Apidologie (2025) 56:40

https://doi.org/10.1007/s13592-025-01171-2

Scientific note

A scientific note on 'Rapid brood decapping'—a method for assessment of honey bee (*Apis mellifera*) brood infestation with *Tropilaelaps mercedesae*

Aleksandar Uzunov[1,2], Irakli Janashia[3], Chao Chen[2], Cecilia Costa[4], and Marin Kovačić[5]

[1] Faculty of Agricultural Sciences and Food, Ss. Cyril and Methodius University in Skopje, Skopje, Macedonia
[2] State Key Laboratory of Resource Insects, Institute of Apicultural Research, Chinese Academy of Agricultural Sciences, Beijing 100193, China
[3] Agricultural University of Georgia, Tbilisi, Georgia
[4] CREA Research Centre for Agriculture and Environment, Bologna, Italy
[5] Faculty of Agrobiotechnical Sciences Osijek, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia

Received 2 November 2024 – Revised 9 February 2025 – Accepted 27 February 2025

Abstract – *Tropilaelaps mercedesae* is a parasitic mite species that negatively affects the health of *Apis mellifera* colonies. Recent reports show that it is spreading westwards, through Central Asia into Europe. Several field and laboratory methods have been proposed to detect *Tropilaelaps* spp. in *A. mellifera* colonies; however, most of them are recognised to be either laborious, costly or ineffective for detecting the mites, and some are even destructive to the colony. Here, we introduce a novel method for detecting and monitoring *T. mercedesae* based on the mite's characteristic biology (reduced feeding as bee pupae mature, brief dispersal phase on adult bees and agility) and the use of wax strips for decapping sealed brood area. Sealed worker brood cells at the development stage of white to purple-eyed pupae are swiftly decapped with wax strips to observe and count surfacing adult mites. The results from our study show supporting evidence of over 90% detection efficacy and brood survival, and ease of application. Therefore, we recommend the novel 'Rapid brood decapping' method as a reliable tool for detecting and monitoring *T. mercedesae* infestation. This method is suitable for beekeeping and research settings, being less invasive and stressful for colonies compared to other existing methods, cost-effective and quick.

1. *TROPILAELAPS* / HONEY BEES / BROOD INFESTATION / METHOD / MONITORING

Tropilaelaps mercedesae (Anderson and Morgan 2007) is one of the four currently described species of the *Tropilaelaps* genus that naturally parasitise Asian giant honey bees, *Apis dorsata*, *A. breviligula* and *A. laboriosa* (Anderson and Roberts 2013; Chantawannakul et al. 2018). This species also infests the Western honey bee *Apis mellifera* in many Asian countries (Delfinado and Baker 1961; Chantawannakul et al. 2018) and is considered one of its most damaging pests (Rinderer et al. 1994; Dainat et al. 2009; Gao et al. 2021; Han et al. 2024).

Recent reports show that the mite is spreading westwards, through Central Asia into Europe, where it has been found in southwestern Russia and Georgia (Namin et al. 2024; Brandorf et al. 2024; Janashia et al. 2024). Thus, it is ever more crucial for the beekeeping sector to be equipped

Corresponding author: A. Uzunov, uzunov@fznh.ukim.edu.mk; C. Chen, chao_chen@outlook.com
Manuscript editor: Peter Rosenkranz

with reliable methods for rapid detection and effective monitoring.

Several field and laboratory methods, mainly adapted from those used for *Varroa destructor*, have been proposed for the detection of *Tropilaelaps* spp. in *A. mellifera* colonies, such as sampling and analysing adult bees (powder sugar roll, soapy water wash, ethanol wash, CO_2) or sealed brood (uncapping with a honey fork or with tweezers/forceps), using the 'comb bump', observing mite fall on bottom boards or analysing hive debris by flotation method in alcohol or by molecular analyses (de Guzman et al. 2017, Kim et al. 2019, Gill et al. 2024). The proposed methods, primarily based on the biology of *V. destructor*, are characterised by being either laborious, costly or ineffective for detecting *Tropilaelaps* spp., and some are even destructive to the colony (Gill 2024; Gill et al. 2024). Gill et al. (2024) found that uncapping infested brood with tweezers, using sticky traps to catch mite drop and rolling adult bees in icing sugar were the most promising methods for detection of *Tropilaelaps* mites.

