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## Queen ringing vs. queen caging for summer brood interruption

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### ABSTRACT

In many temperate regions, increasing temperatures in the autumn and winter result in shorter or even non-existent brood breaks in honey bee (*Apis mellifera* L.) colonies. This facilitates a year-round reproduction of *Varroa destructor* and corresponding pathogen pressure. To address this problem, introducing an artificial brood break during winter might be essential. On the other hand, queen caging in summer, as one of the most prominent brood interruption methods, has already been accepted as an effective way for *Varroa* control in honey bee colonies. In this study, we compared queen caging vs. queen ringing as a novel technique for inducing summer brood interruption.

### ARTICLE HISTORY

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### KEYWORDS

Honey bee queen; brood interruption; colony development; queen ringing

In many temperate regions, increasing temperatures in the autumn and winter result in shorter or even non-existent brood breaks in honey bee (*Apis mellifera* L.) colonies (Le Conte & Navajas, 2008). This facilitates a year-round reproduction of *Varroa destructor* and corresponding pathogen pressure. To address this problem, introducing an artificial brood break during winter might be essential. On the other hand, queen caging in summer, as one of the most prominent brood interruption methods, has already been accepted as an effective way for *Varroa* control in honey bee colonies (Büchler et al., 2020; Gregorc et al., 2017; Jack et al., 2020; Pietropaoli et al., 2012; Uzunov et al., 2023). The technique has also been used by some large-scale Italian beekeepers for winter treatment in combination with oxalic acid (OA), which might be particularly relevant for regions with year-round brood rearing (Giacomelli et al., 2016; Uzunov et al., 2023). However, during winter this confinement of the queen may pose a risk since it restricts her in-hive migration with the bee cluster and continuous exchange of food and pheromones. This limitation may be overcome by the recently presented “Queen Ringing” technique (Uzunov & Chen, 2023), which does not seem to significantly restrict the queen’s locomotive ability and allows free in-hive roaming. Nevertheless, oviposition is efficiently prevented during the whole winter by simply mounting a small ring on her abdomen (Uzunov &

Chen, 2023). In China, where queen ringing was developed and is currently mainly applied, it seems to be mostly used for winter brood interruptions, which may involve the use of acaricides, and little is known about its application in summer.

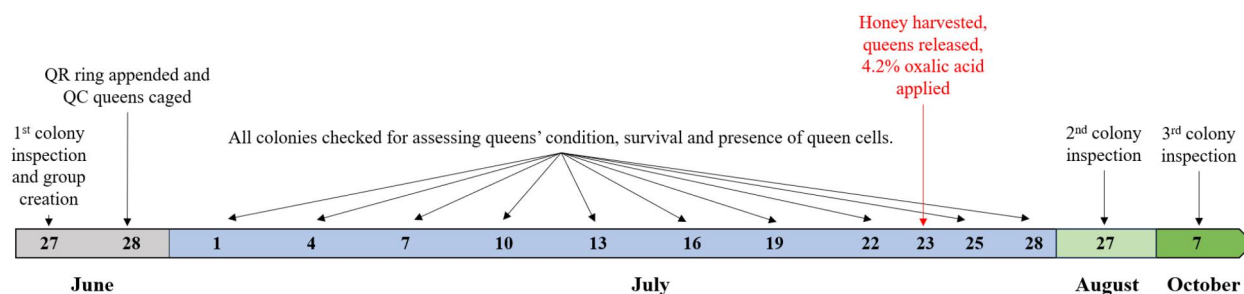
In this study, we compared the novel queen ringing technique (QR,  $N=7$  colonies) to the already known queen caging (QC,  $N=7$  colonies) as a summer brood interruption method. In addition, we compared both groups to a control group (CG,  $N=7$  colonies) without brood interruption, i.e., constant brood rearing.

The experiment was conducted in the IAR, CAAS Puwa experimental apiary ( $39^{\circ}56'35.34''N, 116^{\circ}10'56.63''E$ ) in Beijing, China, from June to the first half of October 2023 corresponding to the main summer nectar flow of Chaste tree (*Vitex negundo*) and scattered flowers. All colonies, managed in two boxes of Langstroth (LR) hives, were headed with one-year-old queens from local *A. m. ligustica* stock. The queens from the QR and QC were ringed or caged, respectively, on 28<sup>th</sup> June and released 25 days later on 23<sup>rd</sup> July when 4.2% OA was trickled according to common *Varroa*-treatment practice (Büchler et al., 2020). The confinement of queens followed the protocols of Büchler et al. (2020) and Uzunov & Chen (2023) which both require the handling of queens. However, two hands are needed to secure the queen and mount the ring according to the latter method.

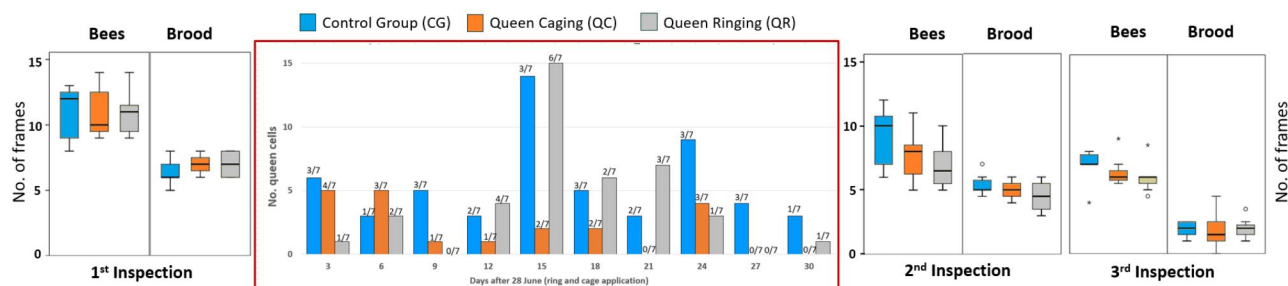
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**Figure 1.** Timetable of activities for study setup, data collection and honey harvesting.



