



Efficacy and trade-offs of an innovative hyperthermia device to control *Varroa destructor* in honeybee colonies

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Abstract

For decades, the honeybee, *Apis mellifera*, has suffered from severe colony losses due to the ectoparasitic mite *Varroa destructor*. Various strategies based on chemicals fail to adequately control varroa mite populations, and often comprise side-effects on the host, parasite resistance and residues in hive products. Reduced temperature tolerance of *V. destructor* compared to its host has long been recognised and accordingly, the potential of hyperthermia to disrupt mite reproduction within honeybee brood cells or even kill adult parasites. Yet, earlier studies on hyperthermia remain largely anecdotal, and readily implementable solutions have so far been lacking. This study investigates autonomously controlled interval heating from within brood combs throughout the season compared to control colonies maintained according to good apicultural practice. We documented treatment-dependent colony growth dynamics and honey production at three apiaries, complemented by regular monitoring of varroa mite levels and comprehensive digital assessments of brood development over time. Our one-year field trial suggests the evaluated hyperthermia device efficiently suppresses mite populations below critical thresholds until autumn. Whilst a general winter reference-curing revealed similar mite infestations of colonies previously treated with hyperthermia versus formic acid (control), only the latter imposed substantial overwintering burdens indirectly through frequent late-season queen supersedure. However, relative to targeted pupae, increased mortality particularly of heat-treated non-target brood stages (eggs and larvae) appeared to trigger compensatory colony-level responses, translating into temporarily decreased numbers of adult workers coupled with increased pollen foraging and overall lower honey harvests. Valuable insights into previously unrecognised side-effects of hyperthermia and mitigation thereof may ultimately permit successful routine applications of this chemical-free approach to combat the major threat to honeybees worldwide.

Keywords Digital brood assessment · Formic acid treatment · Honeybee colony conditions · Hyperthermia · *Varroa destructor*

Key message

- Past reports on hyperthermia-based control of the honeybee parasite *Varroa destructor* remained largely anecdotal.

- Modern in-comb hyperthermia efficiently controls *V. destructor* levels within honeybee colonies throughout the season.
- High-tech hyperthermia devices may partly replace prevalent chemical treatments against varroa mites.
- Hyperthermia-triggered trade-offs require further research and adequate technological improvement.
- Hyperthermia routine application to mitigate global colony losses due to varroa mites seems feasible.

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Introduction

The Western honeybee, *Apis mellifera*, is the globally most important managed pollinator, making vital contributions to food security and essential ecosystem services, thereby

creating considerable wealth (Breeze et al. 2011; Hung et al. 2018). Combined challenges of altered land use, nutritional stress, pesticide exposure, and pathogens and parasites threaten colony resilience and have resulted in massive recurrent colony losses over the past decades (Genersch 2010; Sandrock et al. 2014; DeGrandi-Hoffman and Chen 2015; Goulson et al. 2015; Sánchez-Bayo et al. 2016; Requier et al. 2017; Dolezal and Toth 2018; O'Neal et al. 2018; Steinhauer et al. 2018; Branchiccela et al. 2019). The parasitic mite *Varroa destructor* is a key driver of colony losses, mainly through transmitting several honeybee viruses (Guzmán-Novoa et al. 2010; Rosenkranz et al. 2010; McMenamin and Genersch 2015; Ramsey et al. 2019). Enormous research efforts have been undertaken worldwide to control this major honeybee pest (Rosenkranz et al. 2010; Nazzi and Le Conte 2016; Traynor et al. 2020). While an evolutionary resilient host-parasite relationship would be most favourable in the long-term, causal mechanisms underlying occasionally observed resistance of local honeybee populations appear complex and are understood only slowly (Büchler et al. 2020a, b; Mondet et al. 2020), which so far has hampered systematic breeding progress (Guichard et al. 2020; Gabel et al. 2023). Therefore, in the lack of sustainable co-existence, beekeepers are advised to pursue an integrated varroa mite management strategy building on several seasonal interventions to mitigate overall colony losses (Noël et al. 2020; Jack and Ellis 2021; van der Steen and Vejsnæs 2021).

Due to increasing evidence of unwanted residues of synthetic acaricides in hives, as well as widespread varroa mite resistance induced by their recurrent use, applications of organic acids have become popular not only throughout Europe (Rosenkranz et al. 2010; Noël et al. 2020; Bubnič et al. 2021; van der Steen and Vejsnæs 2021; Brodschneider et al. 2023). However, treatments with organic acids require careful consideration of various aspects to ensure efficacy against *V. destructor* and avoid side-effects on honeybees (Vilarem et al. 2021). For instance, formic acid, uniquely affecting varroa mites within brood cells, shows strong effect dependency on dispenser types and weather conditions (Rosenkranz et al. 2010; van der Steen and Vejsnæs 2021; Vilarem et al. 2021), which raises concerns about its reliability in the view of progressing climate change (Switanek et al. 2017; Smoliński et al. 2021). Similarly, oxalic acid is most effective when applied to broodless colonies but unpredictable brood activity during winter may increasingly compromise optimal timing of treatments because repeating certain application variants causes side-effects (Rademacher and Harz 2006). Temporary queen caging for artificial brood interruption or total brood removal coupled with oxalic acid treatments throughout the season are considered appropriate alternatives (Büchler et al. 2020a, b; Noël et al. 2020), but remain labour intensive for commercial beekeeping

operations, while hobby beekeepers are often reluctant to deliberately destroy brood or risk queen losses (Bubnič et al. 2021).

Recent state-of-the-art reviews advise combined complementary measures to control *V. destructor* in honeybee colonies over the year (Noël et al. 2020; Bubnič et al. 2021; Jack and Ellis 2021; van der Steen and Vejsnæs 2021), optionally including hyperthermia as a possible chemical-free approach. *V. destructor* is more sensitive to high temperatures than honeybees and hyperthermia has been recognised since the 1980s to selectively kill varroa mites (Komissar 1985; Hoppe and Ritter 1987; Rosenkranz 1987; Tihelka 2016), but so far lacks adequate routine implementation. While the optimal honeybee brood temperature is close to 35 °C, developing and adult honeybees seem to tolerate up to 45 °C for short periods. In contrast, 36.5–41 °C can significantly compromise varroa mite reproduction, and temperatures between 41 and 44 °C are lethal to adult *V. destructor* (Rosenkranz 1987; Le Conte et al. 1990; Appel and Büchler 1991). Pioneering studies heat-treated entire colonies either within their hives or as separated artificial swarms (Komissar 1985; Hoppe and Ritter 1987; Tihelka 2016). Later investigations focussed on incubating individual frames containing capped worker brood in separate devices (Rosenkranz 1987; Le Conte et al. 1990; Appel and Büchler 1991; Engels and Rosenkranz 1992; 1993; Marien 1995). The concept of hyperthermia has since fuelled inventiveness within the beekeeping community, but widespread rather anecdotal evidence of its potential to control *V. destructor* in honeybee colonies contrast a modest increase in robust data. Yet, hyperthermia recently experienced an academic revival, including research on improved devices that led to peer-reviewed publications, which altogether suggest that hyperthermia is efficient in combatting varroa mites without severely harming the bees (Goras et al. 2015; Bičík et al. 2016; Kablau et al. 2020a, 2020b; Porporato et al. 2022). Nevertheless, any hyperthermia approach developed to date has suffered from feasibility and/or efficacy constraints, particularly regarding full-sized colonies engaged in honey production. Specifically, incubation of entire hives prompts immediate countermeasures of confined workers, such as increased fanning to cool the brood, which might jeopardize required heat exposure of reproductive varroa mites within brood (Engels and Rosenkranz 1992). Conversely, treating individually collected capped brood frames separately in external devices bypasses some constraints, yet also results in high workload (removing honey supers for thorough inspections of each hive to select relevant frames, brushing off bees and returning frames post treatment). Moreover, the necessity for repeating any such procedure over the season to sufficiently control varroa mites appears impractical and hardly feasible for large-scale beekeeping operations. A promising development in this regard was the use of

electrically heatable wires integrated within combs. Originally intended to exclusively kill drone brood (and therein reproducing mites), however, this seasonally restricted measure appears insufficient in controlling mite levels year-round (Huang 2001). Concurrently with others (Porporato et al. 2022), the underlying principle was extended by integrating isolated coils within wax foundations of all worker cell-sized brood combs, including permanent connectivity to an electricity source equipped with solar panels, to allow autonomous interval heating of individually operable combs throughout the season. Such programmed hyperthermia from within combs, aiming at minimising beekeeper efforts and disturbance of colonies alike, could represent an innovative technology for permitting routine implementation independent of the scale of beekeeping operations.

