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SHORT COMMUNICATION

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Temporal increase of Varroa mites in trap frames used for drone brood removal during the honey bee season

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Abstract

Varroa mites are highly attracted to drone brood of honey bees (*Apis mellifera*), as it increases their chance of successful reproduction. Therefore, drone brood removal with trap frames is common practice among beekeepers in Europe and part of sustainable varroa control. However, it is considered labour-intensive, and there are doubts about the effectiveness of this measure. At present, it is mostly unknown how many mites a drone frame can carry at different times of the season, and how many mites can be removed on average if this measure is performed frequently. Therefore, we sampled a total of 262 drone frames with varying proportion of capped cells (5–100%) from 18 different apiaries. Mites were washed out from brood collected from mid-April to mid-July based on a standard method to obtain comparable results. We found that a drone frame carried a median of 71.5 mites, and with the removal of four trap frames, about 286 mites can be removed per colony and season. In addition, mite counts were significantly higher in June and July than in April and May (Tukey-HSD, $P<0.05$). The number of mites and the proportion of capped cells, however, were not correlated ($R^2<0.01$, $P<0.05$). Our results suggest that drone brood removal is effective in reducing *Varroa destructor* numbers in colonies, supporting the findings of previous studies on the efficacy of this measure. Although mite counts varied, we believe that increasing sample size over different seasons and locations could elucidate infestation patterns in drone brood and ultimately improve drone brood removal as an integrated pest management tool for a wider audience of beekeepers.

KEYWORDS
Apis mellifera, drone brood removal, drone trap frame, Integrated pest management, mite control, *Varroa destructor*, honey bees

1 | INTRODUCTION

More than 70% of *Varroa destructor* mites are found in capped cells of bee brood when brood is present in *Apis mellifera* colonies (Frey & Rosenkranz, 2014). Drone brood is 6–11 times more likely to be infested with mites than worker brood for probably several reasons (Beetsma et al., 1999; Fuchs, 1990); (i) drone development takes 2 days longer, giving mites more time to reproduce (Boot et al., 1995); (ii) drone brood is two to three times more likely to be frequented by nurse bees that may carry phoretic mites (Calderone

& Kuenen, 2003); (iii) the pre-capping period during which drone brood is attractive to mites is two to three times longer than for worker brood (Boot et al., 1992); and (iiii) longer and increased production of kairomones by drone larvae, which make them attractive to mites (Trouiller et al., 1992).

Considering all the reasons above makes drone brood removal (DBR) an effective tool for controlling varroa mites when integrated as a pest management measure (Evans et al., 2016; Whitehead, 2017). Good results can be achieved when 4 to 5 fully capped trap frames are removed per season (Charrière et al., 2003). It is worth noting that DBR is mainly used by small-scale beekeepers in Europe and is considered labour-intensive or not effective enough as a single treatment elsewhere (Evans et al., 2016; Whitehead, 2017). There is also a risk of rapid varroa spread if trap frames are not harvested in time (Jack & Ellis, 2021).

When done properly, the effectiveness of DBR is demonstrated by the fact that the number of mites during colony development in spring and early summer was significantly lower than in untreated colonies (Wantuch & Tarpy, 2009). Final infestation rates of colonies after late summer treatments were also substantially lower than in colonies where DBR was not performed (Calderone, 2005; Charrière et al., 2003). However, to date, there are few data on how many mites a single drone frame can actually carry. Furthermore, it is unknown whether there is a difference in infestation levels over time and to what extent the proportion of capping (i.e. the number of capped drone cells in relation to all drone cells) may influence DBR success. The latter could play a role in practice, since beekeepers may simply have removed the trap frame too early if they do not find the method sufficiently effective. There is also general doubt among beekeepers if this method removes mites at all (Whitehead, 2017).

The aim of this study was, therefore, to determine the number of mites in individual drone frames over the course of a bee season. In addition, we assessed whether there was a correlation between the number of mites and the proportion of capped cells.

