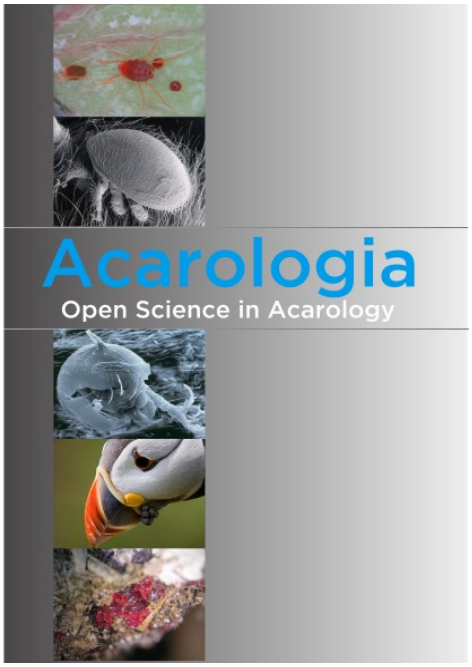

Acarologia

A quarterly journal of acarology, since 1959
Publishing on all aspects of the Acari

All information:
http://www1.montpellier.inra.fr/CBGP/acarologia/
acarologia-contact@supagro.fr

OPEN ACCESS

Acarologia is proudly non-profit,
with no page charges and free open access

Please help us maintain this system by
encouraging your institutes to subscribe to the print version of the journal
and by sending us your high quality research on the Acari.

Subscriptions: Year 2023 (Volume 63): 450 €
http://www1.montpellier.inra.fr/CBGP/acarologia/subscribe.php
Previous volumes (2010-2021): 250 € / year (4 issues)
Acarologia, CBGP, CS 30016, 34988 MONTFERRIER-sur-LEZ Cedex, France
ISSN 0044-586X (print), ISSN 2107-7207 (electronic)

The digitalization of Acarologia papers prior to 2000 was supported by Agropolis Fondation under the reference ID 1500-024 through the « Investissements d'avenir » programme (Labex Agro: ANR-10-LABX-0001-01)

INVESTISSEMENTS D'AVENIR

agropolis fondation
Supporting agricultural research for sustainable development

Acarologia
Open Science in Acarology

A method for semi-field rearing of *Varroa destructor* (Acari: Varroidae) to obtain mites of controlled age and specific life cycle

Irina Muntaabski[a], Romina M. Russo[a], María C. Liendo[a], Lucas Landi[b], Silvia B. Lanzavecchia[a], Alejandra C. Scannapieco[a]

[a] Instituto de Genética 'Ewald A. Favret' - Grupo vinculado al IABIMO (INTA - CONICET), Hurlingham, Buenos Aires, Argentina.
[b] Instituto de Recursos Biológicos - INTA, Hurlingham, Buenos Aires, Argentina.

Short note

ABSTRACT

Varroa destructor is one of the most devastating ectoparasites of the honey bee, *Apis mellifera*, worldwide. Given that *V. destructor* has very low survivability and exhibits no successful reproduction away from their natural environment and host and that the availability of mites for experimental purposes is limited by seasonality, several protocols of mite rearing under laboratory conditions have been developed. However, only one of these rearing systems has been able to yield a fertile second generation with a low mite survival. The aim of this study was to develop a semi-field rearing method to obtain mites of known age and life cycle that can be maintained through several generations. We registered and compared survival and reproductive parameters of mites of controlled age during four generations (P, F1, F2, F3) and evidenced no significant differences between these mite groups for these life-history traits. With present results we demonstrate that it is possible to successfully produce a third generation of mites under semi-field conditions. This study brings useful information about key conditions for the proper reproduction of mites in a controlled rearing system and provides a potential standardized method for *V. destructor* research, especially for host-parasite interaction experiments.