Here, we introduce a novel method for detecting and monitoring *T. mercedesae* based on the mite's characteristic biology (reduced feeding as bee pupae mature, brief dispersal phase on adult bees and agility) and the use of wax strips for decapping sealed brood area, building upon the method proposed by Siceanu (1996) for *Varroa* control. Sealed worker brood cells at the development stage of white to purple-eyed pupae (13–17 days from egg laying) are swiftly decapped with wax strips to observe and count surfacing adult *Tropilaelaps* mites. To evaluate the method's detection efficacy, reliability and practicality, we conducted a study in the summer of 2024 in Georgia, using *A. mellifera* colonies (N =30) in apiaries (N = 3) infested with *T. mercedesae*. A comb of sealed worker brood from each colony, with eye colour ranging from white to purple, was decapped using a commercial wax strip (Veet) covering more than 250 cells. The wax strip was evenly applied to the sealed brood area by pressing gently with a soft paper ball for 10 s. Once adhered, the strip was immediately and swiftly removed from the comb's surface (Figure 1). The entire decapped (stripped) brood area was instantly video recorded using a Honor 90 mobile phone camera on an overhead mount for 60 s to film and detect emerging mites. Following the video recording, the decapped brood area and the sealed worker brood from the comb's backside (control cells) were cut out. Care was taken to register any mites leaving the decapped area while cutting brood samples. The comb cuts were wrapped tightly in a white paper towel, inserted into a Ziploc sample bag and taken to the laboratory, where they were frozen at −20°C before inspection of brood infestation.

For each comb sample, *T. mercedesae* infestation was assessed on both sides by examining cell contents using a binocular stereo microscope (Carl Zeiss Stemi 508, magnification ×6.3). The control brood cells were carefully uncapped using tweezers (Gill et al. 2024), and the data were used to estimate the correlation with the video observed mites. *V. destructor* infestation was assessed only in the stripped cells. Freezing at −20°C eliminated the risk of counting the same mite twice. Only adult *T. mercedesae* and *V. destructor* mites were considered, identified by the brown pigmentation.

Considering the total number of inspected brood samples (N = 28), an average of 200.9 cells (146–241) were checked in the decapped brood and an average of 202.9 previously unopened control cells (151–253). Out of 28 samples, 11 (39.3%) were positive for *T. mercedesae*; of these, 9 samples (81.8%) were co-infested with *T. mercedesae* and *V. destructor*, and 2 samples (18.2%) infested only with *T. mercedesae* (Table I). The *T. mercedesae* infestation level from the decapped cells, including the video-observed mites ('super control'), averaged 3.05%. Multiple species infestation of a single cell was detected in only one sample.

The video recordings were observed by eight (N = 8) persons: an accurate and repeated observation of each recording was conducted by the most experienced person (N = 1, Table I—super control), yielding the ultimate number of observable mites. Then, seven (N = 7) persons 'blindly' checked positive (N = 9) and negative (N = 3) samples. The data was analysed assuming that

Figure 1. Decapped brood area with wax strip with removed brood caps, available for further analysis.

each *T. mercedesae* mite observed emerged from a single cell. While this approach simplifies the analysis, it is important to acknowledge that multiple mites may have originated from the same cell. *T. mercedesae* mites move very fast, and identifying the exact cell of exit is challenging. Thus, for consistency in data interpretation, we proceeded with the one mite per cell assumption.

The video observations detected *T. mercedesae* in 10 ($N = 10$) out of 11 ($N = 11$) subsequently laboratory-confirmed positive samples. A significant positive correlation ($r = 0.89$, Figure 2A) was found between the number of mites detected during 60-s post-decapping video recordings and the number of mites in the decapped cells (excluding video-observed mites) from all positive samples. We also found a significant positive correlation ($r = 0.96$, Figure 2B) between the number of video-detected mites and the number of mites from the control cells.

To evaluate the reliability of the observations, the variation between the different observers was analysed (Table I, excluding samples N6 and N30). All observers successfully distinguished and identified all *T. mercedesae*–positive ($N = 9$) and *T. mercedesae*–negative ($N = 3$) samples. In addition, there was no significant difference ($F(7, 88) = 0.111$, $p > 0.001$, one-way ANOVA) in the number of observed mites between observers, indicating that beekeepers and honey bee experts can effectively use this method with different levels of experience (Figure 2C). No *V. destructor* mites were detected, thus demonstrating the method's unsuitability in detecting this mite species.