2 | MATERIALS AND METHODS

2.1 | Experimental field sites and colonies

The field sites with apiaries (n = 18) were all located in the state of Baden-Württemberg in southern Germany. Apiaries were sampled unevenly due to logistical reasons (1–3 times). Some drone frames were collected only once, others multiple times from these locations.

The total number of honey bee colonies (*A. m. carnica*) sampled was $n = 63$. These colonies belonged to the stock of the Apicultural State Institute and were kept according to good beekeeping practice. This included varroa treatment with 85% formic acid twice in the previous season (August and September) and winter treatment with 3.5% oxalic acid in November/December, the last treatment before drone frames were sampled.

Colonies were housed in Hohenheim standard hives with 10 Zander frames per box. A hive consisted of two boxes for brood and up to two boxes for honey, separated by the use of a queen excluder. One empty frame without foundation was placed next to the brood nest, either as frame no. 2 or 9 in the upper brood box. Bees and brood showed no clinical signs of disease upon inspection throughout the sampling period.

2.2 | Data collection

Whole drone frames ($n = 262$) were collected from mid-April (18 Apr) to mid-July (15 Jul) of the 2011 season. We applied a brood washing method similar to that of Dietemann et al. (2013), chapter 3.1.4.2.2. In brief, the entire brood was uncapped with a sharp knife, and the comb parts were rinsed through a first sieve (5 mm mesh) with a hand shower until all the cell contents were removed. Subsequently, empty comb parts were washed again, and cell caps that were removed and washed separately, as mites can hide under them. All mites were then collected in a second sieve (0.5 mm mesh) and dried on tissue paper. They were counted with the help of a counting grid and a hand counter. Prior to washing, the area of capped cells of each drone frame was measured in 10 × 10 cm squares, which were then converted to percentage using the Liebefeld method (Imdorf et al., 1987). One Zander frame fits exactly 8.1 dm^2 or eight Liebefeld units per side and thus a total of 8 × 230 (1840) drone cells (Aumeier, 2017; Imdorf & Gerig, 1999).

2.3 | Statistical analysis

We fitted a negative-binomial mixed model (estimated using ML and nlminb optimizer) to predict mites with month and location (formula: mites ~ month + location). The model included the proportion of the frame with capped cells as a random effect (formula: ~1|capped_perc). The model's explanatory power related to the fixed effects alone (marginal R^2) was 0.65. To compare groups pairwise, estimated marginal means were calculated and adjusted by the Tukey-HSD method for multiple comparisons for the response variable month (= adjusted means).

In addition, linear regression was performed to identify whether the number of mites per frame, and the proportion of capped cells were correlated (formula: mites ~ capped_perc).

All analyses were performed in R v.4.1.2 (R Core Team, 2021). A significance level of $\alpha = 0.05$ was used for all tests, respectively.

3 | RESULTS

3.1 | Varroa mite count

The model's intercept was at 3.94 (95% CI [3.07, 4.81], $P < 0.001$). To illustrate the effect size, the estimated marginal means (± CL) are shown in Figure 1. The number of mites per drone frame increased each month, as indicated by the higher mean values. The increases from April to June, April to July, May to Jun and May to July were significant (Figure 1, Tukey-HSD, $P<0.05$). Across all samples, a single frame carried a median of 71.5 mites (Mean = 208.49, SD = 344.21, Skewness = 3.31, Figure S2). The number of mites per frame across all 18 apiaries was significantly different (Tukey-HSD, $P<0.05$), as was the number of drone frames removed (Figure S1). Overall, there were only six samples with 0 mites (2.3%) and 40 samples with <10 mites (15.3%) (Figure S2). Note that all data points above 200 are shown in Figure S2 only.