Keywords semi-field rearing; *Varroa destructor*; mite reproduction; *Apis mellifera*; host-parasite interaction

Received 26 April 2022
Accepted 26 February 2023
Published 28 March 2023

Corresponding author
Alejandra C. Scannapieco:
scannapieco.a@inta.gob.ar

Academic editor
Roy, Lise

https://doi.org/10.24349/eyu1-eweg

ISSN 0044-586X (print)
ISSN 2107-7207 (electronic)

Introduction

Varroa destructor (Acari: Varroidae) is an obligate ectoparasite of *Apis mellifera* (Hymenoptera: Apidae) that thrives exclusively in honey bee colonies (Rosenkranz *et al.*, 2010). The life cycle of this mite is divided into two phases: a commonly called phoretic phase on adult honey bees and a reproductive phase that occurs inside the sealed honey bee brood cells (reviewed by Traynor *et al.*, 2020). The process of obtaining *V. destructor* mites for experimental purposes under temperate climate is dependent upon season and weather conditions, time-consuming, and usually costly (Dietemann *et al.*, 2012; 2013). Indeed, the development of an *in vitro* rearing method for *V. destructor* is quite challenging as the mite has highly specific life cycle requirements (Rosenkranz *et al.*, 2010). Several authors have advanced in establishing different *in vitro* and semi *in vitro* rearing methods (Chiesa *et al.*, 1989; Bruce *et al.*, 1988; Beetsma and Zonneveld, 1992; Nazzi and Milani, 1994; Donzé and Guérin, 1994, 1997; reviewed by Vilarem *et al.*, 2021). These approaches showed promising results, but all attempts failed to yield a reproductively mature and fertile second generation of female mites and to obtain

How to cite this article Muntaabski I. *et al.* (2023), A method for semi-field rearing of *Varroa destructor* (Acari: Varroidae) to obtain mites of controlled age and specific life cycle. **Acarologia 63(2): 383-389.** https://doi.org/10.24349/eyu1-eweg

relatively large sample sizes. Here, we designed a semi-field protocol for *V. destructor* rearing and evaluated biological parameters of adult mites across several generations. In particular, the use of a mixed strategy of laboratory conditions alternated with the mite on its natural host, the honey bee, enabled the survey of four generations maintaining high mite survival and reproductive success. Our results provide key information to obtain mite progeny with controlled age and specific generation for biological assays.

Material and methods

Varroa destructor mites were obtained from *A. mellifera* colonies located at the Instituto de Genética, Instituto Nacional de Tecnología Agropecuaria (INTA, Buenos Aires, Argentina), during the summers between 2017 and 2019. All honey bee colonies were maintained under regular beekeeping practices, with the use of synthetic acaricides once a year, during early autumn. Prior to the experiments, phoretic mite loads from these colonies were assessed following the method described by Dietemann *et al.* (2013). Honey bee colonies were categorized according to their mite loads (high: < 5%, low: < 0.5%). Those colonies that exhibited a low mite load (hereafter named LL colonies, a total of 8 colonies) were used as donors of experimental honey bee combs (Figure 1) to prevent experimental brood cells from being naturally parasitized, prior to artificial infestation. Colonies with high mite loads (hereafter named HL colonies, a total of 8) were used as donors of phoretic mites (Figure 1), which were collected through the powdered sugar method (Dietemann *et al.*, 2013) and immediately transported to the laboratory. The mites in the powdered sugar were collected one by one with a fine-tipped paintbrush, rinsed with a drop of distilled water and transferred onto

