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Paper

Effectiveness of a Varroosis treatment in managed apiaries: a pilot study

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Abstract

Varroa destructor is the main parasitic threat to *Apis mellifera* colonies worldwide and represents a major concern for honey bee health and apicultural sustainability. Oxalic acid (OA) is widely used for the control of varroosis; however, its efficacy is strongly influenced by the presence of capped brood. This pilot field study aimed to evaluate the efficacy and safety of a combined treatment protocol based on Api-Bioxal® administered by trickling and sublimation. The study was carried out in a permanent apiary located in the province of Bergamo (Northern Italy) from October 2024 to February 2025 and involved ten *A. mellifera* colonies housed in Dadant-Blatt hives. The experimental protocol consisted of one OA trickling treatment followed by two OA sublimations and a control treatment with amitraz. Treatment-induced mite fall was recorded daily for 117 days; brood presence, ambient temperature and pollen influx were monitored throughout the study period, and colony strength was assessed at the beginning and end of the trial using the Liebefeld method. Differences between pre- and post-treatment measurements were analysed using the Wilcoxon signed-rank test. An overall mean of 563.6 Varroa mites per colony was recorded as treatment-induced mite fall. The highest efficacy of OA was observed when treatments were applied in broodless conditions, whereas the presence of capped brood markedly reduced acaricidal efficacy. No abnormal honey bee mortality or adverse effects on colony health were observed. Colony strength increased in 8 out of 10 colonies, with statistically significant differences between pre- and post-treatment assessments ($p < 0.012$). These results indicate that *Varroa destructor* populations remain susceptible to oxalic acid when treatments are properly timed in relation to brood presence. A combined protocol of OA trickling and sublimation may enhance varroosis control without negative effects on colony health. Further large-scale studies are required to confirm these preliminary findings and to support evidence-based adjustments to national Varroa control guidelines.

Keywords

Apis mellifera, Varroa destructor, oxalic acid, brood interruption, treatment, honey bee health

Introduction

Varroa destructor is considered the main cause of honey bee colony mortality around the world, with losses mostly reported during late autumn and early winter (Carreck et al., 2010, Guzmán-Novoa et al., 2010; Le Conte et al., 2010; van der Zee et al., 2015; Frizzera et al., 2023; Plamondon et al., 2024). Originally restricted to parasitizing the Eastern honey bee, *Apis cerana*, this obligate ectoparasite mite shifted to a novel host *Apis mellifera* becoming the greatest threat for apiculture since its spread across the globe (Rosenkranz et al., 2010; Adjlane et al., 2016; Warner et al., 2024). *Varroa destructor* reproduces in bee capped brood cells and harms *Apis mellifera* in two ways: by feeding on the fat body of larvae and adult honey bees, depriving them of essential nutrients (Rosenkranz et al., 2010; Beyer et al., 2018), and by transmitting several pathogens such as *Varroa destructor* virus-1 and the related deformed wing virus (VDV-1/DWV), acute bee paralysis virus (ABPV), Israeli acute bee paralysis virus (IAPV), Kashmir bee virus (KBV), and black queen cell virus (BQCV). Indeed, the mite acts as a mechanical and biological vector, acquiring viruses during feeding on infected hosts, and facilitating their dissemination within and between colonies (Highfield et al., 2009; McMenamin and Genersch, 2015; Ryabov et al., 2017; Damayo et al., 2023; Prouty et al., 2023; Plamondon