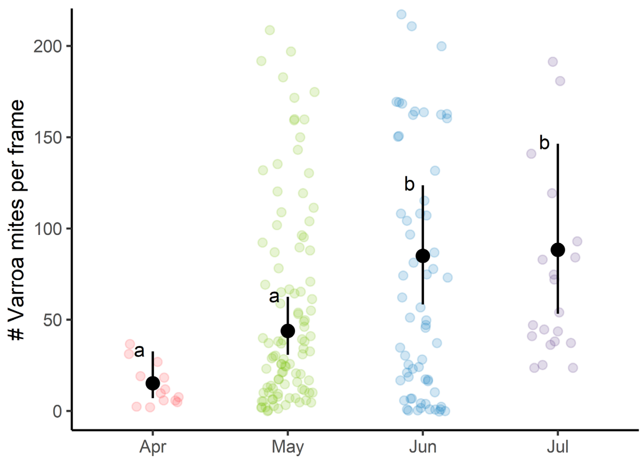

FIGURE 1 Number of varroa mites per drone frame. Black dots and error bars indicate the adjusted means (±CL) of mites per drone frame. Means that follow a common letter are not significantly different (Tukey-HSD, $P>0.05$). Note that all values above 200 mites are not shown in this graph but are available in Figure S2

3.2 | Proportion of capped cells

For linear regression, 12 data points were excluded from the analysis because their capping status was not recorded. Therefore, only $n = 250$ data points were analysed. With $R^2<0.01$, no correlation was found between the number of mites and the proportion of capped cells (Figure 2). On average, the proportion of capped cells was 63% across all samples, with the majority above 50% ($n = 210$ samples or 84%, Figure S3).

4 | DISCUSSION

It is known that drone brood attracts varroa mites on average eight times more than worker brood and is, therefore, an effective means of controlling this pest when removed (Charrière et al., 2003). Due to limited data, it is currently unclear how many mites are removed by a single frame and at what status drone cells were cut. Understanding how a temporal progression can alter drone brood infestation could provide insight into the effectiveness of this measure and further improve it.

In this study, therefore, we evaluated drone frames taken from 18 apiaries over an entire season to determine mite counts and infestation patterns that have not been reported anywhere before. We found a significant increase in mites over time, consistent with mite development in the entire colony (Wantuch & Tarpy, 2009). Less than 3% of our samples contained no mites at all and only ~15% contained <10 mites, demonstrating the effectiveness of this method. Assuming that DBR was performed four times per season and colony, an average of 834 mites could be removed (mean). This agrees with the results of Charrière et al. (2003), who removed 788

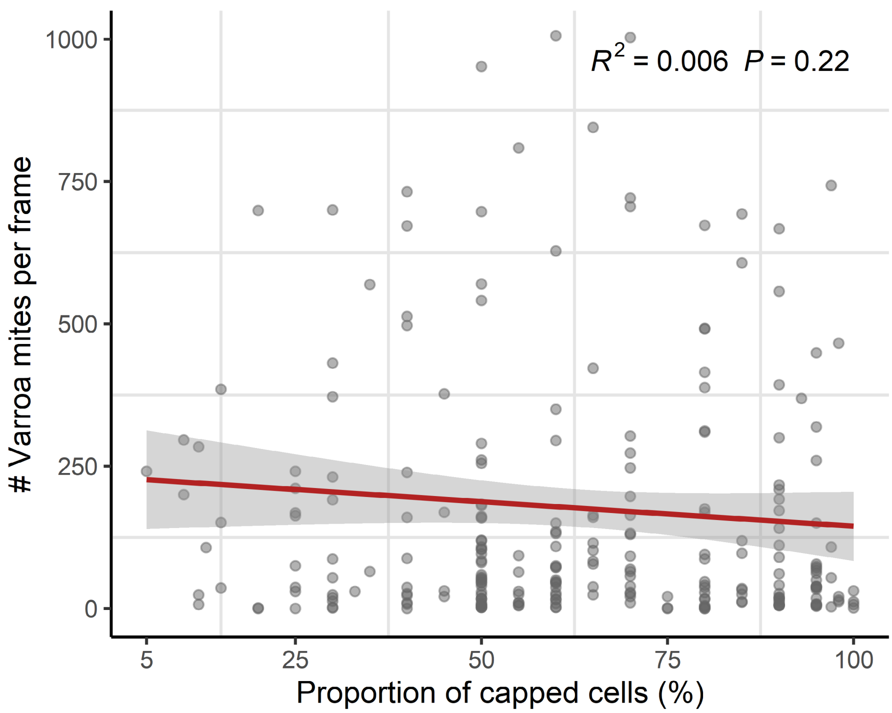

FIGURE 2 Scatterplot of capped cell proportion (*x*-axis) versus varroa mite number per frame (*y*-axis) including 250 valid data points. Linear regression for mite count and proportion of capped cells was not significant ($P = 0.22$)