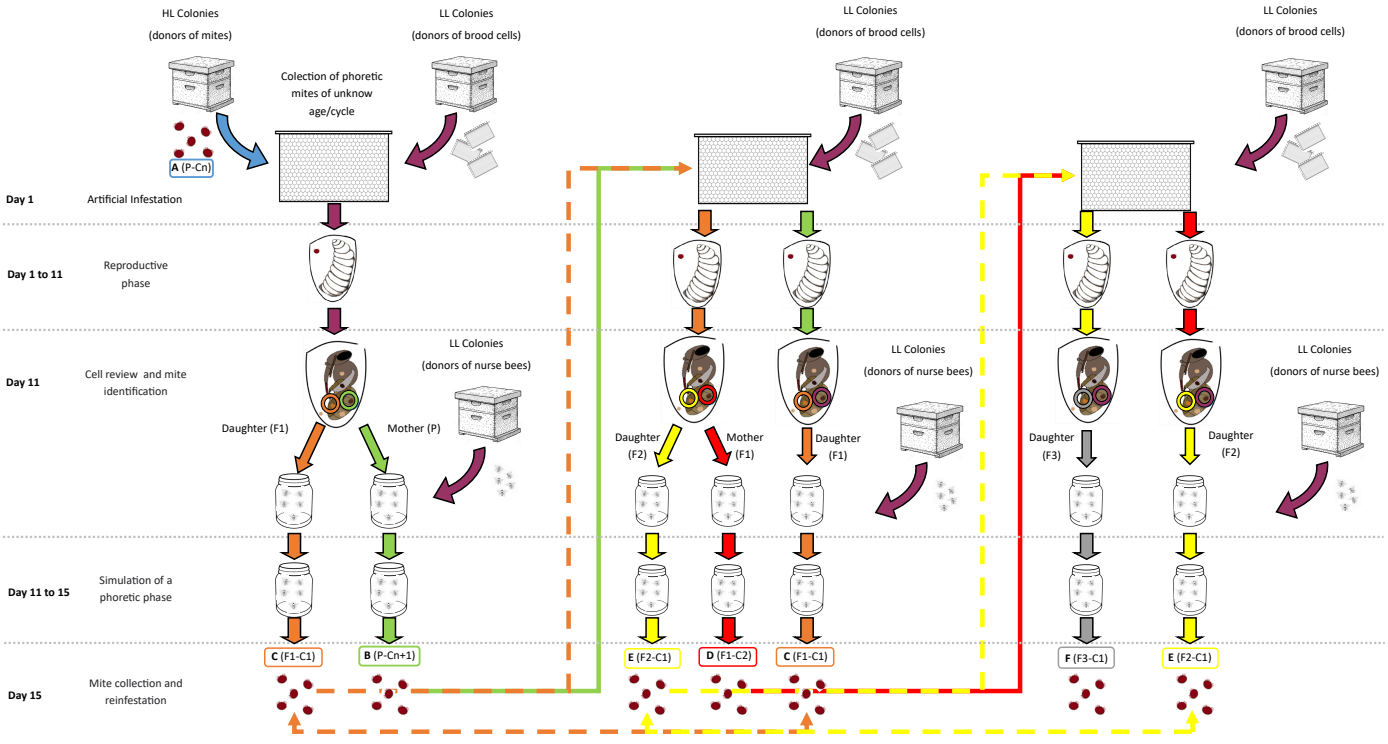

Figure 1 Experimental procedure for obtaining *V. destructor* mites of controlled age and reproductive cycle. Six groups of mites were obtained: group A (P-Cn): P foundress mites with an unknown number of previous reproductive cycles; group B (P-Cn+1): P foundress mites with at least one reproductive cycle within the rearing system; group C (F1-C1): F1 daughter mites in their first reproductive cycle; group D (F1-C2): F1 foundress mites in their second reproductive cycle; group E (F2-C1): F2 daughter mites in their first reproductive cycle; and group F (F3-C1): daughter mites in their first reproductive cycle.

a wet paper towel placed in a petri dish with a lid. The mites were placed in a chamber with controlled conditions of temperature and humidity (35 ± 1 °C and 60 ± 2%, respectively).

Frames with uncapped brood cells of LL colonies were chosen, and these cells were marked on a transparent acetate sheet (map). After the maps were drawn, the frames were placed back in the colonies. Five hours later, recently capped worker brood cells were identified, opened with a scalpel at one edge of the wax cap, and artificially infested with a mite (foundress) using a fine-tipped paintbrush. Subsequently, the cells were closed by gently pushing the capping back on the cell edge (Liendo *et al.*, 2021). Mites were used within approximately 1 h after collection, and those that could not clutch the paintbrush bristles were considered unhealthy and discarded. A total of 1954 mites were artificially infested in honey bee brood cells.

The frames containing the artificially-infested brood cells were placed in a chamber with controlled conditions of temperature and humidity (35 ± 1 °C and 60 ± 2%, respectively) for eleven days (Figure 1). Next, the capped brood cells were carefully opened with a scalpel, and the foundress and the presumably sexually mature daughters were collected. The daughter (F1) and foundress (P) mites were identified by their pigmentation (Rosenkranz *et al.*, 2010). Once identified, female mites were separated, immediately mounted on nurse bees obtained from LL colony brood frames, and placed in glass flasks (3L) (Figure 1). Each glass flask of P or F1 mites harbored thirty nurse bees and ten mites. The flasks were provided with containers with honey and water *ad libitum* and placed in a chamber with controlled conditions of temperature and humidity (35 ± 1 °C and 60%, respectively) in darkness for 4 days (Figure 1). This period was previously described as the time needed for spermatozoa maturation inside the spermatheca, simulating a phoretic phase (Häußermann *et al.*, 2016; 2020). Then, the mites were recovered from the bees and re-infested in newly-capped brood cells from the LL colonies, restarting a new reproductive cycle (Figure 1). This procedure was repeated for three consecutive generations and, as a handling control, the removal of artificially-mite-infested cells by worker bees and mite mortality were registered at day 11 post-infestation in each generation.