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et al., 2024). The parasitism results in an incomplete development of adult bees which show reduced weight, malformations, low vitality and less survival rate. Moreover, these conditions weaken their immune system making colonies more susceptible to viral infections (Plamondon et al., 2024). Hence, incisive and effective acaricide treatments are needed to mitigate the devastating impact of *Varroa* on bee colonies (Rosenkranz et al., 2010; Plamondon et al., 2024). Oxalic acid (OA) is a natural acaricide commonly used to control mite infestation showing an optimal efficacy in the absence of brood (>90%) and low risk of hive contamination, low toxicity on bees and low mite resistance (Adjlane et al., 2016; Maggi et al., 2016; Berry et al., 2023; Prouty et al., 2023). It can be applied by trickling and spraying, typically in sugar-based or glycerol-containing solutions to increase its efficacy, or by sublimation. Usually, the first two methods are preferred during winter, in the absence of brood and when bees are clustered while sublimation is increasingly used also in warm seasons (Charrière and Imdorf, 2002; Rademacher and Harz, 2006; Berry et al., 2023; Prouty et al., 2023). The effectiveness of oxalic acid is much reduced during the reproductive phase of *Varroa*, due to the OA inability to cross the operculum of capped cells (Rademacher and Harz, 2006; Adjlane et al., 2016; Berry et al., 2023; Plamondon et al., 2024). Hence, a correct temporal strategy is essential to treat hives during the phoretic phase of *Varroa* (Prouty et al., 2023). Combining summer and fall treatments, before the appearance of winter brood and in the presence of *Varroa* mites in phoretic phase, allows the achievement of better results with lower infestation rates and increased survival of colonies during winter, compared to the sole summer treatment (van der Zee et al., 2015; Beyer et al., 2018). Moreover, a comprehensive strategy that combines induced brood interruption and OA treatments may boost the overall efficacy of controlling *Varroa* infestation (Gregorc et al., 2017; Berry et al., 2023).

In Italy, ministerial guidelines recommend two oxalic acid treatments: one following the mail honey harvest and one during winter under broodless conditions (Istituto Zooprofilattico Sperimentale delle Venezie, 2025). Api-Bioxal® (632.7 mg/g OA anidro, Alveis, Chemicals Laif S.p.A.) is the most common OA-based veterinary medicinal product used in Italy. According to the manufacturer's instructions, Api-Bioxal® should be administrated once during winter by trickling, with a maximum of two treatments per year. However, a field study conducted in 2023 in Northern Italy by our research group revealed that those who had not strictly followed the OA manufacturer's instructions did not report increased colonies losses (*unpublished data*). Based on these observations, a pilot field study was conducted in 2024 to evaluate the impact of repeated Api-Bioxal® administrations, combining trickling and sublimation, on *Varroa* mite drop and colony strength under real apiary conditions. This study aims to provide preliminary evidence to support the refinement of therapeutic strategies against *Varroa destructor* and contribute to the development of evidence-based monitoring protocols for assessing Api-Bioxal® efficacy in honey bee colonies.

Materials and methods

Experimental design

The study was conducted in a permanent apiary located in the province of Bergamo, at approximately 350 meters above sea level, from 24 October 2024 (T1) to 17 February 2025 (T117), for a total duration of 117 days. All hives Involved had previously been treated with Api-Bioxal® during summer, in accordance with Italian ministerial guidelines. Colonies were housed in 10-frame Dadant–Blatt hives and headed by queens of unknown origin.

In July 2024, six colonies (identified as 1a, 2a, 3a, 4a, 5a, and 6a) were induced to swarm, and their broods were used to establish four orphaned nuclei (identified as 1b, 2b, 3b, and 4b). The six broodless colonies were treated with Api-Bioxal® by the trickling method, following the Italian Ministry of Health guidelines. Twenty-four days after nucleus formation, to ensure complete brood emergence prior to treatment, the orphaned nuclei were also treated using the same protocol. In the meantime, the four nuclei reared new queens, which subsequently initiated ovoposition. In all hives, the wooden hive cover was replaced with a polyethylene cover to enable visual monitoring of cluster size and bee movement on the combs, even during very cold days. At the beginning (T1) and at the end (T117) of the trial, colony strength was assessed using the Liebefeld method adapted for the Dadant-Blatt hive (Imdorf et al., 1987; Dainat et al., 2020). According to this method, each comb face was visually divided into six nearly equal sections ("sixths"). Therefore, the area covered by bees, brood, honey, or pollen is expressed as the number of "sixths" of the comb face. As these elements generally occupy irregular areas, their surfaces are visually re-estimated as fitting within these sections and quantified accordingly, rounding to the nearest half-sixth when appropriate.