Here we evaluated the ‘*Varroa Kit*’, (4th generation by Vatorex AG) as a novel hyperthermia device to control *V. destructor* in a comprehensive honeybee field study. Over one year, mature colonies placed at different apiaries were monitored to assess its efficacy in maintaining mite loads below critical thresholds, but also its tolerability by the bees and putative trade-offs at the colony level compared to a control subjected to varroa mite management based on widely used formic acid summer treatments.

Material and methods

Experimental design, honeybee colony management and differential varroa mite treatment

Three experimental apiaries, referred to as A1, A2 and A3, were located near Winterthur, north-eastern Switzerland, with pairwise distances ranging between 2.9 and 6.5 km. Swiss landrace colonies (mixed population of *A.m. carnica*, *A.m. ligustica* and *A. m. mellifera*) were kept in Swiss-format hives within bee houses. Hives were allocated to two groups after initial colony assessment by ensuring comparable strength within apiaries, i.e. nearly equal means for numbers of adult workers and total brood. There were 4 and 5 (A1), 6 and 6 (A2), and 7 and 7 (A3) colonies per hyperthermia and control group, respectively, and 35 experimental colonies in total.

Between March 2020 and April 2021, the control group was maintained according to good apicultural practice. Given foreseeable limitations of including untreated negative controls, positive controls were subjected to one of various recommended varroa mite management concepts based on organic acids for practice-oriented comparison. Specifically, controls were treated twice with formic acid after honey harvest during early August and mid-September, respectively, using FAM-dispensers (Imdorf et al. 2003). These are frequently used in Swiss-format hives across Switzerland, and

we applied the recommended dose of 130 mL of 70% formic acid. To enable a 1-week long-term treatment, initial opening settings for evaporation surface were adjusted according to actual temperature regime and increased after a few days. Notably, the first formic acid treatment stretched into a beginning hot spell (see discussion). A winter treatment was performed during early December by sublimating 1 g of oxalic acid per colony (Rademacher and Harz 2006) using the commercial device ‘VarroxEddy’ (Andermatt BioControl, Grossdietwil, Switzerland). To ensure high efficacy of winter treatment, absence of brood was verified by prior inspections of most colonies (at least half per group at each apiary). In contrast, in the hyperthermia colonies all combs of the brood chamber were subjected to heat-treatments using the *Varroa Kit* (4th generation; Vatorex AG, Winterthur, Switzerland) in 16-days intervals during early April 2020 and late October 2020. Heat-treatments were not synchronised across brood combs, individual combs were heated independently, evenly staggered over time. Specifically, isolated iron coils integrated into wax foundations of the brood combs, covering 50% of its area, were heated up linearly over 20 min and then held at 42.5 °C for 130 min. This temperature profile was chosen to kill both juvenile and adult *V. destructor* within capped brood cells during the pupal phase of developing workers. Repeated heat-treatments of all brood combs throughout the season aimed at steadily interrupting varroa mite reproduction to keep parasite loads below critical thresholds (see ‘varroa mite monitoring’ below). The investigated prototype (Supplementary Figure 1) was not able to explicitly detect capped brood within a given frame, meaning that both targeted (i.e. capped) and non-targeted (i.e. eggs and larvae) areas of the brood nest were heated at random. Individual brood frames were controlled based on system-specific identifiers, with energy supply via contacts to metal bearing rails connected to a battery powered by solar panels. At any time, proper functioning of the system could be inspected for each frame via app-access, including warnings if a given frame failed its routine interval heating (e.g. heavily propolised contacts). For convenience of pinpointed scientific interventions (see ‘digital assessment of developing honeybee brood’ below), manual programming could bypass regular interval heating of individual frames temporarily and independent of all others (thereafter being suspended as needed, i.e. for max. 22 days, and then reset), with logged data allowing to track individual frames. In the hyperthermia group, no other treatment against *V. destructor* was applied over the season, except the winter treatment using oxalic acid applied to colonies of both groups, thus serving as a reference treatment. No other measure against varroa mites, such as drone brood removal, was taken for any group.

Apart from differential varroa mite treatments, colonies of both groups were equally managed, including adjustments

of comb numbers in brood and honey chambers as indicated by colony strength and actual honey flows. Honey was harvested several times throughout the season, with the final harvest taking place in late July. Thereafter, all colonies were gradually provisioned in the same way with approximately 30 L sugar syrup (table sugar diluted in tap water in a 3:2 ratio) before October. Swarming was prevented by breaking swarm cells and providing more space, if applicable, but no colony was cupped or split on purpose. In case they occurred, swarms were documented but not returned to their colonies. Instead, all but one swarm cell were removed to prevent subsequent swarms and allow colonies to continue with a daughter queen upon successful mating. Further, if laying queens were lost for other (treatment-related) reasons, respective colonies depended on rearing emergency queens on their own, and in no case queenless colonies were rescued. All original queens were individually marked, as were egg-laying replacement queens, in order to track possible treatment effects on queen fate. Colonies were considered dead upon collapsing due to irreversible *V. destructor* damage, but also if queens or workers were found to exclusively lay unfertilised eggs with no more possibility for re-queening.

Honeybee colony strength parameters

Colony conditions of each hive were assessed using the Liebefelder method (Imdorf et al. 1987; Delaplane et al. 2013), including numbers of adult honeybees, numbers of open brood cells (eggs and larvae), numbers of capped brood cells (spinning larvae, pre-pupae and pupae) and numbers of pollen cells. Number of cells were inferred based on one square-centimetre comb surface covering four cells on average (Delaplane et al. 2013). Assessments were performed five times over one year in order to track seasonal colony dynamics with regard to differential varroa mite treatments: (i) late March 2020 (prior to group allocation), (ii) late May 2020, (iii) Mid-July 2020, (iv) late October 2020 and (v) late April 2021. Control and hyperthermia colonies were alternately evaluated at individual apiaries to mitigate correlating effects of daytime. Cumulative honey yields per colony were obtained by weighing honeycomb before and after extraction across multiple harvests over the season.

Varroa mite monitoring

Varroa mite infestation levels were tracked throughout the study using two complementary approaches. First, nursing workers, as preferred by non-reproducing *V. destructor* (Ramsey et al. 2019), were sampled in duplicate batches from each colony on frames adjacent to the brood nest and subjected to the powdered sugar method to separate varroa mites as detailed elsewhere (Dietemann et al. 2013).

Per duplicate sample an average of 38 g, corresponding to approximately 319 workers (Pietropaoli et al. 2021), were included, and returned to their hives after the procedure. Second, natural varroa mite fall was tracked on bottom sheets inserted for three to four days periods (Dietemann et al. 2013). Sheets were thoroughly protected from honeybees by a grid and covered the entire hive floor. Varroa mite assessments using powdered sugar and bottom sheets were synchronised on the following dates: (i) early April 2020, (ii) mid-May 2020, (iii) early July 2020, (iv) late July 2020 (shortly before the first formic acid treatment of controls), (v) late September 2020 (2.5 weeks after the second formic acid treatment of controls), (vi) late October 2020, (vii) late November 2020 (shortly before oxalic acid treatment of all colonies; bottom sheets only) and (viii) late April 2021.

After winter treatment with oxalic acid varroa mites were counted on bottom sheets over 2 weeks, thereby serving as a reference evaluation for differential treatments.

While there is no consensus on treatment thresholds for *V. destructor* in the scientific literature, these thresholds could be considered 3 and 10 varroa mites per day detected on bottom sheets in May and July, respectively, and 5% infested workers throughout the season (Genersch et al. 2010; Guzmán-Novoa et al. 2010; Rosenkranz et al. 2010; Dietemann et al. 2013; Bubnič et al. 2021; Jack and Ellis 2021; van der Steen and Vejsnæs 2021).

Digital assessment of developing honeybee brood

Next to overall treatment-mediated impact on colony performance and relative parasite dynamics, we were interested in investigating possible undesired side-effects of hyperthermia on developing honeybee brood. Therefore, relevant brood stages were evaluated according to OECD Guidance Document 75 (Oomen et al. 1992; OECD 2007; Medrzycki et al. 2013). Assessments were performed for three to four colonies of both groups per apiary during mid-June 2020 and late September 2020 to account for putative seasonal effects. Relevant brood stages were eggs, young larvae and old larvae as non-targets possibly unintentionally exposed to heat-treatments. Although not part of the OECD evaluation, we assessed capped brood, the target developmental stage for hyperthermia here, as detailed below. Frames for brood development assessments were selected based on the presence of high numbers (ideally hundreds) of one or multiple relevant brood stages, and initial photos corresponding to day zero were taken (OECD 2007). Hyperthermal treatments of selected combs were then manually activated to heat-treat within 24 h, success thereof being confirmed based on the logged data. Subsequent brood assessments were performed by taking photos of the same combs conforming to the guideline's intervals, i.e. days 5, 10, 16 and 22 (\pm one day each) (OECD 2007). Successive photos of all combs were

analysed using the software HiveAnalyzer (Visionalytics, Pleidelsheim, Germany) to determine brood termination rates (BTRs), i.e. the proportion of initially selected cell contents that at any occasion fail matching expected brood stages pursuant to regular development (OECD 2007; Medrzycki et al. 2013). Further, the programme provides brood and compensation indices, with values of both ranging from 0 to 5, which corresponds to complete failure and success, respectively. Compensation indices extend brood indices, which correlate with BTRs, by reflecting if and how fast previous losses are compensated by new brood within the evaluated period (OECD 2007).