**Figure 2.** Colony development (frames occupied with bees and frames with brood) at the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> inspection (box plots). Total number of observed queen cells per group are presented in coloured columns. Numbers above the columns show the number of colonies with queen cells compared to all colonies in the respective group. The red box shows when the queens in the QR and QC groups were kept confined.

Likewise to paint marking techniques for queens, the ringing method thus requires a little more practice than the sole caging.

The following parameters were of primary interest: colony development, queen survival and performance (i.e., egg laying capacity), queen cell incidents and honey production (Büchler et al., 2013; Uzunov et al., 2021). Initially, during the first inspection, colony strength (bees and brood) was assessed according to standard protocols (Uzunov et al., 2021) for all 21 colonies and their values were used for formation of equalised groups by colony strength (bees  $F(2,20)=0.012$ ,  $p=0.988$ ; brood  $F(2,20)=0.873$ ,  $p=0.435$ , one-way ANOVA). Honey production was measured by weighting supers before and after extraction, from where amount of extracted honey was calculated (Uzunov et al., 2021). The field trial concluded on 7<sup>th</sup> October (Figure 1).

Frames with built combs or foundations were added to the colonies in need of space for nectar storage, and when observed, queen cells were recorded and destroyed. No further beekeeping practices were applied. A detailed timetable of the activities related to the study setup, data collection and honey harvesting is given in Figure 1.

At the second inspection (27<sup>th</sup> August) groups showed no significant differences in colony strength (bees  $F(2,19)=1.803$ ,  $p=0.195$ ; brood  $F(2,19)=1.556$ ,  $p=0.240$ , one-way ANOVA). The same was true for the third (7<sup>th</sup> October) final inspection (bees  $F(2,18)=0.909$ ,  $p=0.421$ ; brood  $F(2,18)=0.031$ ,  $p=0.969$ , one-way ANOVA) indicating that the QR

and QC colonies compensated for the oviposition interruption (Figure 2), a pattern already reported by Büchler et al. (2020). However, the CG colonies tended to be stronger, followed by the QC colonies. In other words, there were no losses or malfunctions in colony development due to caging and ringing, even though constant brood rearing slightly increased the bee population as expected. Nevertheless, after the queens were released, a single queen from QR group was lost and during the final inspection, another one from the same group was found to exist alongside her daughter. We excluded possible effects of the queens' age by using only one year old queens. However, the after-ringing loss and possible superseding might be the consequence of the ring's removal when queens can be injured. An additional risk factor could be damage caused by the ring during the ringing period (queen physiology, injuries etc.).

During the regular inspections (at 3-day intervals) we observed queen cells in some of the CG colonies with free-laying queens as expected for this time of the season (Figure 2). The peak of the swarming tendency in the study area is in May and June, and still persist in July unless a new queen replaces the original; in our study, the queen remained unchanged, leading to the expected observation of queen cells. Intriguingly, in the QR and QC colonies (i.e., groups with inhibited oviposition), eggs, larvae and drawn queen cells were found in some colonies even 9 days after the queen confinement (Figure 2). The question about the origin of eggs and larvae remains elusive.

However, the pattern of how the eggs are laid suggests that they were deposited by workers (eggs on the cell walls, two or more eggs in a cell) but the drawn queen cells challenge such suggestion. Yet, no increased queen losses, e.g., as a result of drone-laying workers or supersedure, were observed in the respective colonies. Interestingly, in colonies where queens were caged eggs were found close to the cage, while in colonies where queens were ringed, eggs were found randomly on the frames. No such observations were reported in the earlier studies with brood interruption methods (Büchler et al., 2020; Giacomelli et al., 2016; Kovačić et al., 2023; Uzunov et al., 2023) which might be due to a lack of such regular inspections in the studies design. Nevertheless, Giacomelli et al. (2009), reported a significant number of eggs found in cells when the queen was caged in Scalvini© cage. Additional cytological, molecular and behavioural studies are required to identify the origin of the eggs, larvae and pupae.

There were significant differences in the amount of harvested honey ( $F(2,20)=3.911$ ,  $p=0.039$ ). Significantly more ( $p<0.05$ ) honey was harvested from colonies with brood interruption compared to the CG ( $\bar{x} = 9.3$  kg). In addition, no significant differences ( $p>0.05$ ) between QR ( $\bar{x} = 12.8$  kg) and QC ( $\bar{x} = 13.1$  kg) were observed. These findings align well with the results reported by Büchler et al. (2020) and Kovačić and Uzunov et al. (2023) and may be related to the decreased food requirement for brood rearing.

Considering the findings of our study and the practical aspects reported by Uzunov & Chen (2023), the queen ringing technique reflects a promising alternative as a method for summer brood interruptions. However, further investigations on the queen's performance and health as well as other beekeeping aspects under different beekeeping conditions seem to be recommended. To address this gap of knowledge, a large-scale pancontinental study has been initiated to verify the reported mainly anecdotal positive experience in China with queen ringing for extended winter brood interruption as a method administered for control of *Varroa* as well as *Tropilaelaps* infestations. Moreover, the study will also address other relevant beekeeping aspects such as overwintering food consumption, possible queen's injuries as well over all colony development.

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No potential conflict of interest was reported by the authors.

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