite lays eggs after 70h of operculum closure (Steiner et al., 1994), there were no offspring in the brood cell.

The amount of mites attached to the bodies of adult bees, in the control treatment and in hives without the queen bee, did not show significant differences ($P=0.287$ Paired t-test $T= 1.19 - 5$ df). In five of the six hives analyzed, the rate of mites parasitizing adult bees was higher in the hive with the queen bee (Table 2).

Table 3. 2 - Number of mites and number of adult bees collected. Column 1 indicates the identification of the hive and which treatment the hive underwent (Q = with queen; no Q = without queen).

Colony	n Varroa	n Adult bees	%
1 Q	7	290	2,4
1 no Q	9	456	2
2 Q	24	467	5,1
2 no Q	8	365	2,2
3 Q	5	112	4,5
3 no Q	6	170	3,5
4 Q	4	311	1,3
4 no Q	4	328	1,2
5 Q	9	272	3,3
5 no Q	3	220	1,4
6 Q	2	322	0,6
6 no Q	6	260	2,3

Discussion

There are few studies in the literature that seek to investigate the influence of the presence/absence of the queen bee on the infestation of the mite *V. destructor*. Those that exist, despite following methodologies very similar to this work, present data that are different from those described in this article.

De Jong (1981) describes an increase in the rate of mite invasion of brood cells immediately after construction of queen brood cells. In his experiment, the queen was removed and the invasion of worker cells followed in the search for varroas. The author observes that, with the construction of royal cells, invasion rates increase considerably (64 mites in 79 cells before royal cell construction as opposed to 721 mites in 374 cells after royal cell construction), mainly in the cells around the royal cells and in the comb that contained them, different from the opposite comb (without royal cells). These results are contrary to those described in this article, which follows approximately the same methodology. This fact may indicate that one of the predominant factors for the antagonism in the results may be the difference between haplotypes of *V. destructor* that occurred in the two experiments. In 1981, the dominant haplotype was Japanese (Thai), whereas in this article (2023), the haplotype is Korean (Russian), which in addition to its molecular differences (mtDNA) is more virulent (De Guzman & Rinderer, 1999; Garrido et al., 2003) and may have a higher transmissive viral load (Vetharianian & Barlow, 2006).

V. destructor was introduced in Brazil in 1972, coming from colonies infested in Paraguay (Crane, 1978; De Jong et al., 1982) with the Japanese-type mite (haplotype J) (De Jong & Gonçalves, 1981b). This type of varroa persisted with its parasitic relationship for a few decades in Brazil until investigations carried out in 2001 revealed the substitution and prevalence of the K haplotype, according to Garrido et al. (2003). Strapazzon et al. (2009) and Guerra Jr. et al. (2010) through genetic characterizations also confirmed the existence of the K haplotype in samples collected in the states of São Paulo and Santa Catarina, proving the replacement of the type of mite parasite of *A. mellifera* bees in Brazil.

The cited difference in haplotypes may influence the results, however, as shown by Calderón et al. (2010) and Garrido et al. (2003), the haplotype alone does not interfere with infestation levels; the observations made by the two authors when comparing levels of infestation in Mexico and Brazil, with the same haplotype of varroa (K) and with the same type of bee (Africanized), showed different results, where in Brazil the levels of infestation were lower, so it must be concluded that there are other

factors that, together, coordinate the parasite/host relationship between *A. mellifera* and *V. destructor*.

Mendonça (2017), carried out a study similar to that of De Jong (1981), investigating whether the absence of the queen bee influences the rate of infestation of the mite *V. destructor*. The research methodology is also similar to the present work, but the results are different from those found by De Jong (1981), and from the results found in this research in question. According to Mendonça (2017), after removing the queen bee from the hive, the level of mite infestation did not show significant differences when compared to the levels in control hives. Despite this difference, Mendonça (2017) and De Jong (1981) described the same observation in relation to the increase in infestation near to royal cells, a fact that was also not found in this work.