For eggs and larval stages, these parameters were evaluated after completing a regular brood cycle on day 22 (± 1). Our supplementary assessment of freshly capped brood provided a reference point to determine the developmental stage of pupae and allow for the assessment of failure vs. regular development prior to regular honeybee worker emergence. Therefore, we exclusively selected supposedly freshly capped brood cells directly adjacent (within a maximum of three cell rows) to old larvae to maximise chances effectively selected capped cells contained early pupal stages. Moreover, capped brood was monitored only up to day 5 (± 1) because by day 10 regular emergence vs. failure could not be distinguished anymore. Accordingly, calculations of compensation indices for capped brood were omitted. Yet, irrespective of these assessment-specific precautions, capped brood, and therein reproducing varroa mites, would be target to hyperthermia during the entire pupal phase.

Average numbers (SD) of selected brood cells (across dates) per colony were 326 ± 56 (eggs), 326 ± 101 (young larvae), 299 ± 101 (old larvae) and 293 ± 60 (capped brood), whereby a minimum of 100 cells per replicate was ensured throughout.

Statistical analyses

All data were analysed and visualised in R v.4.2.2 (R Development Core Team 2019), including standard packages such as *lme4*, *lmerTest*, *glmmTMB*, *lsmeans*, *phia* and *ggplot2*. Initially, full models were fitted including twofold or threefold interaction terms of the explanatory factors apiary, treatment and, if applicable, date. Model simplification was evaluated for all variables by hierarchically removing interaction terms and consulting, e.g., the Akaike information criterion, and model diagnostics of residuals were inspected during all steps.

Colony strength parameters were analysed using linear mixed effect models including colony as a random effect. Numbers of adult honeybees and pollen cells per colony were square-root transformed to conform to model assumptions of homogeneity. Numbers of open and capped brood cells were divided by maximum respective scores to

account for the upper-bounded egg-laying rate of honeybee queens under the present conditions and to generate binomially distributed variables. Obtained ratios were arcsine square-root transformed to stabilise residual variances. Total honey harvest (kg) was analysed using a weighted linear model to account for different residual variances across factor combinations, with weights being set as the inverse of the residual variances. The general parameters swarming, queen supersedure and colony survival were evaluated across apiaries using two-sided fisher tests.

Varroa mite counts obtained from powdered sugar monitoring and estimated numbers of sampled workers were averaged across duplicate samples per colony and assessment date. Infestation rates were analysed using a generalised linear mixed effect model (GLMM) with binomial error distribution and logit link including numbers of sampled workers per colony as weights. Naturally fallen mites per day and colony, as monitored on bottom sheets, were subjected to a GLMM with poisson error distribution with log link. Both GLMMs included an observation-level random effect (OLRE) to account for overdispersion, and convergence of final models was ensured by implementing appropriate optimizers ('nlminb' and 'bobyqa' for models on varroa mites detected in worker samples and on bottom boards, respectively). Total numbers of varroa mites detected after the winter reference treatment were analysed with a generalised linear model (GLM) with log link and quasipoisson error distribution to account for overdispersion.

Brood assessment data were evaluated by means of GLMMs. Proportional brood termination data were fitted based on models with binomial error distribution and logit link. Likewise, brood and compensation indices were evaluated based on binomial responses as calculated by quotients between effectively obtained and maximum possible scores. By definition, both indices reach a maximum score of 5 for eggs and larvae upon 100% successful development (OECD 2007; Medrzycki et al. 2013). However, since (early) pupae were only monitored until day 5 and not anticipated to emerge regularly, this response was fixed to a maximum score of 4. BTR responses of all brood stages were modelled including numbers of evaluated cells as weights, and OLREs to account for overdispersion. By implementing appropriate optimizers, convergence was verified for all models (eggs and old larvae: 'BFGS'; young larvae and capped brood: 'nlminb').

Next to contrasting treatments for all variables, additional nested contrasts were performed in the presence of significant interaction terms by comparing treatments within apiaries on individual assessment dates. If the best-fitting model on a given response included main effects only, nested contrasts based on models including twofold interaction terms were still computed for overall comparison. Since seasonal

effects on virtually all responses were a priori expected, no contrasts between dates were performed.

Results

Swarming, queen supersedure and colony survival

During the season 2020, 11% and 6% of the control and hyperthermia colonies, respectively, swarmed (Odds Ratio: 1.96 [95%CI: 0.09–125.15]; $p = 1$). Frequent replacement of original queens was observed across colonies by the last assessment, being significantly more common in the control compared to the hyperthermia group (78% vs. 35%; Odds Ratio: 6.04 [95%CI: 1.19–37.90]; $p = 0.018$). Particularly queen loss as a direct consequence of formic acid treatments in 50% of the control colonies was striking. In this regard, although overall colony losses did not differ between treatments (22% vs. 18% of the control and hyperthermia colonies, respectively; Odds Ratio: 1.32 [95%CI: 0.19–10.75]; $p = 1$), respective reasons appeared to differ. While colony failure in the control was exclusively due to insufficiently mated or lost replacement queens, this was the case in only one of the three lost hyperthermia colonies, whereas the other two probably collapsed due to irreversible *V. destructor* damage.

Colony conditions and honey harvest

Relevant colony parameters generally exhibited expected dynamics throughout the trial (Fig. 1). Date significantly affected numbers of adult honeybees, amounts of open and capped brood as well as pollen stores (Table 1A). While apiary had no effect on any of these responses, hyperthermia significantly decreased numbers of adult workers and significantly increased pollen stores, but had no effect on the amounts of open and capped brood (Table 1A). Dynamics of capped brood (pupae) was the sole parameter for which the retained model included main effects only, whereas best-fitting models for the other responses involved all twofold interaction terms (but no threefold interaction). Significant interactions between apiary and date were prevalent (adult honeybees, open brood and pollen), and for open brood also the interaction between treatment and date was significant (Table 1A). However, interaction effects between apiary and treatment were absent for all responses.

Upon equal starting conditions for both treatments, numbers of adult workers in the control grew significantly larger than in the hyperthermia group during spring 2020, remained increased during summer (non-significantly) and autumn (significantly), but showed no difference in spring 2021 (Fig. 1a, Table 2A). These patterns of significance were also present when comparing treatments within apiaries on

individual dates, yet consistent significant effects across apiaries were detected only during peak colony growth in May 2020. Across the season 2020, one apiary showed consistent significant differences between treatment groups (A3) (Table 2B).

Numbers of eggs and larvae were similar in both treatments throughout, except during May 2020, where significantly increased amounts of open brood in the hyperthermia group opposed patterns of adult honeybees (Fig. 1b, Table 2A) largely consistently across apiaries (Table 2B).

Numbers of pupae did not differ between treatments at any date (Fig. 1c, Table 2A), and, except for peak growth during May 2020, largely reflected patterns of open brood. Contrasts between treatments within apiaries revealed the absence of significant differences throughout the study, except for apiary A3, where during May 2020 increased numbers of pupae in the control opposed trends at the other apiaries (Table 2B).

Pollen stores were not considered for initial group allocation, and were by chance significantly increased in the hyperthermia group at experimental start. Relative effect size continued to increase during May 2020 and pollen stores converged between groups only later in the season (Fig. 1d, Table 2A). Trends were similar across apiaries, except for A1 where pollen stores of hyperthermia colonies remained significantly increased throughout the season 2020 (Table 2B).

Across apiaries, mean cumulative honey harvest per colony (Fig. 2) was significantly lower in the hyperthermia (42.7 kg) compared to the control group (55.0 kg). Control colonies were more productive at all sites, so a simple model without interaction terms could be used for the analysis (Table 1A). However, contrast-based estimates (control vs. hyperthermia) within apiaries still revealed local variation for effect size: 8.16 kg for A1, 0.92 kg for A2 (both not significant, $p > 0.19$), and 25.14 kg for A3 ($p < 0.01$).