mites under similar conditions. It is important to note that our data are left-skewed, which requires a cautious interpretation of mean values. A more reasonable interpretation, in this case, is provided by the median of 71.5 mites per drone frame (von Hippel, 2005). Removing four trap frames during the season, therefore, yields a more realistic estimate of 286 mites removed (median).

Furthermore, the proportion of capped cells of the drone frame did not affect the mite count. When the frames were evaluated, an average of 63% of the cells were capped. This indicates that all open cells containing larvae were in an appropriate condition to be infested (i.e. <60h before capping) (Calderone & Kuenen, 2003; Frey et al., 2013). In practical terms, this means that DBR does not require fully capped frames to be effective. Thus, frames could be removed earlier to minimize removal intervals and maximize removal frequency to extract more mites. Likewise, Licek et al. (2004) suggest overwintering colonies with drawn trap frames to promote drone rearing in the early season and extend the removal period. Some beekeeping magazines also recommend using two trap frames and collecting them in alternating order to maximize mite extraction (Bienen & Natur, 2022).

Since we have only presented a small data set on this subject, a better insight into the infestation pattern of drone brood and ultimately an increase in the effectiveness of DBR could be the result if studied in more detail. This is why we encourage data collection from different countries to enable future region-specific recommendations for DBR as an integrated pest management measure in beekeeping.

AUTHOR CONTRIBUTIONS

G.L. and D.d.C. contributed to conceptualization; R.O. and D.d.C. contributed to Data curation; D.d.C. contributed to investigation and methodology; G.L. contributed to funding acquistition, project administration, resources and supervision; R.O. contributed to formal analysis, software, visulalization and writing—original draft; R.O. and F.O. contributed to validation; F.O., G.L. and D.d.C. contributed to writing—review and editing.

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CONFLICT OF INTEREST

The authors declare that no competing interests exist.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the 'Open Science Framework' under DOI: https://doi.org/10.17605/OSF.IO/ZJS4X (Odemer et al., 2022).

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REFERENCES

Aumeier P (2017) Sie dürfen nicht alles glauben, was Sie denken!, Die neue Bienenzucht (DNB) 02/2017: 48-51.

Beetsma, J., Boot, W. J., & Calis, J. (1999). Invasion behaviour of *Varroa jacobsoni* Oud.: from bees into brood cells. *Apidologie*, *30*, 125–140. https://doi.org/10.1051/apido:19990204

Bienen & Natur (2022) Drohnenrahmen: Varroa bekämpfen und Wachs ernten (English title: Drone Frame: Fighting Varroa and Harvesting Beeswax), dlv Deutscher Landwirtschaftsverlag. https://bit.ly/3L2D3XC. Accessed May 11, 2022.

Boot, W. J., Calis, J. N. M., & Beetsma, J. (1992). Differential periods ofVarroa mite invasion into worker and drone cells of honey bees. *Experimental & Applied Acarology*, *16*, 295–301. https://doi.org/10.1007/BF01218571

Boot, W. J., Schoenmaker, J., Calis, J. N. M., & Beetsma, J. (1995). Invasion of *Varroa jacobsoni* into drone brood cells of the honey bee, *Apis mellifera*. *Apidologie*, *26*, 109–118. https://doi.org/10.1051/apido:19950204

Calderone, N. W., & Kuenen, L. P. S. (2003). Differential tending of worker and drone larvae of the honey bee, *Apis mellifera*, during the 60 hours prior to cell capping. *Apidologie*, *34*, 543–552. https://doi.org/10.1051/apido:2003054