In this investigation, the amount of mites invading worker brood cells was lower in hives whose queen bee was absent. In addition to the previously mentioned differences in mite haplotypes, two hypotheses can be raised to explain this change in infestation levels. The first hypothesis is based on the fact that mites prefer adult bees in the nursing phase to complete their phoretic cycle (Del Piccolo et al., 2010). According to Pankiw et al. (1998) and Hoover et al. (2003), the pheromone of the queen's mandibular gland (QMP) interferes in the ontogeny of worker bees, delaying their development into foragers when the hives are supplemented with this pheromone, in the absence of the QMP (situation where the hive is without the queen, for example), development to the foraging stage is accelerated, so with more foragers than nursing bees, the mites are left without the appropriate adult individuals to start their phoretic cycle. Winston & Slessor (1998), Slessor et al. (2019) and Kaatz et al. (1992) also confirmed this suppression in the ontogeny of workers, as the QMP inhibits the synthesis of the juvenile hormone in them, delaying development and, consequently, the incorporation of new tasks in the nest. With the accelerated development of workers in hives without the queen bee, the number of bees in the foraging phase is greater, this situation interferes with the host selection performed by the mites, since the unsaturated hydrocarbons ((Z)-8-heptadecene) found in the cuticle of foragers that carry pollen act as repellents for mites (Del Piccolo et al. 2010; Nazzi & Le Conte, 2016). This atypical condition in the hive may also be one of the factors that explain the drop in the level of acarine invasion.

The second hypothesis is related to the increase in production and maintenance of brood cells of queen bees (royal cells), a situation found after, on average, 48 hours

after the removal of the queen bee from the nest. Octanoic acid, abundant in royal jelly and queen cells, but scarce in worker cells, acts as a varroa repellent (Calderone et al., 2002; Nazzi et al., 2009), thus, the excessive amount of royal cells in the queenless hive can repel the invasion of mites in worker cells.

The interpretation of the data from this work makes it possible to confirm that the presence/absence of the queen bee in the nest interferes with the levels of infestation of the mite *V. destructor* in bee brood, revealing higher rates of varroa invasion in hives with the queen present. Despite this result, there are still numerous works in the literature that reveal other factors that also influence infestation levels, such as: chemical stimuli from the cuticle of larvae and from larval food (Nazzi, F. et al. 2001; Calderoni, N.W. & Lin, S. 2001); the distance from the edge of the brood cell (Boot, W.J. et al., 1995) and depth in relation to the larva (De Ruijter, A. & Calis, J., 1988); the physical structure of the alveoli and the age of the comb (Piccirillo, G.A. & De Jong, D., 2004); the amount of brood cells with larvae of adequate age for mite invasion (Calis, et al. 1991); the environment climate (Moretto et al. 1991; De Jong et al., 1984); the type of bee (Gonçalves et al., 1995; Piccirillo & De Jong, 2003).

Despite the decrease of mites invading and parasitizing the bee brood in hives without a queen bee, the analysis of adult bees with mites did not reveal significant differences between the two treatments. Regardless, there was a higher rate of mites in samples of adult bees from hives with queen bees, but the difference was too small to consider an important factor in reducing infestation in hives.

There are more than fifty years of studies on varroaosis and much has already been revealed, but there are still many obscure points to understand in the search for a more sustainable management of hives, which avoids the intensive use of acaricides and preserves bee products from contamination and the health of bees and ecosystems.

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6. DISCUSSÃO GERAL

A Varroatose, há mais de 60 anos, desafia os apicultores e pesquisadores na busca por controle e manejos que possam beneficiar a criação de abelhas e formação dos produtos da colmeia de maneira segura e sustentável. Patologia apícola causada pelo ácaro ectoparasita *Varroa destructor* atinge a espécie de abelha mais requerida na produção de alimentos na agricultura mundial, a espécie *Apis mellifera*.

O conhecimento científico e empírico se amplia a cada dia, novos tratamentos, instrumentos e protocolos são desenvolvidos, testados e divulgados, entretanto a evolução e a adaptação do ácaro ao controle humano dificultam a erradicação desta parasitose, que gera inúmeros prejuízos à atividade de criação de abelhas.