Varroa mite monitoring

Dynamics of *V. destructor* infestation rates based on mite counts with the powdered sugar method and recordings of natural mite fall on bottom sheets were highly consistent (Fig. 3a, b). Unsurprisingly, season had the strongest effect in both models (Table 1B). Treatment main effects were absent, yet modelling varroa mite dynamics based on both approaches (powdered sugar method: threefold interaction; bottom sheets: all twofold interactions) revealed all interaction terms were significant, except between apiary and treatment. A stronger increase in varroa mite levels in the control compared to the hyperthermia treatment over the season 2020 (Fig. 3a, b) resulted in significant differences during early and late July for each one of the two complementary monitoring approaches (Table 3A).

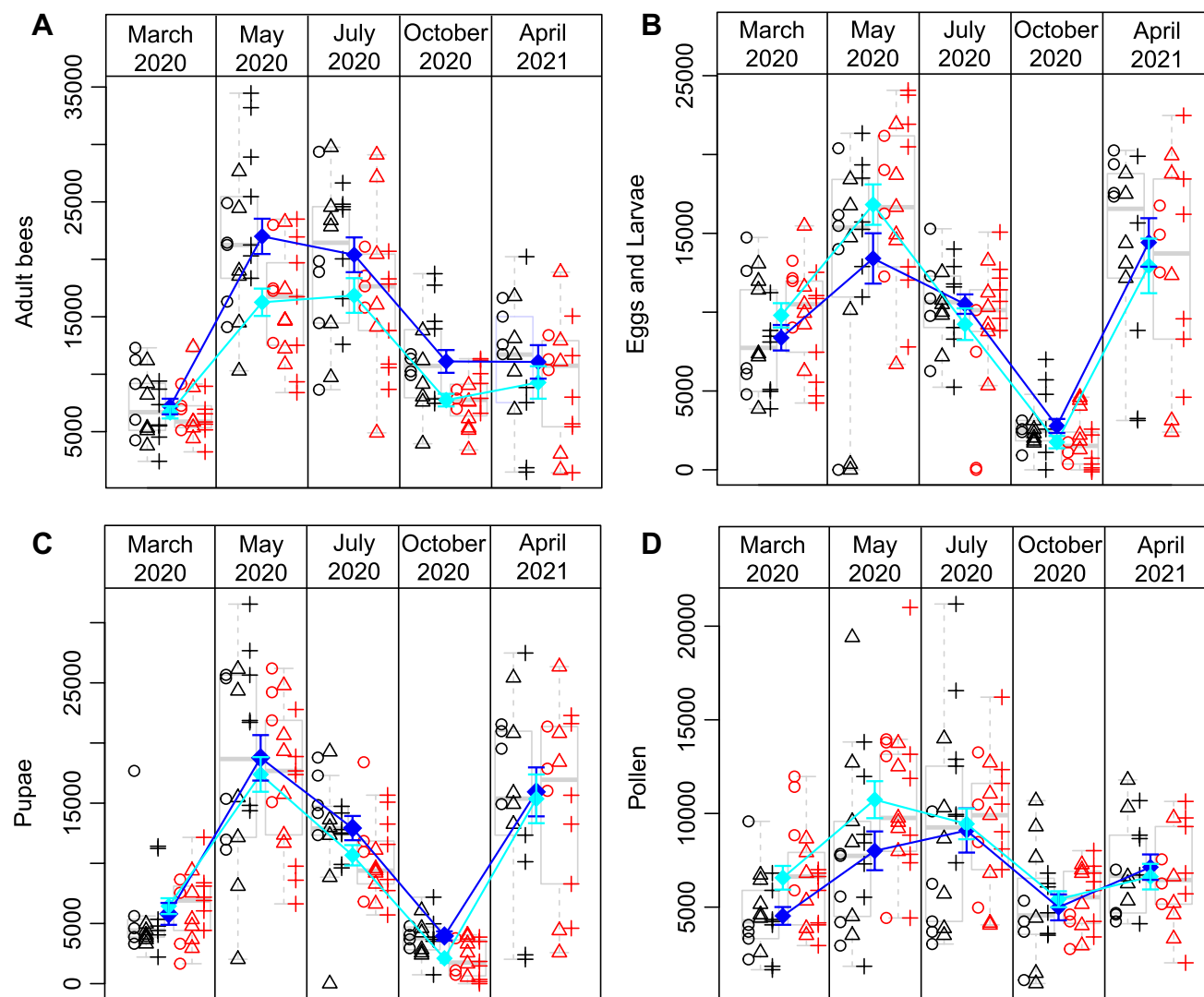


Fig. 1 Colony parameters throughout the experiment. Population estimates of adult honeybees (a), eggs and larvae (b), pupae (c) and comb cells containing pollen stores (d) are indicated for experimental groups (X-axis), i.e. control (black) and hyperthermia (red) colonies per apiary denoted as symbols (A1: circles; A2: triangles; A3:

crosses) on successive assessment dates. Boxplots for each treatment (across apiaries) within dates are shown in grey. Adjusted means (SE) over time are highlighted for control (dark blue) and hyperthermia (light blue) colonies. For overall statistical evaluations see Table 1, for specific contrasts see Table 2

Subsequent to formic acid treatments between early August and mid-September in the control, autumn patterns were temporarily reversed (Fig. 3a, b), with controls showing significantly decreased varroa mite levels compared to hyperthermia colonies until October 2020 for one or both *V. destructor* monitoring approaches (Table 3A).

Natural mite fall on bottom sheets during late November 2020 revealed significantly increased varroa mite levels in the control compared to the hyperthermia group, a pattern that persisted even beyond the winter treatment with oxalic acid as a non-significant trend the following spring (Fig. 3a, b, Table 3A).

Contrasts between treatments within apiaries confirmed largely consistent local dynamics during critical

phases, although effect sizes of different monitoring approaches reflected some stochasticity on individual dates (Table 3B).

Numbers of varroa mites recorded after the winter reference treatment were significantly increased in the control (Table 1B; $p=0.002$), and a significant apiary effect was driven by apiary A3 (Fig. 3c, Table 1B). Within apiaries contrast-based estimates for ratios of varroa mites (control vs. hyperthermia, response scale) were 1.27 for A1, 1.77 for A2 ($p>0.37$), and 2.59 for A3 ($p<0.01$). Between-apiary-within-treatment differences were detected for the control but not the hyperthermia group.

Table 1 Summary of statistical analyses. Effect sizes, i.e. *F*- and Wald-Chi-square values obtained for parameters relevant for colony parameters (A), varroa mite monitoring (B) and brood termination rates (C), are indicated for each factor and interaction term (according to specifically retained models)

Response	Factor						
	Apiary (A)	Treatment (T)	Date (D)	A × T	A × D	T × D	A × T × D
<i>(A) Colony parameters</i>							
Adult bees	0.77	7.27*	48.3***	0.44	2.28*	1.15	na
Eggs and larvae	0.01	0.02	41.79***	0.49	2.57*	3.17*	na
Pupae	1.56	1.69	52.93***	na	na	na	na
Pollen	1.11	4.53*	15.43***	2.29	2.72**	1.9	na
Honey harvest	3.9*	6.09*	NA	na	NA	NA	NA
<i>(B) Varroa monitoring</i>							
Infestation rate	0.23	0.84	320.07***	2.11	29.18**	45.73***	28.58**
Mite fall/day	12.14**	0.28	146.18***	2.09	26.28*	39.69***	na
Winter treatment	29.03***	10.69**	NA	na	NA	NA	NA
<i>(C) Brood termination rate</i>							
Eggs	0.5	22.56***	7.89**	na	na	na	na
Young larvae	0.23	40.01***	6.74***	3.27	7.22*	0.62	11.18**
Old larvae	0.28	40.21***	5.97*	3.68	3.59	3.08	na
Pupae	11.45**	9.91**	35.81***	1.49	1.68	3.97*	23.85***

Significant effects are highlighted in bold (***) < 0.001 ; ** < 0.01 ; * < 0.05 ; < 0.1). NA—not applicable (owing to design); na—not relevant according to retained model

Digital assessments of developing brood

Across treatments, brood termination rate (BTR) was generally increased later in the season, and independent of the season, significantly higher for all developmental stages exposed to hyperthermia (Fig. 4; Table 1). While hyperthermia effects were stronger relative to seasonal effects for open brood stages, the opposite was found for pupae (Table 1C).

For BTR of eggs, the retained model excluded interaction terms, and significant main effects for treatment and season were detected (Fig. 4a, Table 1C). Contrasts between treatments within apiaries revealed prevalent significantly increased egg BTR of the hyperthermia group on both assessment dates (Table 4).

The best-fitting model for BTR of young larvae was based on the threefold interaction (Table 1C). Post hoc contrasts across apiaries revealed significantly increased brood mortality in the hyperthermia group on both dates ($p \leq 0.002$). However, while young larvae BTR of the control were significantly increased later in the season ($p = 0.022$), season had no additional effect on hyperthermia-treated colonies ($p = 0.281$; Fig. 4b). Post hoc contrasts within apiaries mostly detected significantly increased BTR of hyperthermia-treated young larvae on both dates but no consistent change in effect sizes across seasons (Table 4). These patterns overall translated into significant two- and threefold interactions including apiary and date (Table 1C).