Reproductive parameters of *V. destructor* females were also recorded at day 11 post-infestation in six groups of mites that differed in generation (P: parental; F1: filial 1 mites; F2: filial 2 mites; F3: filial 3 mites) and reproductive cycle (C1: first or C2: second life cycle) (Figure 1). The mite groups were named and defined as follows: group A (P-Cn): P foundress mites with an unknown number of previous reproductive cycles; group B (P-Cn+1): P foundress mites with at least one reproductive cycle within the rearing system; group C (F1-C1): F1 daughter mites in their first reproductive cycle; group D (F1-C2): F1 foundress mites in their second reproductive cycle; group E (F2-C1): F2 daughter mites in their first reproductive cycle; and group F (F3-C1): daughter mites in their first reproductive cycle (Figure 1). Reproductive parameters were calculated according to Harbo and Harris (2009) and Häußermann *et al.* (2020): fertility (proportion of female mites with at least one male and/or female offspring); non-reproductive *V. destructor* (NRVD, proportion of female mites without offspring); non-viable offspring (NVO, proportion of female mites for which the entire offspring was male or the first female descendant emerged too late to allow reaching its sexual maturity before the bee emergence); and abnormal reproduction (AR, sum of NRVD and NVO). Data of reproductive parameters were analyzed by using the general linear mixed model (GLMM) with a binomial distribution and logit link function (NRVD *vs.* reproductive mites, NVO *vs.* viable offspring, and AR *vs.* normal reproduction) considering mite group (A, B, C, D, E, F) as a fixed factor and colonies as random factors. Similarly, data of cell removal and mite mortality were analyzed by using GLMM with a binomial distribution and logit link function (cell removed *vs.* cell not removed; dead mite *vs.* live mite). Odds ratios were used to compare mite mortality, reproductive parameters, and cell removal. Multiple comparisons were performed using Fisher LSD ($\alpha = 0.05$). In all cases, the dispersion of the residues was tested and analyzed. Random residues were analyzed with the Shapiro-Wilks test. To obtain the most appropriate structure of variance, the Akaike information criterion was used. All analyses were performed using the glmer function in R package "lme4" (Bates, 2015).

Results and discussion

From a total of 1954 mites artificially infested in honey bee brood cells, 707 (36.18%) were removed from cells by worker bees and 83 (4.25%) were registered dead (Supplementary Table S1). Statistical analysis showed no significant differences between P, F1, F2, and F3 generations for these variables ($p > 0.05$, GLMM results, Supplementary Table S2; Figure 2). As expected, the mean mite survival (95%) registered in our semi-field rearing experiment is higher than the values reported in *in vitro* rearing systems (80.5% in Jack *et al.* (2020), 78.5 and 50% for the first and the second mite generations, respectively, in Piou *et al.* (2020)).