Calderone, N. W. (2005). Evaluation of drone brood removal for management of *Varroa destructor* (Acari: Varroidae) in Colonies of *Apis mellifera* (Hymenoptera: Apidae) in the Northeastern United States. *Journal of Economic Entomology*, *98*(3), 645–650. https://doi.org/10.1603/0022-0493-98.3.645

Charrière, J.-D., Imdorf, A., Bachofen, B., & Tschan, A. (2003). The removal of capped drone brood: an effective means of reducing the infestation of varroa in honey bee colonies. *Bee World*, *84*, 117–124. https://doi.org/10.1080/0005772X.2003.11099587

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. *Journal of Apicultural Research*, *52*, 1–54. https://doi.org/10.3896/IBRA.1.52.1.09

Evans, J., Müller, A., Jensen, A. B., Dahle, B., Flore, R., Eilenberg, J., & Frøst, M. B. (2016). A descriptive sensory analysis of honeybee drone brood from Denmark and Norway. *Journal of Insects as Food and Feed*, *2*, 277–283. https://doi.org/10.3920/JIFF2016.0014

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*). *Journal of Invertebrate Pathology*, *113*, 56–62. https://doi.org/10.1016/j.jip.2013.01.007

Frey, E., & Rosenkranz, P. (2014). Autumn invasion rates of *Varroa destructor* (Mesostigmata: Varroidae) into honey bee (Hymenoptera: Apidae) colonies and the resulting increase in mite populations. *J Econ Entomol*, *107*, 508–515. https://doi.org/10.1603/EC13381

Fuchs, S. (1990). Preference for drone brood cells by *Varroa jacobsoni* Oud in colonies of *Apis mellifera* carnica. *Apidologie*, *21*, 193–199. https://doi.org/10.1051/apido:19900304

Imdorf, A., Buehlmann, G., Gerig, L., et al. (1987). Überprüfung der Schätzmethode zur Ermittlung der Brutfläche und der Anzahl Arbeiterinnen in Freifliegenden Bienenvölkern. *Apidologie*, *18*, 137–146. https://doi.org/10.1051/apido:19870204

Imdorf, A., & Gerig, L. (1999). *Lehrgang zur Erfassung der Volksstärke*. Schweizerisches Zentrum für Bienenforschung.

Jack, C. J., & Ellis, J. D. (2021). Integrated pest management control of *Varroa destructor* (Acari: Varroidae), the Most Damaging Pest of (*Apis mellifera* L. (Hymenoptera: Apidae)) Colonies. *Journal of Insect Science*, *21*(5). https://doi.org/10.1093/jisesa/ieab058

Licek, E., Moosbeckhofer, R., & Pechhacker, H. (2004). *Varroa destructor*, a parasitic mite of the honeybee (*Apis mellifera*)—a survey of biology and control strategies. *Wiener Tierärztliche Monatsschrift*, *91*, 311–316.

Odemer R., Odemer F., Liebig G., de Craigher D. (2022) Data: Temporal Increase of Varroa Mites in Trap Frames Used for Drone Brood Removal during the Honey Bee Season. OSF. March 22. https://doi.org/10.17605/OSF.IO/ZJS4X

R Core Team. (2021). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Trouiller, J., Arnold, G., Chappe, B., le Conte, Y., & Masson, C. (1992). Semiochemical basis of infestation of honey bee brood by *Varroa jacobsoni*. *Journal of Chemical Ecology*, *18*, 2041–2053. https://doi.org/10.1007/BF00981926

von Hippel, P. T. (2005). Mean, median, and skew: correcting a textbook rule. *Journal of Statistics Education*, *13*, 3. https://doi.org/10.1080/10691898.2005.11910556

Wantuch, H. A., & Tarpy, D. R. (2009). Removal of drone brood from *Apis mellifera* (Hymenoptera: Apidae) colonies to control *Varroa destructor* (Acari: Varroidae) and retain adult drones. *J Econ Entomol*, *102*, 2033–2040. https://doi.org/10.1603/029.102.0603

Whitehead HR (2017) Varroa mite management among small-scale beekeepers: Characterizing factors that affect IPM adoption, and exploring drone brood removal as an IPM tool. Master Thesis. The Ohio State University.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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