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Acaricide treatment protocol

All ten hives were subjected to the same acaricide treatment protocol (Prouty et al., 2023) as follows: trickling of 5 ml of Api-Bioxal® between each pair of combs at T1, following the manufacturer's instructions; sublimation of 2.2 g of Api-Bioxal® powder using the BioLetal *Varroa* sublimator and following the manufacturer's instructions (3 November 2024, T11; Fig. 1a); a second sublimation of Api-Bioxal® powder (28 November 2024, T36); insertion of two Apitraz® strips (Calier Italia) per hive (4 January 2025, T73; Fig. 1b), which were left in place for 45 days as control.

Metal trays previously placed under the hives were replaced with honeycomb-structured polycarbonate panels, which provided improved insulation (Fig. 1c). Throughout the 117-day trial period, a daily count of treatment-induced *Varroa* fall was performed, and the presence of residual fresh pollen introduced by the bees was assessed. After each inspection, the panels were cleaned using a metal spatula. Weekly, flight boards were removed to inspect the screened bottom in order to determine whether OA administration had caused a significant increase in bee mortality (Jack et al., 2020). In addition, a daily check of the flight boards and concrete flooring placed in front of the hives was performed to monitor the presence of any dead bees carried outside by scavenger bees. Inspection of all colonies was conducted eight times during the trial period to verify the presence of capped brood and to assess the eventual reduction in the efficacy of Api-Bioxal®. Two inspections were carried out in the absence of bee flight; therefore, the hives were transported to a partially heated room adjacent to the apiary, allowing the combs to be gently moved — with the bees forming tight clusters — in search of capped brood. Moreover, fresh pollen importation and ambient temperature (minimum and maximum) were monitored daily.

Figure 1. Pictures of instruments and hives treated during the trial. 1a) BioLetal Varroa used for the sublimation of Api-Bioxal® at T11 and T36. 1b) Apitraz® strips inserted at T73. 1c) Honeycomb-structured polycarbonate panels placed under the hives.

Statistical analyses

Differences between pre- and post-treatment measurements were assessed using the Wilcoxon signed-rank test for paired samples, and the corresponding p value was calculated. Differences were considered statistically significant when p value was ≤0.05. All analyses were performed with R software version 4.3.1 (R Core Team, 2023; Siegel and Castellan, 1988).

Results

Count of treatment-induced *Varroa* drop

Daily counts showed an average treatment-induced *Varroa* drop of 563.6 mites (min. 238, max. 859) across all hives at the end of the trial. Most of the mites were detected within 10 days after the first trickling Api-Bioxal® application. However, satisfactory results were obtained after the first sublimation treatment as well, with an average *Varroa* drop of 60.2 mites (min. 8, max. 206). Two peaks of *Varroa* fall were recorded within 5 days following the second sublimated treatment (means: 65 mites at T38 and 76 mites at T42) (Figure 2). For each hive, the count of the total number of fallen *Varroa* was grouped into four time intervals, covering the period between two consecutive treatments. The first time interval spanned the 10-day period between the trickling OA treatment and the first OA sublimation (T1-T11). The second period covered 25 days between the two OA sublimations (T11-T36). The following 37-day time interval extended from the second OA sublimation to the application of Apitraz® strips (T36-T73). The final count was performed at the end of the trial, 45 days after the last treatment (T117; Table I). During the whole trial period, no ants were observed that could have removed fallen mites from the panels.