Uma das formas de controle da Varroatose é a aplicação de produtos químicos nas colmeias, existem compostos orgânicos como ácido fórmico, oxálico e óleo de timol, e substâncias sintéticas, como formamidina, piretróides, flumetrina (Jack & Ellis, 2021). Este procedimento vem sendo usado há décadas e permite, se aplicado corretamente, o desenvolvimento e a manutenção de colmeias a ponto de possibilitar a atividade de apicultura, contudo provoca efeitos colaterais indesejáveis, como a contaminação dos produtos da colmeia (Wallner, K. 1999) e a possibilidade de fortalecimento da população acarina, através do desenvolvimento de resistência ao acaricida (Milani, N. 1999).

Outras maneiras de controlar a infestação do ácaro nas colmeias, como citado anteriormente, são: supressão da cria no verão, para diminuir a população da abelha quando chegarem as estações mais frias; monitoramento da infestação com a observação da queda de ácaros mortos no fundo da colmeia; criação de populações de abelhas mais resistentes, com foco no comportamento higiênico. Esses procedimentos geram menos efeitos adversos, são mais sustentáveis por não utilizarem produtos químicos tóxicos, mas exigem maior conhecimento por parte de quem realiza os manejos, além de gastar mais tempo do que a aplicação de substâncias nas colmeias despende.

Os experimentos realizados neste trabalho mostraram que a ausência da rainha na caixa ninho diminui o nível de infestação do ácaro *V. destructor*. Quando a rainha foi isolada em uma das duas caixas que formavam a colmeia do tipo sobreninho, alguns sobreninhos analisados na caixa sem a rainha apresentaram

menores níveis de infestação. Uma hipótese foi traçada para explicar essa diferença de quantidades de ácaros encontrados. A hipótese se baseia no fato de que o número de abelhas em caixas do tipo sobreninho é muito elevado (aprox. 100.000), essa situação interfere negativamente na transmissão dos feromônios liberados pela abelha-rainha (Trhlin et al. 2011). Esses sinais químicos controlam a homeostase social, a maturação dos ovários das operárias e o desenvolvimento etário das mesmas (Slessor et al. 2005). O ácaro necessita de abelhas operárias em fase de nutriz para seu ciclo dispersivo (Del Piccolo et al., 2010), entretanto, com a diminuição (ou ausência) do feromônio real na caixa sem a rainha, ocorre um aceleração no amadurecimento até a fase de forrageira (Hoover et al. 2003; Slessor et al. 2019) diminuindo, assim, as abelhas nutrizas do ninho, dificultando, ou impedindo, a continuidade do ciclo de vida do *Varroa*, situação que pode ter gerado uma redução da infestação de ácaros nesta caixa.

Houve sobreninhos que não apresentaram diferenças significativas entre as quantidades de ácaros encontrados na caixa com e sem rainha, nesses sobreninhos, a abelha-rainha, livre em uma das caixas ninho, pode ter transitado com maior frequência próximo à tela excludora, ou seja, no limite entre as duas caixas, facilitando assim a transmissão de seus feromônios para a caixa que não obtinha acesso, simulando a normalidade de controle real sobre as operárias.

O experimento no qual a abelha-rainha foi totalmente retirada do ninho obteve resultados mais significativos, no quesito de diminuição da quantidade de ácaros encontrados na colmeia sem a rainha. Este procedimento utilizou apenas uma caixa ninho, em contrapartida, a rainha foi extraída da colmeia, diferentemente de apenas isolá-la em uma das duas caixas tipo sobreninho. As hipóteses que podem explicar a diminuição dos ácaros na caixa sem a rainha são: como citado acima, em relação ao experimento de isolamento da rainha na colmeia tipo sobreninho (recria), a ausência do feromônio real pode acelerar o amadurecimento das operárias até a fase de forrageira, diminuindo as nutrizas da população, interferindo negativamente no ciclo dispersivo dos ácaros, essa situação fica ainda mais patente com a retirada total da rainha do ninho. Outra hipótese está relacionada à repelência que o ácido octanóico gera no ácaro (Calderone et al., 2002; Nazzi et al., 2009). Este ácido está em abundância nas células de cria de rainhas e após a retirada da abelha-rainha da colmeia houve grande produção de tais células de cria, fator que pode ter inibido a invasão dos ácaros nos alvéolos, diminuindo a infestação.