BTR of old larvae was modelled including all twofold interaction terms, but only the two main effects treatment and date were found significant (Table 1C). The interaction between treatment and date remained non-significant

(Table 1C) despite the lack of season-mediated differences for old larvae BTR in the control ($p = 0.622$) compared to a significant increase in BTR for hyperthermia-treated old larvae during autumn ($p < 0.005$, Fig. 4c), as reflected by contrasts between treatments for each apiary within seasons (Table 4).

BTR of pupae was evaluated based on the full model including the threefold interaction and found to be significantly increased by hyperthermia, although at strongly relaxed effect size compared to younger brood stages (Table 1C). Across apiaries, hyperthermia significantly affected pupae only later in the season, but not during June (Fig. 4d, Table 4). However, contrasting treatments within apiaries on individual dates documented some divergent dynamics at A1 relative to A2 and A3 (Table 4) that drive an overall significant threefold interaction effect (Table 1C).

Importantly, interactions between apiary and treatment were absent in BTR models for all developmental stage (Table 1C), as they were for colony parameters and varroa mite monitoring.

To evaluate brood (Supplementary Table 1A, Supplementary Figure 2) and compensation indices (Supplementary Table 1B, Supplementary Figure 3), retained models for all developmental stages included only main effects, and in no case apiary or date were significant. For open brood stages, hyperthermia resulted in significantly decreased brood indices (eggs: $p = 0.007$; young larvae: $p = 0.003$; old larvae: $p = 0.029$), however, no effects were found for pupae

Table 2 Contrasts between treatments (control vs. hyperthermia) for relevant colony condition responses on successive assessment dates (A) across apiaries, and (B) for apiaries within dates

Factor	Response			
	Adult bees	Eggs & larvae	Pupae	Pollen
A Date				
March 2020	1.27	-0.06	-0.02	-15.73*
May 2020	19.96**	-0.21**	0.03	-16.67**
July 2020	12.75	0.10	0.06	-4.47
October 2020	15.81*	0.09	0.12	-7.56
April 2021	7.09	0.05	0.01	1.83
B Apiary within date				
March 2020 (A1)	-3.14	0.00	0.07	-28.63**
March 2020 (A2)	0.62	-0.10	-0.06	-8.59
March 2020 (A3)	6.34	-0.08	-0.06	-9.97
May 2020 (A1)	15.54	-0.15	-0.13	-29.57**
May 2020 (A2)	19.31*	-0.26**	-0.10	-9.53
May 2020 (A3)	25.02**	-0.23*	0.31**	-10.91
July 2020 (A1)	8.33	0.16	0.12	-17.37
July 2020 (A2)	12.10	0.06	0.03	2.67
July 2020 (A3)	17.81*	0.08	0.05	1.29
October 2020 (A1)	11.40	0.16	0.11	-20.45*
October 2020 (A2)	15.16	0.05	0.06	-0.42
October 2020 (A3)	20.88*	0.08	0.17	-1.80
April 2021 (A1)	2.68	0.11	0.01	-11.06
April 2021 (A2)	6.44	0.00	0.14	8.97
April 2021 (A3)	12.16	0.03	-0.12	7.59

Estimates of the control relative to the hyperthermia treatment are shown in specifically transformed scales (see *statistical analyses*), with significant differences highlighted in bold (***<0.001; **<0.01; *<0.05; <0.1). Contrasts across apiaries may be interpreted with caution in the presence of significant interactions (see Table 1A). Nested contrasts for retained models including only main effects (pupae) are based on the alternative model including all two-fold interaction terms

($p=0.385$). Somewhat relaxed but still significant decreases

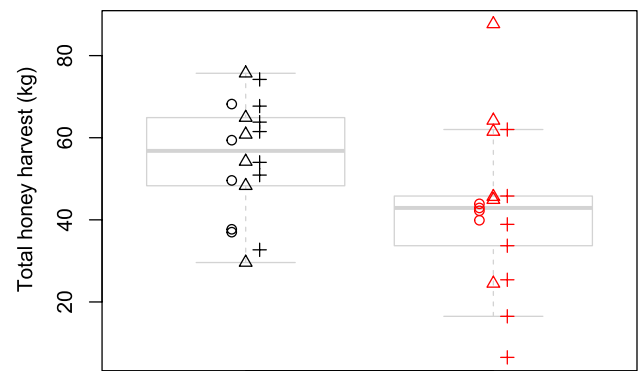


Fig. 2 Total honey harvest of all experimental colonies during the season 2020. Boxplots for each experimental group (X-axis; across apiaries) are shown in grey. Black (control) and red (hyperthermia) symbols refer to different apiaries (A1: circles; A2: triangles; A3: crosses). For statistics see Table 1

in the hyperthermia group were detected for compensation indices (eggs: $p=0.040$; young larvae: $p=0.015$; old larvae: $p=0.049$).

Discussion

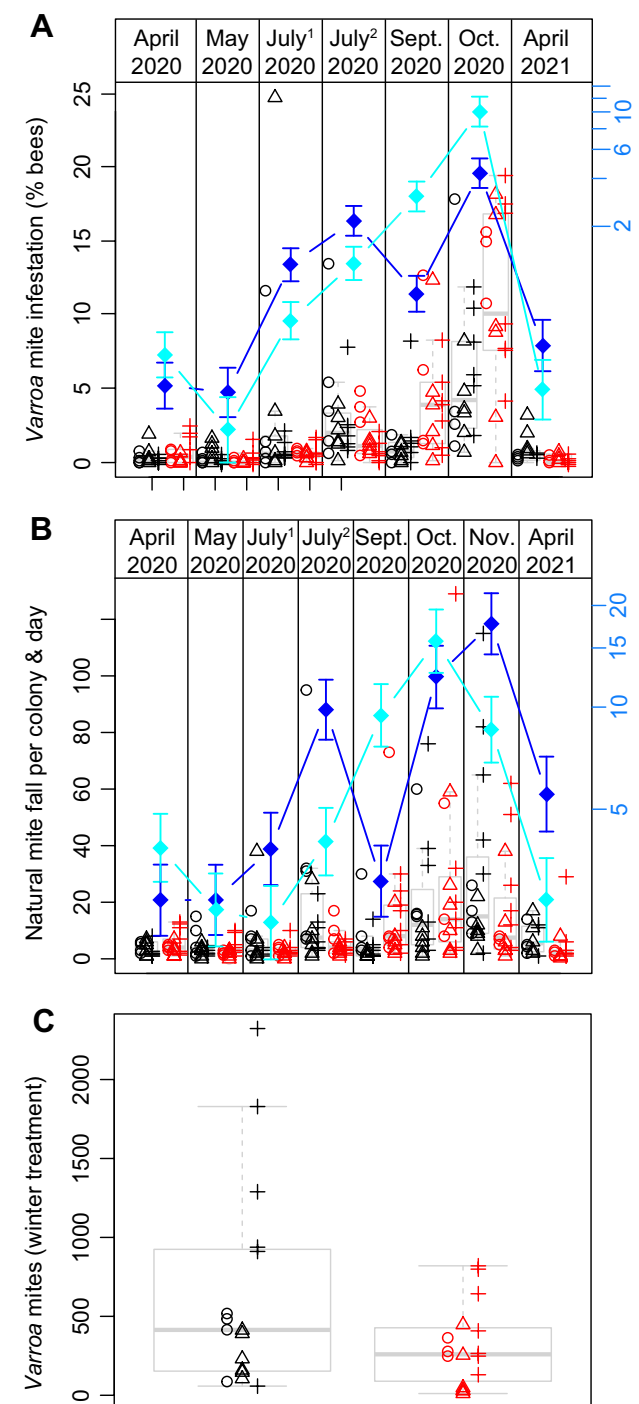
This study documents the most comprehensive scientifically monitored honeybee field trial on hyperthermia treatment against *V. destructor* to date. Thereby, it substantially advances our understanding of the previously recognised but largely anecdotal potency of hyperthermia treatment. In the following, the efficacy of an innovative hyperthermia device for control of *V. destructor* based on heatable coils integrated into brood comb is evaluated throughout the season and compared to recommended summer treatments with formic acid. Both treatment options are discussed in an apicultural context, including an emphasis on relative trade-offs at the colony level.