For video observations, simple equipment (monitor and PC) is needed. Due to the high mobility of *T. mercedesae* mites within the first 10 s after uncapping, the video review can be improved with particular focus during that time

Table I The outcome of the laboratory inspection of brood and observations of the video recordings post decapping in 11 ($N = 11$) *T. mercedesae*–positive worker brood samples and 3 ($N = 3$) negative samples, which were used as controls in the 'blind' video observations

Colony ID	No. of inspected cells		No. of infested decapped cells		No. of infested control cells	Observed T by video reviewers (60 s)							
	Decapped[1]	Control[2]	Adult T[3]	Adult V[4]	Adult T	1	2	3	4	5	6	7	8[5]
N1	205	196	5	0	7	7	6	5	6	5	6	5	**6**
N3	196	151	3	9	4	3	2	3	2	3	4	3	**3**
N5	202	190	2	6	2	1	1	1	1	1	1	1	**2**
N6	238	182	2	4	0								**1**
N7	146	202	3	0	8	5	3	4	3	6	2	4	**5**
N8	241	253	2	11	3	1	1	1	1	1	1	1	**1**
N11*	195	178	0	8	0	0	0	0	0	0	0	0	**0**
N13*	203	188	0	17	0	0	0	0	0	0	0	0	**0**
N22	175	188	1	8	0	1	1	1	1	1	1	1	**1**
N23*	178	175	0	46	0	0	0	0	0	0	0	0	**0**
N25	198	205	1	2	1	1	1	1	1	1	1	1	**1**
N26	170	193	1	1	0	1	1	1	1	1	1	1	**1**
N27	175	195	12	1	14	6	6	6	5	5	4	5	**8**
N30**	188	194	1	3	0								**0**

[1]Field test using wax strip, [2]laboratory test with tweezers, [3]T—*T. mercedesae*, [4]V—*V. destructor*, [5]super control, *negative sample, **failed video detection

interval. In an average of 19.8 s (median 25, SD 12.3, 1 to 55 s), the observers reported the definitive number of *T. mercedesae* from the recorded videos. Nevertheless, to ensure accuracy and avoid double counting, we recommend a 30-s observation period. Additionally, assessing the uncapped brood area in two segments/halves may improve detection accuracy in cases of higher infestation.

Additionally, we checked for brood survival 24 h after the application of the 'Rapid brood decapping' method on 29 *T. mercedesae* uninfested *A. mellifera* colonies in Italy ($N = 6$), Croatia ($N = 13$) and Georgia ($N = 10$). The method was found to have low adverse effects on brood survival, with an average of 93.8% brood survival (SD 10.7, 54.4 to 100%). Siceanu (1997) reported a similar post-decapping survival (emerging bees) rate of 97.4% using waxed cloth decapping as a *V. destructor* control method. Variations in our study may have been due to differences in strip quality and/or in brood age (e.g. prepupae present in the treated area).

Based on supporting evidence of over 90% detection efficacy and brood survival, together with ease of application, we recommend the novel 'Rapid brood decapping' method as a reliable tool for detecting and monitoring *T. mercedesae* infestation. This method is suitable for beekeeping and research settings, being less invasive and stressful for colonies compared to other existing methods, cost-effective and quick (around 10 min per colony, including colony manipulation and video reviewing). As many pupae are uncovered, this method can also be used to assess covert forms of brood diseases. Furthermore, the brood cappings on the wax strip may be used for evaluation of recapping events.