De Jong (1981) realizou um experimento semelhante, entretanto obteve resultados antagônicos. Em seu experimento, a abelha-rainha foi removida da colmeia, posteriormente à remoção, as operárias, como de praxe, construíram células de cria para rainhas (realeiras). O autor então observou um grande aumento na infestação após a construção das realeiras (na ausência da rainha), constatação contrária aos resultados deste trabalho. Uma explicação para essa diferença nos níveis de infestação pode ser o haplótipo de *Varroa* que parasitava as colmeias nos dois experimentos. Em 1981, o haplótipo era do tipo japonês, menos virulento do que o haplótipo coreano (De Guzman & Rinderer, 1999), que estava presente no trabalho em questão, essa diferença entre os haplótipos do *Varroa* pode ter determinado o antagonismo entre os resultados dos dois trabalhos, considerando principalmente que no período que De Jong (1981) realizou sua pesquisa, as abelhas estavam ainda se adaptando ao ácaro, por isso os níveis de infestação eram altos, se comparados ao presente.

São necessários maiores aprofundamentos na questão da influência que a rainha exerce na Varroatose. Fatores como: sinergia entre sinais químicos da rainha, imaturo e alimento larval precisam ser explorados, bem como fatores interferentes que a presença da rainha influi sobre a invasão dos ácaros nos alvéolos precisam ser melhor compreendidos. Manejos de controle do *Varroa* utilizando a rainha são interessantes e visam a diminuição, ou interrupção, do uso de acaricidas nas colmeias, possibilitando tratamentos mais sustentáveis na apicultura.

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8. Anexo – Fotografias dos materiais e procedimentos utilizados

Foto 1. Colmeia tipo sobreninho (recria)