Congruent with the literature on seasonal varroa mite thresholds (Genersch et al. 2010; Guzmán-Novoa et al. 2010; Rosenkranz et al. 2010; Bubnič et al. 2021; Jack and Ellis 2021; van der Steen and Vejsnæs 2021), Swiss beekeepers are advised to avoid exceeding 1.2 (July), 2.4 (August) and 3.6 (September) mites per 100 workers, with 6% infestation rate considered a season-wide alarming damage threshold (*apisuisse*; www.bienen.ch). Accordingly, natural mite fall per colony and day should not exceed 3 (May), 5 (October/November) or 10 (any other time of the year). In this study, mean varroa mite numbers in both treatments ranged below damage thresholds during most seasons, except for late autumn, when potentially alarming infestation rates were reached (Fig. 3a, b). During July controls approached critical varroa mite levels suggesting that formic acid treatments were timely, whereas at that time no measures were

Fig. 3 *Varroa destructor* monitoring throughout the experiment. ▶ Infestation rates per 100 workers inferred from the powdered sugar method performed on seven successive occasions (a), natural mite fall per colony and day recorded from bottom sheets on eight successive occasions (b), and total numbers of varroa mites documented within 2 weeks post oxalic acid winter reference treatment (c). Experimental groups (X-axis), i.e. control (black) and hyperthermia (red) colony replicates are denoted per apiary as symbols (A1: circles; A2: triangles; A3: crosses) on successive assessment dates and box-plots for each treatment (across apiaries) within dates are shown in grey (collectively referring to left-hand Y-axes). Panels A and B further highlight adjusted means (SE) over time for control (dark blue) and hyperthermia (light blue) colonies plotted on geometric scales of respective link functions (see statistical analyses) on the right-hand Y-axis for better accessibility. Superscript numbers in panels A and B refer to screenings in ¹early and ²late July, respectively. For overall statistical evaluations see Table 1, for specific contrasts (panels A and B) see Table 3

indicated for hyperthermia colonies, which yet approached concerning infestation rates until September despite ongoing heat-treatments (Fig. 3a, b). Comparatively high numbers of varroa mites recorded after the oxalic acid winter treatment (Fig. 3c) indicate several colonies across treatment groups may have been at risk by October/November (Fig. 3a, b; Imdorf et al. 2003). However, both treatment options (including a winter treatment with oxalic acid) can be considered successful, as a moderate colony loss rate of 20% was observed on average, lower than the 25.8% recorded for Swiss lowlands over the winter 2020/21 (Reihl and Charrière 2021). Therefore, the investigated heat-treatment appears suitable to safeguard colonies over the productive season, replacing formic acid summer treatments which may be increasingly difficult to implement in beekeeping practice in various regions under progressing climate change (Pietropaoli and Formato 2019; Smoliński et al. 2021; van der Steen and Vejsnæs 2021; Vilarem et al. 2021). Nevertheless, hyperthermia, as applied in this study, should not be considered an exclusive approach for combatting *V. destructor*. Instead, as an integral chemical-free pillar, it could support apicultural concepts of integrated pest management becoming less dependent on organic acids (Jack and Ellis 2021). Particularly the combination of hyperthermia with a comparatively low-impact oxalic acid treatment during broodless winter periods to ensure efficient resetting of varroa mite loads once per year appears feasible.

Honeybees drifting between hives, including robbers, facilitate the exchange of *V. destructor*, whereby reinvasions of mites from untreated (or collapsing) colonies into naïve or previously treated ones can mediate problematic snowballing effects especially in areas with high colony densities (Rosenkranz et al. 2010; Frey et al. 2011; Frey and Rosenkranz 2014; Peck and Seeley 2019), as is the case for north-eastern Switzerland (von Büren et al. 2019). In our setting, with colonies of both treatments sharing the same apiaries, putative experimental interference due to mutual



parasite reinvasion cannot be dismissed, the extent of which, however, was likely characterised by seasonally reversed invasion pressures. Between spring and end of July, slopes of growing varroa mite populations in hyperthermia colonies were relaxed compared to until then untreated controls (Fig. 3a, b). Possibly, inferred hyperthermia efficacy was underestimated owing to varroa mites constantly reinvading from controls over this period. Even numerically low varroa mite flow to the hyperthermia group could have

Table 3 Contrasts (control vs. hyperthermia) for two varroa mite monitoring approaches on successive assessment dates A) across apiaries, and B) for apiaries nested within dates

Factor	Response	
	Varroa infestation rate/bee (powdered sugar)	Natural mite fall/day (bottom sheets)
A Date		
March 2020	-0.46	-0.36
May 2020	0.55	0.07
July ¹ 2020	0.83*	0.50
July ² 2020	0.62	0.90**
September 2020	-1.45***	-1.13***
October 2020	-0.91**	-0.24
November 2020	NA	0.72*
April 2021	0.65	0.72
B Apiary within date		
March 2020 (A1)	-1.04	-0.04
March 2020 (A2)	1.61	-0.38
March 2020 (A3)	-1.94**	-0.65
May 2020 (A1)	0.02	0.39
May 2020 (A2)	2.85*	0.04
May 2020 (A3)	-1.22	-0.22
July ¹ 2020 (A1)	0.59	0.82
July ¹ 2020 (A2)	1.73**	0.48
July ¹ 2020 (A3)	0.16	0.21
July ² 2020 (A1)	0.24	1.21**
July ² 2020 (A2)	0.38	0.87*
July ² 2020 (A3)	1.23*	0.61
September 2020 (A1)	-1.94**	-0.81
September 2020 (A2)	-1.64**	-1.15**
September 2020 (A3)	-0.76	-1.42***
October 2020 (A1)	-1.43*	0.07
October 2020 (A2)	-0.71	-0.27
October 2020 (A3)	-0.59	-0.53
November 2020 (A1)	NA	1.04*
November 2020 (A2)	NA	0.69
November 2020 (A3)	NA	0.43
April 2021 (A1)	-0.53	1.04*
April 2021 (A2)	1.13	0.69
April 2021 (A3)	1.34	0.43

Estimates of the control relative to the hyperthermia treatment are shown in specifically transformed scales (see *statistical analyses*), with significant differences highlighted in bold (*** < 0.001; ** < 0.01; * < 0.05; < 0.1). Superscript numbers refer to ¹early and ²late July, respectively. NA—not applicable. Contrasts across apiaries may be interpreted with caution in the presence of significant interactions (see Table 1B)

boosted later parasite dynamics between October, when heat-treatments were stopped, and the winter treatment with oxalic acid (Fig. 3a, c). Indeed, residual varroa mite loads observed in hyperthermia colonies during winter conform

to modelling outcomes considering moderate to strong reinvasion by Porporato et al (2022) who used a comparable hyperthermia device. Conversely, the reverse direction of potential reinvasions at even higher levels might have contributed to rapidly increasing *V. destructor* populations in the controls after two formic acid summer treatments, thereby partly negating initially high efficacies until late autumn (Fig. 3a, b). Nevertheless, such strong dynamics may well occur naturally (Wilkinson and Smith 2002; Harris et al. 2003; Smoliński et al. 2021), and were possibly reinforced by prolonged breeding to build up winter bee populations upon pronounced late-season re-queening in our control colonies (Mattila et al. 2001). No control failed due to *V. destructor*, but exclusively inadequate queen replacement following formic acid mediated queen losses, as opposed to some of the hyperthermia treatment cases. A combination of factors might have shaped these queen losses; applying relatively high concentrated formic acid (70%) during an unexpected hot spell (daily max. temperatures up to 30.3 °C on the last days of the first treatment) could have been decisive. Furthermore, Swiss-format hives are characterised by combs oriented in parallel to, rather small-sized, entrances, potentially leading to compromised ventilation. They can also show a lack of noteworthy food crowns in single brood chambers post-harvest. Formic acid mediated queen losses are well known in beekeeping practice (Underwood and Currie 2007; Vilarem et al. 2021; Bachert et al. 2022) and likely add to the various reasons for ‘queen problems’ that continue making a major contribution to overall yearly colony losses (van der Zee et al. 2014; Brodschneider et al. 2016, 2018; Gray et al. 2020).

We document common apiary effects as well as interactions with date, particularly regarding *V. destructor* dynamics (Table 1), as expected (Korená Hillayová et al. 2022). However, the absence of treatment-by-apiary interactions for all relevant responses (Table 1) indicates that hyperthermia effects observed here are independent of location, suggesting stable efficacy and allowing robust extrapolation to comparable settings. Conversely, efficacy of formic acid treatments strongly depends on weather, microclimate, and dispenser and hive peculiarities (Imdorf et al. 2003; Rosenkranz et al. 2010; Steube et al. 2021; van der Steen and Vejsnæs 2021; Vilarem et al. 2021). This may explain strong apiary effects on residual varroa mite loads revealed upon the winter reference treatment (Table 1) combined with higher variation across apiaries in the control (Fig. 3c), and highlights that the evaluated hyperthermia device is expected to exhibit at least equally consistent efficacy across sites compared to formic acid treatments. However, large-scale testing across different locations, hive systems, control treatments and operators, as well as monitoring colonies over multiple years, is encouraged to corroborate tentative conclusions on hyperthermia efficacy and reliability.