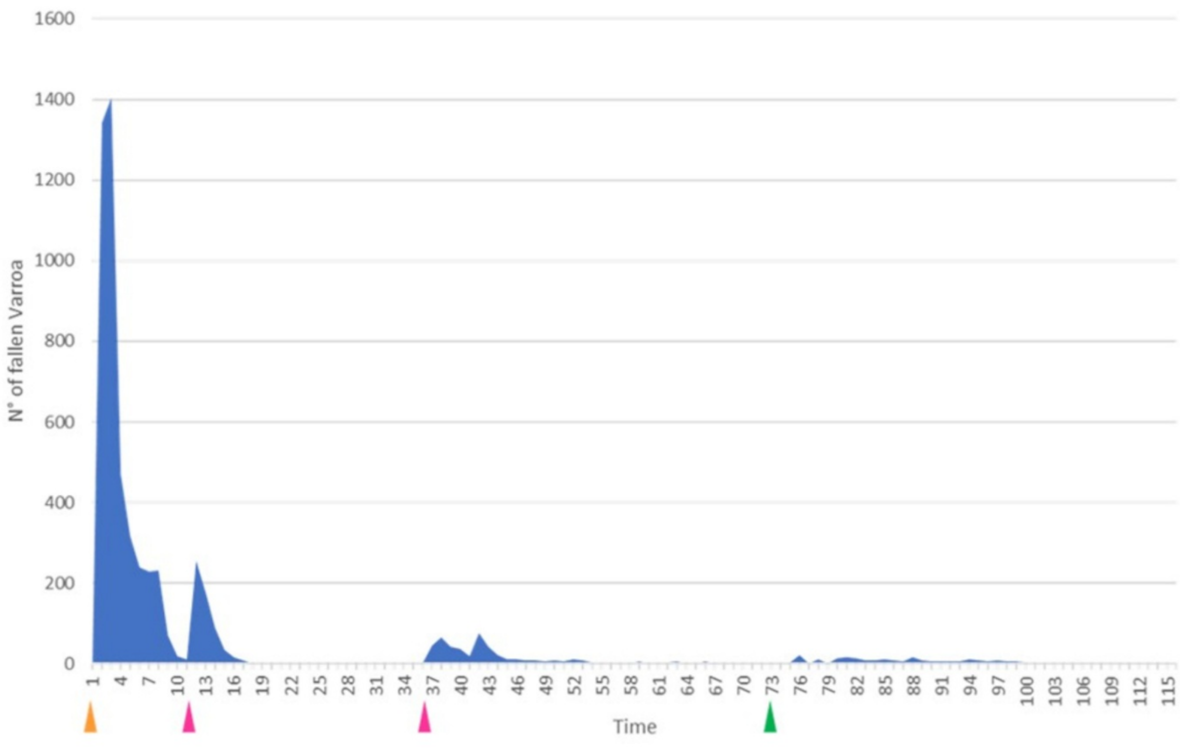

Figure 2. Schematic representation of treatment-induced *Varroa destructor* fall spanning the entire study period. Orange arrow: T1(24/10/2024), trickling of Api-Bioxal®; pink arrows: T11 (03/11/2025) and T36 (28/11/2024), administrations of sublimated Api-Bioxal® green arrow: T73 (04/01/2025), application of Apitraz® strips.

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Bee hive ID	T1 - T11 trickling Api-Bioxal®	T11 - T36 sublimated Api-Bioxal®	T36 - T73 sublimated Api-Bioxal®	T73 - T117 Apitraz® strips	Total *Varroa* units
1a	408	16	23	16	463
2a	804	8	3	2	817
3a	622	131	18	5	776
4a	452	42	26	66	586
5a	211	13	12	2	238
6a	217	18	18	0	253
1b	171	112	245	111	639
2b	530	206	97	26	859
3b	368	41	14	11	434
4b	540	15	14	2	571
Mean	432	60	47	24	563

Table I. Number of treatment-induced *Varroa* units drop within each time interval (T1-T11; T11-T36; T36-T73; T73-T117).

Colonies survival

At the end of the trial (T117), the number of bees increased in almost all families. No evident adverse effects on colony health were observed following three Api-Bioxal® applications over a one-month period. Indeed, no bee mortality rate exceeding normal levels was recorded. In fact, according to the Liebefeld method adapted to the Dadant-Blatt hive, the strength of honey bee colonies increased in 8 out of 10 families. A P value < 0.012 was obtained, confirming the statistically significance of results (Figure 3 and Table II).