The step-by-step procedure of the method application is photo and video visualised and described in Uzunov et al. (in press). We also

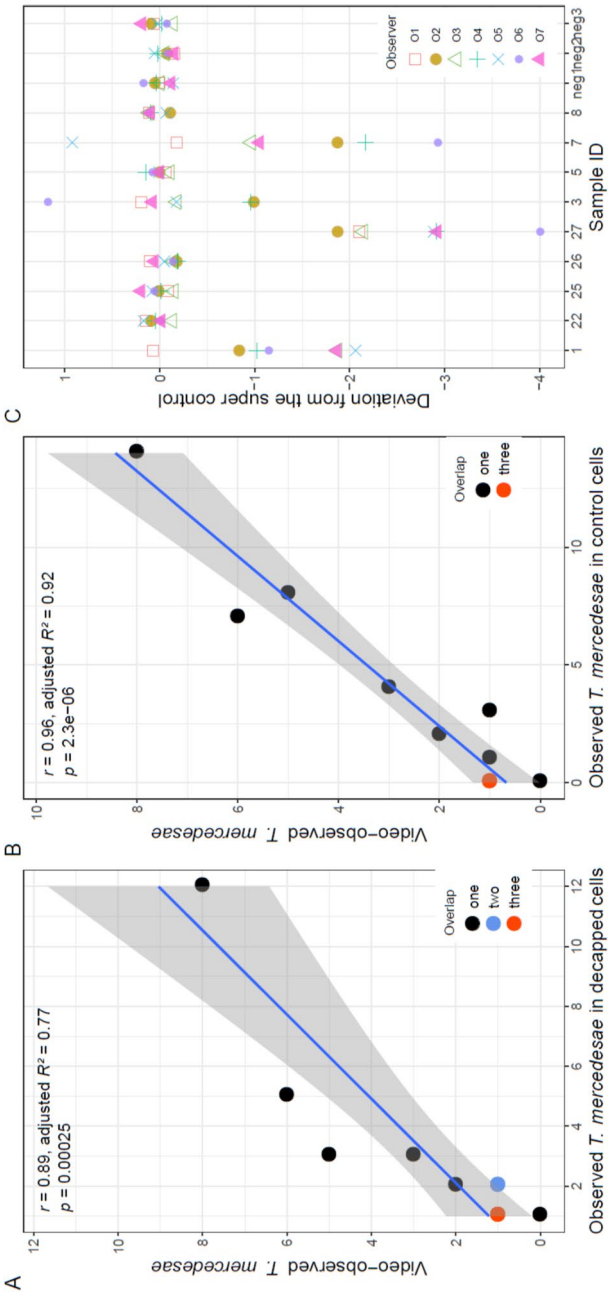

Figure 2. Scatterplot charts presenting (A) the correlation between the number of video observed (60 s) mites and the total number of mites for the positive samples (decapped brood cells, excluding the mites from the video observations), (B) correlation for the number of video-observed mites and the mites from the control side for the positive samples, (C) video observers' deviations from the super control values (*Y*-axis, 0 mark). The grey area indicates 95% confidence interval.

see a potential for further enhancement of the method through machine learning and mobile device integration. Given the biological similarities among *Tropilaelaps* species, it is likely that the method can be applied across the genus. Further research is needed to validate and refine this method which promises to be a key tool for monitoring and managing *T. mercedesae*'s westward spread.

ACKNOWLEDGEMENTS

Credit is due to Adrian Siceanu from Romania and Ralph Büchler from Germany for previously suggesting and using this method for *Varroa* monitoring and control. We thank the students Ruiyi Cheng (程瑞益), Qi Xu (徐齐) and Yufei Zou (邹禹妃) and the Georgian beekeepers for their support.

AUTHOR CONTRIBUTION

AU conceptualised and designed the study. IJ did laboratory analyses. IJ, CeC and MK did the fieldwork. AU, IJ, ChC, CeC, MK and the students did video reviews. All authors contributed to data processing and interpretation. AU wrote the first draft of the manuscript, and all authors commented on previous versions. All authors read and approved the final manuscript.

FUNDING

This research was supported by the Agricultural Science and Technology Innovation Program (CAAS-ASTIP-2024-IAR) and by the Horizon Europe research and innovation programme BeeGuards [Grant Agreement No. 101082073].

DATA AVAILABILITY

The datasets generated during and/or analysed during the current study are available from the corresponding author upon reasonable request.

DECLARATIONS

Ethics approval No approval of research ethics committees was required to accomplish the goals of this study because experimental work was conducted with an unregulated invertebrate species.

Conflict of interest The authors declare no competing interests.