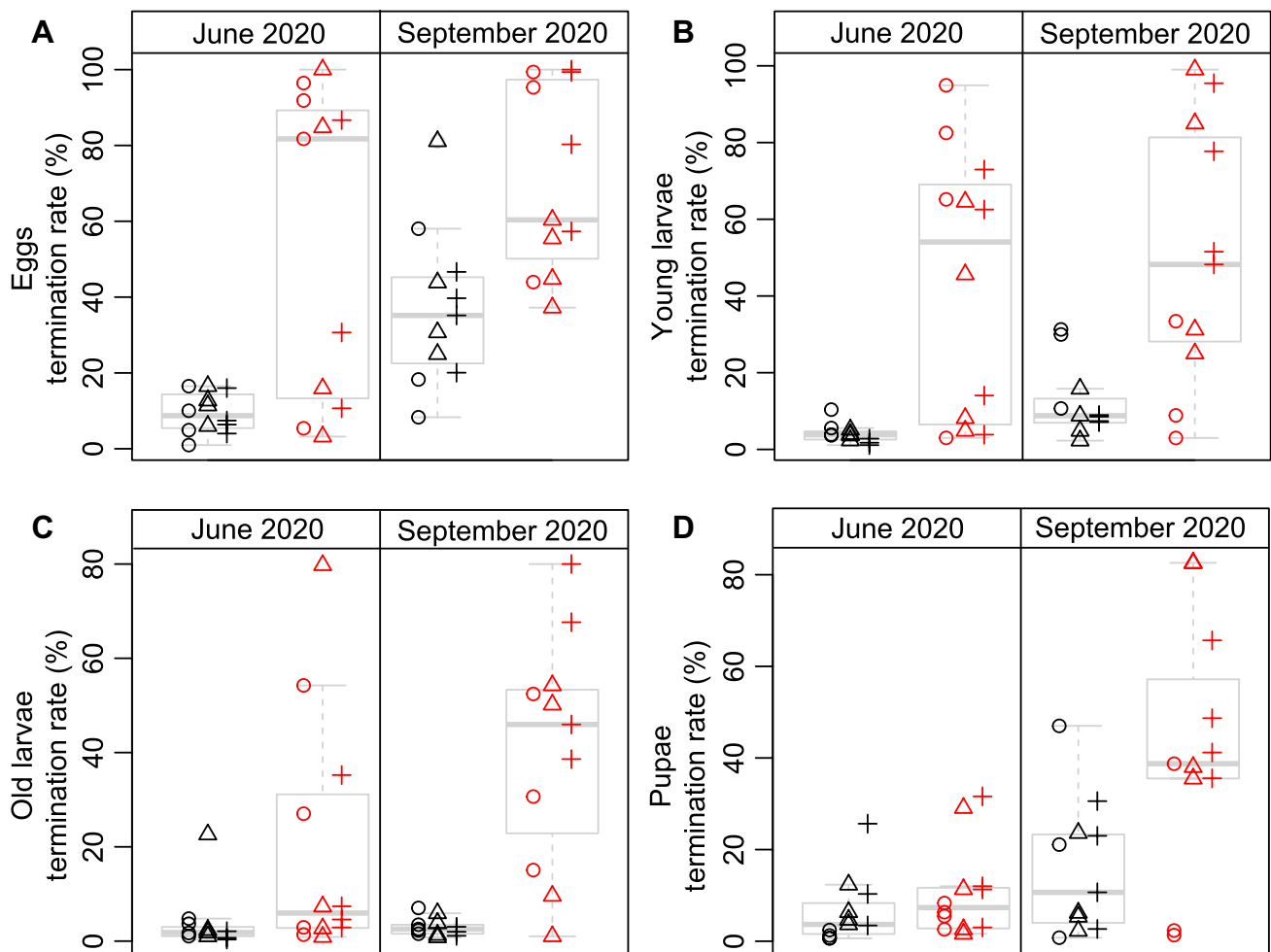


Fig. 4 Brood termination rates during summer and autumn. Percentages of failed development are shown for the relevant brood stages eggs (a), young larvae (b), old larvae (c) and pupae (d). Responses for experimental groups (X-axis), i.e. control (black) and hyperthermia (red) colony replicates are denoted per apiary as symbols (A1: circles; A2: triangles; A3: crosses). Boxplots for each treatment (across apiaries) within dates are shown in grey. For overall statistical evaluations see Table 1, for specific contrasts see Table 4

As an obvious side-effect of hyperthermia increased losses particularly of non-target brood stages (Fig. 4a–c) plausibly translated directly into decreased numbers of adult honeybees during spring and summer (Fig. 1a), and consequently lower honey yields (Fig. 2). Some associated patterns indicate this colony-level trade-off might partly reflect altered resource allocation in hyperthermia colonies. Specifically, increased amounts of eggs and larvae during spring growth in the hyperthermia group (Fig. 1b) point at higher brood rearing investment to compensate for respective losses (Fig. 4a–c). Although inferred indices suggest rather time-lagged compensation (Supplementary Figure 3), numbers of pupae comparable to controls throughout the season (Fig. 1c) show these efforts were overall successful. Due to the low susceptibility of pupae to heat exposure during summer (Fig. 4d), similar numbers of constantly emerging adult honeybees would be expected in both groups.

However, prolonged lifespans of workers subjected to hyperthermia during brood development, as found previously in cage experiments (Kablaw et al. 2020b), could hardly be reconciled with comparatively decreased numbers of adult honeybees in our field setting (Fig. 1a). Indeed, conflicting findings of reduced life expectancy of workers exposed to elevated temperatures during brood development were reported under more realistic conditions (Appel and Büchler 1991). Therefore, we hypothesise that the documented lower numbers of adult honeybees (Fig. 1a) and honey harvests (Fig. 2) of the hyperthermia group are best explained by colony-level responses related to compensatory brood rearing. First, temporarily increased pollen foraging (Fig. 1d) triggered by excessive brood cues (Fig. 1b) (Camazine 1993; Pankiw et al. 1998; Dreller et al. 1999) likely conflicts with simultaneous nectar foraging. Second, relative to honey production, increased nursing activity and pollen

Table 4 Contrasts between treatments (control vs. hyperthermia) for brood termination rates during summer and autumn across apiaries (first line within each date), and for individual apiaries (A1, A2 and A3) within dates

Date (Apiary)	Brood termination rate			
	Eggs	Young larvae	Old larvae	Pupae
<i>June 2020</i>	-3.22***	-2.86***	-1.76**	-0.59
A1	-4.38***	-3.36***	-1.53*	-1.85*
A2	-2.10**	-2.02*	-0.98	-0.03
A3	-3.17**	-3.21**	-2.75**	0.12
<i>September 2020</i>	-2.53***	-2.16**	-3.09***	-1.39**
A1	-4.13***	0.81	-2.87***	0.86
A2	-0.26	-3.74***	-2.31**	-3.29***
A3	-3.19**	-3.54***	-4.08***	-1.73*

Estimates of the control relative to the hyperthermia treatment are shown in specifically transformed scales (see *statistical analyses*), with significant differences highlighted in bold (***) <math><0.001</math>; ** <math><0.01</math>; * <math><0.05</math>; <math><0.1</math>. Contrasts across apiaries may be interpreted with caution in the presence of significant interactions (see Table 1C). Nested contrasts for retained models including only main effects (eggs) are based on the alternative model including all twofold interaction terms

foraging (Fig. 1b, d) are both considered major drivers for accelerating honeybee ageing (Amdam et al. 2009; Münch and Amdam 2010). Exacerbation thereof may occur if brood exposure to higher temperatures triggers earlier onset of foraging in adults (Becher et al. 2009), although such causalities could not be confirmed particularly for hyperthermia-treated pupae (Kablaui et al. 2020b). Nonetheless, altered division of labour due to demographic changes appears likely (Huang and Robinson 1996). Consequently, brood compensation in the hyperthermia group (Fig. 1b, c) may not have counterbalanced both increased losses of non-target brood (Fig. 4a–c) and turnover rates of workers (Fig. 1a). In addition to temporarily decreased numbers of adult workers (Fig. 1a), restricted honey yields of hyperthermia colonies (Fig. 2) could be influenced by reduced sucrose responsiveness of workers emerging from hyperthermia-exposed brood (Kablaui et al. 2020b). Consequences of reduced sucrose responsiveness may not constrain resource exploitation in the presences of floral visual and scent cues (van der Kooi et al. 2019; Bisrat and Jung 2022). However, during honeydew flow, as observed here throughout summer 2020, supposedly lacking floral cues could increase honeybee dependency on sucrose responsiveness, or if compromised, possibly translate into less efficient exploitation of this resource.