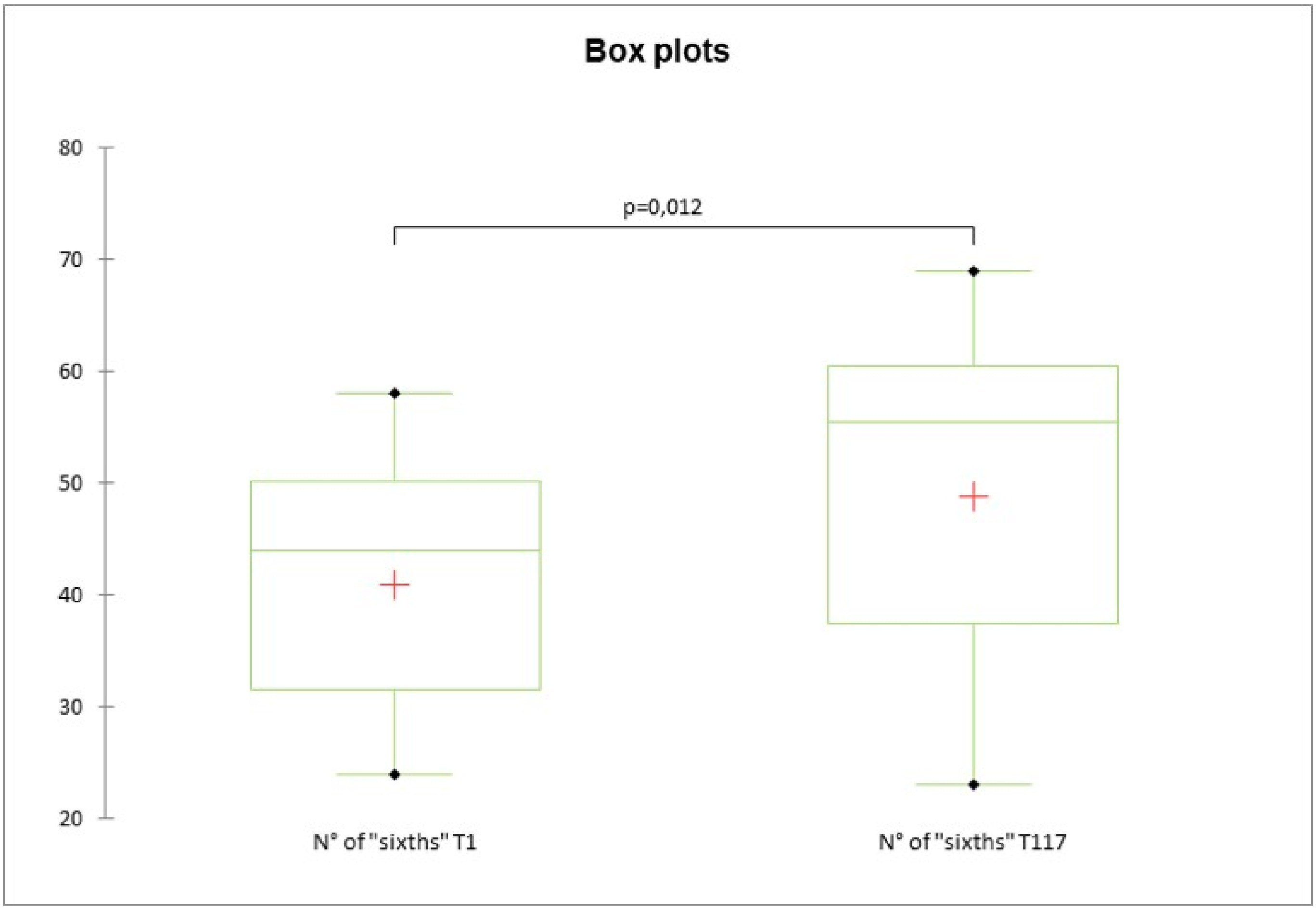

Figure 3. Statistical analyses and the p value confirm the increase of the strength of honey bee colonies at the end of the trial compared to T1.