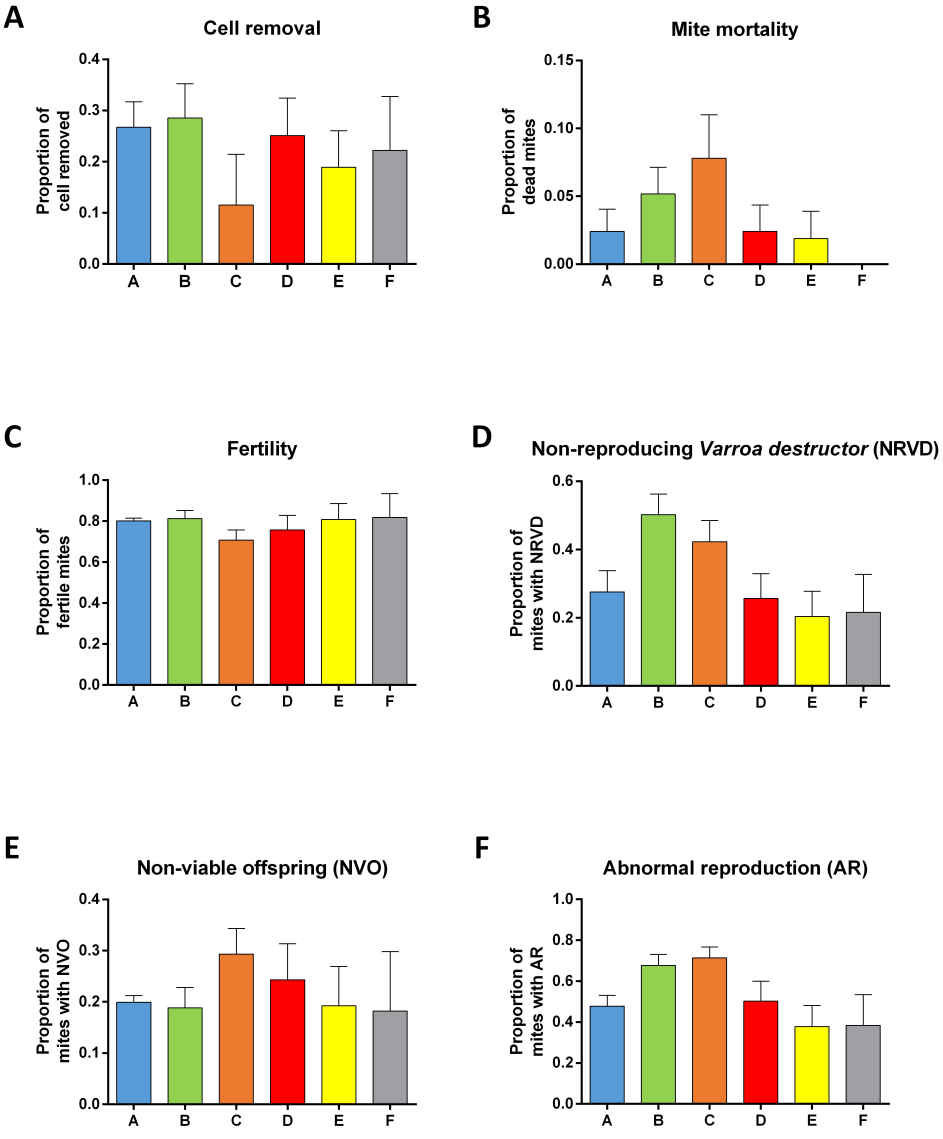

Figure 2 Mean proportion of artificially-infested *V. destructor* mites registered dead (A) or removed (B) from the honey bee brood cells for the six mite groups that differed in generation (P: parental; F1: filial 1 mites; F2: filial 2 mites; F3: filial 3 mites) and reproductive cycle (C1: first or C2: second life cycle). Reproductive parameters of *V. destructor* are shown in lower panels: fertility (C), non-reproductive *V. destructor* (D), non-viable offspring (E), and abnormal reproduction (F). The bar colors indicate mite group as follows: group A (P-Cn) in light blue, group B (P-Cn+1) in green, group C (F1-C1) in orange, group D (F1-C2) in red, group E (F2-C1) in yellow and group F (F3-C1) in gray. The error bars represent the standard deviation.

Regarding reproductive parameters analyzed on the remaining 1164 healthy female mites, we observed that 927 (79.64%) were fertile and 237 (20.36%) were non-fertile (Supplementary Table S1). This fertility value was similar to those obtained by Häußermann *et al.* (2020) and Piou *et al.* (2020) for *V. destructor* mites artificially infesting honey bee brood cells, and it was within the fertility range of 75–95% previously established for naturally reproducing *Varroa* females in temperate climate (Martin *et al.*, 1997; Garrido *et al.*, 2003; Fries *et al.*, 2011; Frey *et al.*, 2013). The relatively high mite fertility might indicate that the host cues and/or physiological status required for *V. destructor* reproduction were maintained in our system regardless of mite handling, as previously described by Kirrane *et al.* (2011) and Frey *et al.* (2013).

Of the 927 fertile mites, 548 (59.12%) produced viable offspring (normal reproduction) and 379 mites (40.88%) produced non-viable offspring (NVO). The mean percentage of mites that exhibited abnormal reproduction (NVO + non-reproductive mites) was 52.92% (616 mites) (Supplementary Table S1). A relatively high percentage of successful reproducing mites (mean value of 47.1% of mites exhibited normal reproduction) was obtained in our system. Even though previous studies reported similar percentages of 24, 37, and 48% of reproductive F1 mites (Beetsma and Zonneveld, 1992; Häußermann *et al.*, 2020; Piou *et al.*, 2020), these values were estimated by considering only mite fertility and not the actual *V. destructor* reproductive success, as in the present study.