REFERENCES

Anderson DL, Morgan MJ (2007) Genetic and morphological variation of bee-parasitic *Tropilaelaps* mites (Acari: Laelapidae): new and re-defined species. Exp Appl Acarol 43:1–24. https://doi.org/10.1007/s10493-007-9103-0

Anderson DL, Roberts JMK (2013) Standard methods for *Tropilaelaps* mite research. J Apic Res 52(4):1–16. https://doi.org/10.3896/IBRA.1.52.4.21

Brandorf A, Ivoilova MM, Yañez O, Neumann P, Soroker V (2024) First report of established mite populations. *Tropilaelaps mercedesae*. In: Europe J Apic Res 1-3 https://doi.org/10.1080/00218839.2024.2343976

Chantawannakul P, Ramsey S, Vanengelsdorp D, Khongphinitbunjong K, Phokasem P (2018) Tropilaelaps mite: an emerging threat to European honey bee. Curr Opin Insect Sci 26:69–75. https://doi.org/10.1016/j.cois.2018.01.012

Dainat B, Ken T, Berthoud H, Neumann P (2009) The ectoparasitic mite *Tropilaelaps mercedesae* (Acari, Laelapidae) as a vector of honeybee viruses. Insect Soc 56:40–43. https://doi.org/10.1007/s00040-008-1030-5

de Guzman L, Williams GR, Khongphinitbunjong K, Chantawannakul P (2017) Ecology, life history, and management of Tropilaelaps mites. J Econ Entomol 110(2):319–332. https://doi.org/10.1093/jee/tow304

Delfinado MD, Baker EW (1961) Tropilaelaps, a new species of mite from the Philippines (Laelapidae [s. lat]: Acarina). Fieldiana Zoology 44:53–56. https://doi.org/10.5962/bhl.title.3028

Gao J, Ma S, Wang X, Yang Y, Luo Q, Wang X et al (2021) *Tropilaelaps mercedesae* parasitism changes behaviour and gene expression in honey bee workers. PLoS Pathog 17(7):e1009684. https://doi.org/10.1371/journal.ppat.1009684

Gill MC, Chuttong B, Davies P, Etheridge D, Panyaraksa L, Tomkies V et al (2024) Assessment of the efficacy of field and laboratory methods for the detection of *Tropilaelaps* spp. PLoS ONE 19(9):e0301880. https://doi.org/10.1371/journal.pone.0301880

Gill M (2024) Tropilaelaps: an underestimated threat?. Bee Culture. https://www.beeculture.com/tropilaelaps-3. Accessed 28 June 2024

Han B, Wu J, Wei Q et al (2024) Life-history stage determines the diet of ectoparasitic mites on their honey bee hosts. Nat Commun 15:725. https://doi.org/10.1038/s41467-024-44915-x

Janashia I, Uzunov A, Chen C, Costa C, Cilia G (2024) A scientific note on the first report on *Tropilaelaps mercedesae* presence in Georgia: the mite is on the move westward! J Apicult Sci 68(2):183–188. https://doi.org/10.2478/jas-2024-0010

Kim B, Kim S, Kim M, Kim J, Tai AT, Kim S, Yoon B (2019) Quantitative detection of *Tropilaelaps* in

hive by specific gene detection from hive debris. J Apic Res 34(1):27–37. https://doi.org/10.17519/apiculture.2019.04.34.1.27

Namin MS, Joharchi O, Aryal S, Thapa R, Kwon S-H, Kakhramanov BA, Jung C (2024) Exploring genetic variation and phylogenetic patterns of *Tropilaelaps mercedesae* (Mesostigmata: Laelapidae) populations in Asia. Front Ecol Evol 12:1275995. https://doi.org/10.3389/fevo.2024.1275995

Rinderer TE, Oldroyd BP, Lekprayoon C, Wongsiri S, Boonthai C, Thapa R (1994) Extended survival of the parasitic honey bee. J Apic Res 33:171–174. https://doi.org/10.1080/00218839.1994.11100866

Siceanu A (1996) The artificial decapping of the honeybee brood for the control of the *Varroa jacobsoni* parasite. Apiacta 31:45–50

Siceanu A (1997) Effect of the artificial decapping of brood on the quality of bees (*Apis mellifera* L.). Pszczelnicze Zeszyty Naukowe, Rok XLI, 109-112

Uzunov A, Janashia I, Chen C, Costa C, Kovačić M (in press) Rapid brood decapping for *Tropilaelaps mercedesae* Detection. Bee World 102(1)