Moreover, variable effect sizes of treatment-mediated differences for numbers of adult honeybees as well as equalising pollen stores later during summer (Table 2) might point to extended brood compensation as a seasonally limited plastic response of hyperthermia colonies that possibly faded when increasing varroa mite levels (Fig. 3a,

b) became an additional stressor (Schott et al. 2021). Yet, respective dynamics could have also been masked by coinciding compensatory brood rearing in controls (Fig. 1b, c) due to late-season emergency re-queening following formic acid treatments, and/or relatively increased susceptibility even of later brood stages to hyperthermia exposure during autumn (Fig. 4). Importantly, regardless of possibly different natures of treatment-specific challenges before the winter treatment, these had no differential impact at the colony level the following spring (Fig. 1a–d).

Our findings regarding *V. destructor* dynamics and colony parameters resemble those of the merely descriptive field study by Porporato et al. (2022) who used a similar hyperthermia device at slightly larger treatment intervals synchronised across brood frames. Their study complements varroa mite dynamics with mechanistic effects of hyperthermia on its reproduction and survival. However, besides honeybee brood dynamics, no data on brood mortality nor comparisons to numbers of adult honeybees are provided, combined with a general lack of statistical analyses (Porporato et al. 2022).

With respect to brood mortalities in our study, the control exhibited expected patterns, including generally decreasing rates for older brood stages and generally increasing rates later in the season (Pistorius et al. 2011; Lückmann and Schmitzer 2019). Development of pupae was not negatively affected by hyperthermia during summer (Fig. 4d). This is in line with previous studies, which subjected pupae to comparable or even higher temperatures, and found notable effects only upon extended exposure for several hours (Le Conte et al. 1990; Appel and Büchler 1991). However, we detected undesired side-effects on the target brood stage in autumn (Fig. 4d). Causal mechanisms could include *i*) physiological changes in pupae becoming summer vs. winter bees (Kunert and Crailsheim 1988; Knoll et al. 2020), possibly rendering the latter more susceptible to high temperatures (Zhao et al. 2021) particularly shortly after pupal moulting (Rosenkranz 1987), and overall similarly vulnerable like drone brood (Kablaui et al. 2020a); *ii*) more variably composed comb contents later in the season, including more frequent simultaneous occurrence of distinct brood stages on individual frames, as well as enlarged food crowns after the removal of honey chambers and subsequent feeding, possibly altogether affecting comb-wide temperature profiling; or *iii*) altered countermeasures of hive bees while perceiving changing temperature regimes on the brood. The latter may further be influenced by increasing worker-to-brood ratios later in the season (Fig. 1a–c). Nevertheless, generally higher varroa mite levels during September plus substantially higher loads in hyperthermia colonies compared to controls (Fig. 3a, b) might simply have biased late-season BTR via featuring increased brood damage by *V. destructor* (Fig. 4).

Irrespective of the uncertainty whether seasonal variation of BTR of heat-treated pupae is directly related to hyperthermia or merely reflects additive effects of increasing varroa mite levels towards autumn, the more striking issue of hyperthermia was the generally severe impact on non-target brood stages (Fig. 4a–c). While devastating brood damage is not uncommon with formic acid (Noël et al. 2020; Vilarem et al. 2021), this was clearer for the here investigated hyperthermia device than the available yet largely anecdotal literature suggested. Essentially, however, no study investigating hyperthermia-based *V. destructor* treatments to date has systematically addressed mortality of different honeybee brood stages, which hampers overall comparison. Some studies provide elusive, barely data-supported hints of low to largely absent effects on worker brood, and only few (Rosenkranz 1987; Goras et al. 2015) specifically reported larval mortality of up to 40–50%, similar to those observed here, upon comparable heat-treatments, i.e. > 40 °C for more than 2 h. In our setting, the intention to render hyperthermia a time-saving routine by controlling individual combs autonomously and independently of their contents inevitably led to probability-based mis-matches with non-targeted brood stages. Thus, here documented losses of brood stages not explicitly target to hyperthermia, represent a constant trade-off of this approach. Nevertheless, substantial BTR variation for non-target brood stages (Fig. 4a–c), either showing excessive mortalities or coinciding with low to moderate losses of controls, was puzzling and certainly deserves further research.

In this regard, promising technical avenues to mitigate side-effects on honeybee brood while maintaining high potency against varroa mites could include parameter adjustments, such as a slightly lower treatment temperature (e.g. 41 °C rather than 42.5 °C) and/or a slightly reduced exposure time (e.g. 70–100 min rather than 130 min). According to current knowledge (Le Conte et al. 1990; Kablau et al. 2020a; Porporato et al. 2022), such refinements would shift focus away from killing both immature and mother varroa mites towards retaining high efficacy only against the former, ideally accompanied by more tolerable impact on non-target honeybee brood stages. Moreover, sophisticated algorithms capable of sensing differentiated temperature-feedback from developing brood could improve accuracy of hyperthermia treatments beyond stochasticity to actually relevant events. For instance, within core brood areas relative amplitudes around more stable mean temperatures (Becher and Moritz 2009) could permit predicting the presence of pupae within comb centres. Hence, temperature profiling may not solely serve for controlling hyperthermia treatments themselves, but also for diagnosing the effectively targeted brood stage. Enhancing likelihoods for pinpointed heating of comb partly or predominantly comprising capped brood would jointly increase efficacy and minimise side-effects.

Conclusions

This paper presents a uniquely comprehensive honeybee field study on hyperthermia-based treatment against *Varroa destructor*, the main culprit of colony losses, compared to control colonies maintained according to good apicultural practice. Our one-year monitoring of varroa mite loads suggests that the here investigated innovative heat-treatment applied during the season, complemented by a single winter treatment with oxalic acid, can control varroa mites below critical thresholds year-round equally successful as officially recommended formic acid summer treatments. At shared apiaries differential *V. destructor* population dynamics might have fostered temporarily reversed cross-group parasite reinvasions, possibly eroding maximum efficacy of either treatment, and ultimately resulting in generally concerning infestation rates by the winter treatment. Nevertheless, overall colony survival (80%) was acceptable, with no difference between treatments. These tentative inferences yet require further validation from long-term field monitoring, including different locations and hive formats. The novel hyperthermia approach may not only reduce risks for introducing chemical contaminants to the hive matrix, but also for colony collapses during honey production when other measures are not feasible. In contrast to earlier hyperthermia devices the here evaluated high-tech solution building on heat-treatments from within combs circumvents a number of practical disadvantages, notably disproportionate workload and material supply. We show that hyperthermia has side-effects, akin to conventional varroa mite management procedures, although hyperthermia-mediated trade-offs differ in nature, temporal occurrence, and their potential to lead to colony failure. Formic acid applications in the control partly coinciding with a hot spell provoked substantial queen supercedure, occasionally translating into losses where late-season replacement queens were insufficiently mated. Conversely, hyperthermia revealed increased mortality particularly of non-target brood stages that triggered altered resource allocation towards compensatory brood rearing, and accordingly comparatively decreased total honey harvests, as well as occasional varroa mite-mediated winter losses. Detrimental responses were either lethal or transient, next to comparable losses, no colony-level differences between experimental groups were observed the following spring. Finally, our thorough survey provides valuable insights for refining this automated system. Further improvements of this promising hyperthermia tool may permit exploiting the long-known potential of heat-treatments against *V. destructor* in widespread routine applications, and contribute to mitigation of global declines in ecosystem services and economic value provided by honeybees.

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Author contributions CS and PB conceived and designed research. CS, JW, WB and PB conducted field work and colony maintenance: colony conditions and initial allocation [CS]; varroa mite monitoring with powdered sugar [CS, JW, PB] and bottom boards [JW, WB, PB]; comb selection for digital brood assessment [CS] and photographing [JW]; comb handling throughout, hive/comb adjustment, swarm prevention, honey harvest, feeding and organic acid treatments [WB, CS]; recording of field data [JW]. WB and PB provided technological equipment and surveillance. JW processed data. CS analysed data and prepared figures. CS wrote the manuscript. All authors read and approved the manuscript.

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Data availability All data generated and analysed during the current study are included in this article. Specific raw data may be available from the corresponding author on reasonable request.

Declarations

Conflict of interest CS and JW have no competing interests to declare that are relevant to the content of this article, including no affiliation or involvement in organisations with any financial or non-financial interest in the matter of subject. Mention of trade names or commercial products in this publication is solely for providing specific information and does not imply recommendation or endorsement by the institution of CS and JW. WB and PB are shareholders and employees of Vatorex AG, the developer of the investigated hyperthermia device. Apart from the involvement of PB and WB in outlining this work and supporting its practical conduct, they declare not to have influenced in any way the collection, analysis and interpretation of the data for independent scientific publication.

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent to publish Not applicable.

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