Fonte: Arthur Nascimento

Foto 2. Sobreninho dividido por tela excludora de rainhas



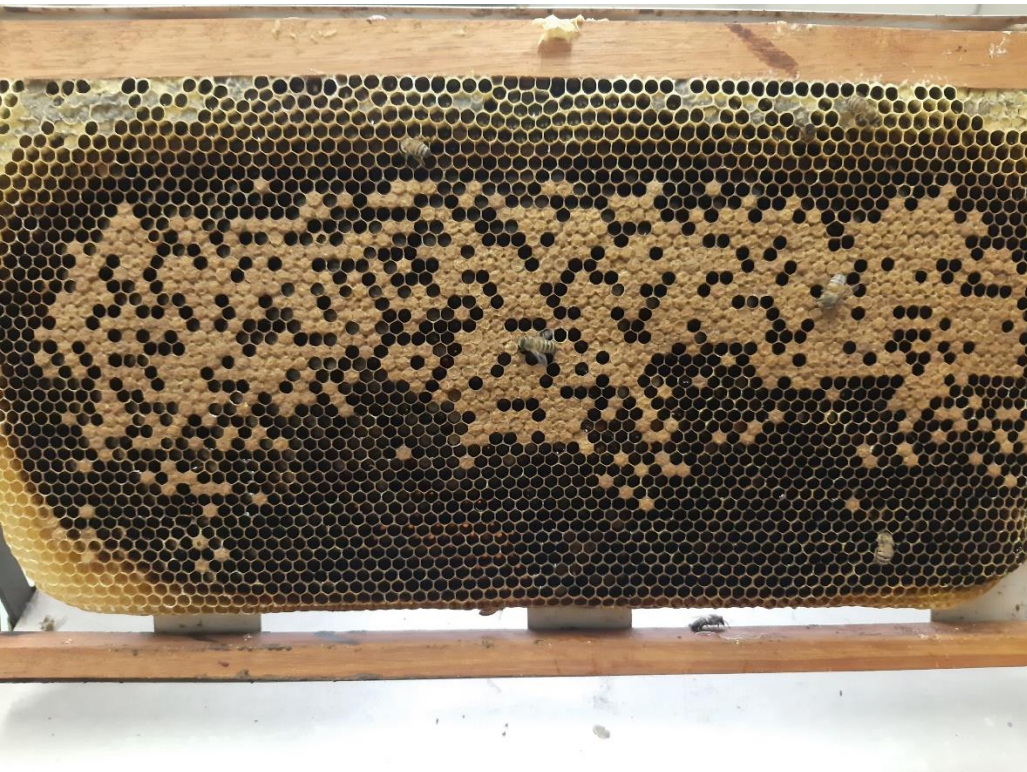
Fonte: Arthur Nascimento

Foto 3. Tela excludora de rainhas



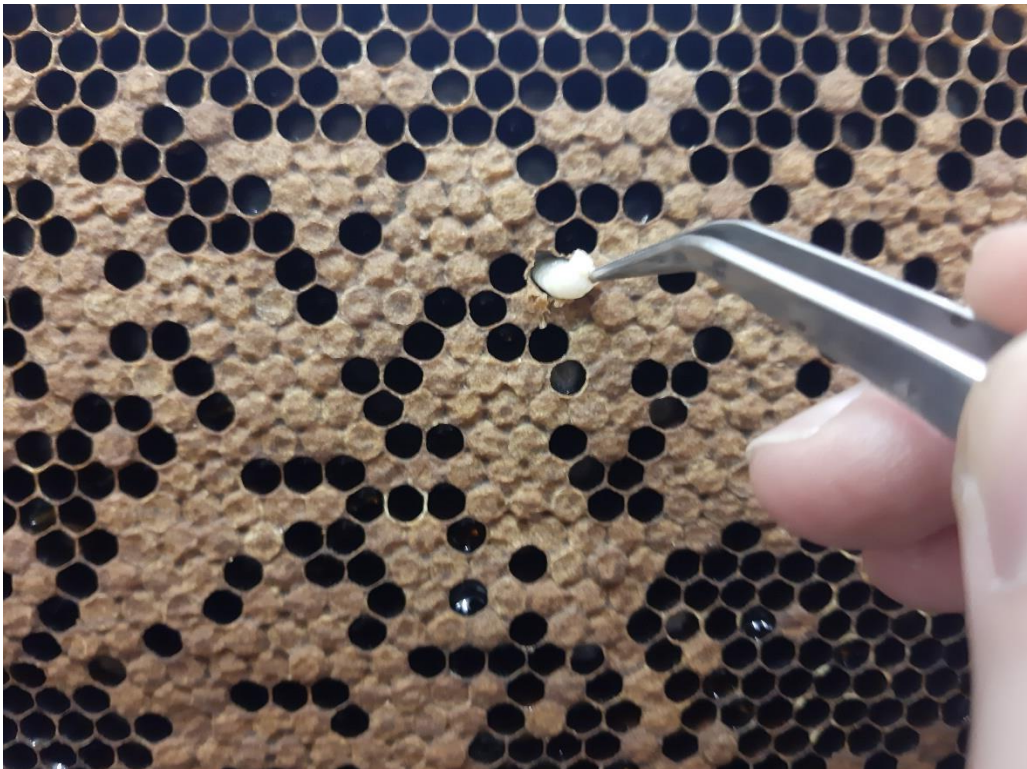
Fonte: Arthur Nascimento

Foto 4. Quadro com favo de cria operculada



Fonte: Arthur Nascimento

Foto 5. Desoperculação do alvéolo de cria e remoção de larva com pinça



Fonte: Arthur Nascimento

Foto 6. *Varroa* fêmea imersa no alimento larval



Fonte: Arthur Nascimento

Foto 7. Gaiola para abelha-rainha



Fonte: Arthur Nascimento