Paired comparisons between mite groups revealed no significant differences for mite fertility, NRVD, NVO, or AR ($p > 0.05$; GLMM results, Supplementary Table S2; Figure 2). Previous developed systems for maintaining *in vitro* populations of *V. destructor* on its natural host showed that female mites exhibited very limited reproduction (Egekwu *et al.*, 2018) and that *V. destructor* reproduction *in vitro*, specifically past F1 generation, continues to be a challenge (Piou *et al.*, 2020). The use of our semi-field protocol would allow researchers to overcome this difficulty and, at the same time, enable a relatively large volume of rearing mites to be obtained and maintained for several generations. In addition, our results show that artificially introduced *V. destructor* females can reproduce at a rate similar to that of naturally-invaded mites informed in the bibliography. Hence, this semi-field protocol is a promising, effective, and inexpensive strategy to be included in standard methods for *V. destructor* research. As artificial infestation includes handling and physiological manipulation of *V. destructor* females, the analysis of other reproductive parameters, such as mite fecundity, must be further evaluated for our rearing system. More studies are also needed to clarify the factors involved in the potential differences observed in survival between *V. destructor* foundress and daughter mites beginning their first reproductive cycle, because these are crucial data for developing a large-scale rearing system for this mite species.

In conclusion, the high and similar survival values and reproductive success registered across mite generations indicate that this semi-field rearing method is suitable to analyze biological parameters of *V. destructor* in studies that involve a relatively high number of adult mites and several generations and reproductive cycles.

Acknowledgments

We thank Dr. Maria Alejandra Palacio for her contributions on drafting the manuscript and Luciano Landi for his support in honey bee colony management.

Funding

This study was funded by the Agencia Nacional de Promoción Científica y Tecnológica of Argentina through the project Foncyt-PICT 2020-2564, and by Instituto Nacional de Tecnología Agropecuaria (INTA) through Grant PE-E1-I017.

ORCID

Irina Muntaabski https://orcid.org/0000-0002-0631-791X
Romina M. Russo https://orcid.org/0000-0001-6888-9123
María C. Liendo https://orcid.org/0000-0002-8464-5652
Silvia B. Lanzavecchia https://orcid.org/0000-0001-5965-4082
Alejandra C. Scannapieco https://orcid.org/0000-0002-4228-2996

References

Bates D., Mächler M., Bolker B.M., Walker S.C. 2015. Fitting Linear Mixed-Effects Models Using lme4. J Stat Softw 67:1-48. https://doi.org/10.18637/jss.v067.i01

Beetsma J., Zonneveld K. 1992. Observations on the initiation and stimulation of oviposition of the *Varroa* mite. Exp Appl Acarol 16:303-312. https://doi.org/10.1007/BF01218572

Bruce W.A., Chiesa F., Marchetti S., Griffiths D.A. 1988. Laboratory feeding of *Varroa jacobsoni* Oudemans on natural and artificial diets (Acari: Varroidae). Apidologie 19:209-218 https://doi.org/10.1051/apido:19880209

Chiesa F.M., Milani N., D'Agaro M. 1989. Observations on the reproductive behaviour of *Varroa Jacobsoni* Oud. Techniques and preliminary results. In: Cavalloro R (ed) Proceedings of a meeting of the ECExperts' Group, Udine, Italy, 28-30 November 1988. Commission Of The European Communities, Brussels, Luxembourg, pp 213-222

Dietemann V., Nazzi F., Martin S.J., Anderson D.L., Locke B., Delaplane K.S., Wauquiez Q., Tannahill C., Frey E., Ziegelmann B., Rosenkranz P., Ellis J.D. 2013. Standard methods for *Varroa research*. J Apic Res 52:1-54. https://doi.org/10.3896/IBRA.1.52.1.09

Dietemann V., Pflugfelder J., Anderson D., Charriere J.D., Chejanovsky N., Dainat B., Neumann P. 2012. *Varroa destructor*: research avenues towards sustainable control. J Apic Res 51:125-132. https://doi.org/10.3896/IBRA.1.51.1.15

Donze G., Guerin P.M. 1994. Behavioral-attributes and parental care of *Varroa* mites parasitizing honey bee brood. Behav Ecol Sociobiol 34:305-319. https://doi.org/10.1007/BF00197001

Donzé G., Guerin P.M. 1997. Time-activity budgets and space structuring by the different life stages of *Varroa jacobsoni* in capped brood of the honey bee, *Apis mellifera*. J Insect Behav 10:371-393. https://doi.org/10.1007/BF02765605

Egekwu N.I., Posada F., Sonenshine D.E., Cook S.C. 2018. Using an in vitro system for maintaining *Varroa destructor* mites on *Apis mellifera* pupae as hosts; studies of mite longevity and feeding behavior. Exp Appl Acarol 74:301-315. https://doi.org/10.1007/s10493-018-0236-0

Frey E., Odemer R., Blum T., Rosenkranz P. 2013. Activation and interruption of the reproduction of *Varroa destructor* is triggered by host signals (*Apis mellifera*). J Invertebr Pathol 113:56-62. https://doi.org/10.1016/j.jip.2013.01.007

Fries I., Lindström A., Rosenkranz P., Frey E., Odemer R., Schroeder A., de Miranda J.R., Yanez O., Paxton R.J. 2011. The principal parasites and pathogenes of honeybees. Bees in Europe and Sustainable Honey Production, ISBN: 978-1-61209-336-9

Garrido C., Rosenkranz P., Paxton R.J., Gonçalves L.S. 2003. Temporal changes in *Varroa destructor* fertility and haplotype in Brazil. Apidologie 34:535-541. https://doi.org/10.1051/apido:2003041

Harbo J.R., Harris J.W. 2009. Responses to *Varroa* by honey bees with different levels of Varroa Sensitive Hygiene. J Apic Res 48:156-161. https://doi.org/10.3896/IBRA.1.48.3.02

Häußermann C.K., Giacobino A., Munz R., Ziegelmann B., Palacio M.A., Rosenkranz P. 2020. Reproductive parameters of female *Varroa destructor* and the impact of mating in worker brood of *Apis mellifera*. Apidologie 51:342-355. https://doi.org/10.1007/s13592-019-00713-9

Häußermann C.K., Ziegelmann B., Rosenkranz P. 2016. Spermatozoa capacitation in female *Varroa destructor* and its influence on the timing and success of female reproduction. Exp Appl Acarol 69:371-387. https://doi.org/10.1007/s10493-016-0051-4 https://doi.org/10.1007/s10493-016-0051-4

Jack C.J., Dai P.L., van Santen E., Ellis J.D. 2020. Comparing four methods of rearing *Varroa destructor* in vitro. Exp Appl Acarol 80:463-476. https://doi.org/10.1007/s10493-020-00488-0

Kirrane M.J., De Guzman L.I., Rinderer T.E., Frake A.M., Wagnitz J., Whelan P.M. 2011. Asynchronous development of honey bee host and *Varroa destructor* (Mesostigmata: Varroidae) influences reproductive potential of mites. J Econ Entomol 104:1146-1152. https://doi.org/10.1603/EC11035

Lourenço S., Palmeirim J.M. 2008. Which factors regulate the reproduction of ectoparasites of temperate-zone cave-dwelling bats? Parasitol Res 104:127-134. https://doi.org/10.1007/s00436-008-1170-6

Martin S., Holland K., Murray M. 1997. Non-reproduction in the honeybee mite *Varroa jacobsoni*. Exp Appl Acarol 21:539-549. https://doi.org/10.1023/A:1018492231639

Nazzi F., Milani N. 1994. A technique for reproduction of *Varroa jacobsoni* Oud under laboratory conditions. Apidologie 25:579-584. https://doi.org/10.1051/apido:19940608

Piou V., Vétillard A. 2020. *Varroa destructor* rearing in laboratory conditions: importance of foundress survival in doubly infested cells and reproduction of laboratory-born females. Apidologie 51:968-983. https://doi.org/10.1007/s13592-020-00775-0

Rosenkranz P., Aumeier P., Ziegelmann B. 2010. Biology and control of *Varroa destructor*. J Invertebr Pathol 103:S96-S119. https://doi.org/10.1016/j.jip.2009.07.016

Traynor K.S., Mondet F., de Miranda J.R., Techer M., Kowallik V., Oddie M.A., Chantawannakul P., McAfee A. 2020. Varroa destructor: A complex parasite, crippling honey bees worldwide. Trends Parasitol. 36 (7): 592-606. https://doi.org/10.1016/j.pt.2020.04.004

Vilarem C., Piou V., Vogelweith F., Vétillard A. 2021. *Varroa destructor* from the laboratory to the field: Control, biocontrol and ipm perspectives-A review. Insects 12:800. https://doi.org/10.3390/insects12090800