Brood presence and pollen importation

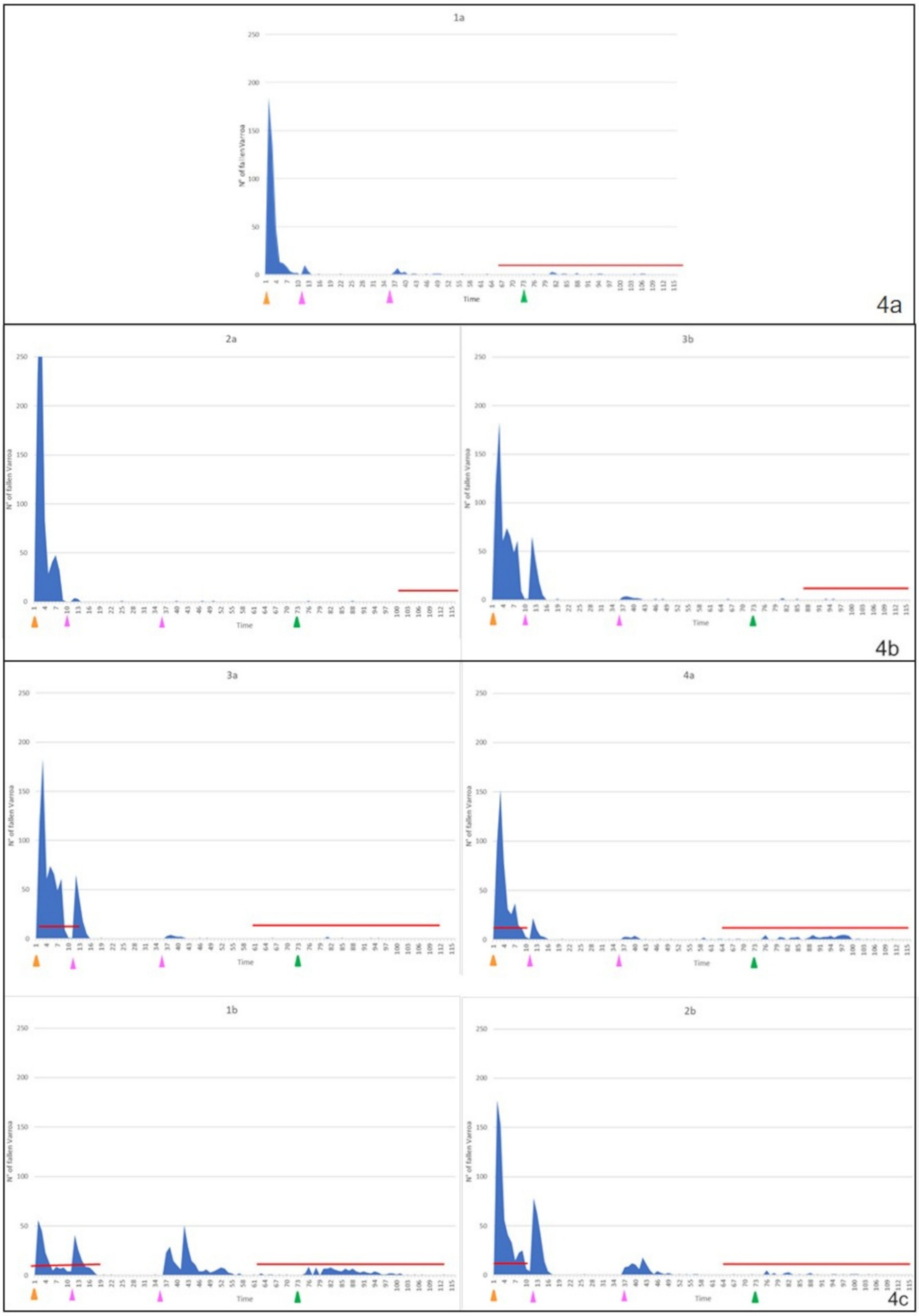

Figure 4. Schematic representation of treatment-induced *Varroa destructor* fall spanning the entire study period. 4a) *Varroa* drop recorded in hives 1a as well as in 5a, 6a, and 4b. 4b) *Varroa* drop recorded in hives 2a and 3b, characterised by the lowest presence of brood. 4c) *Varroa* drop recorded in hives 3a, 4a, 1b, and 2b, characterised by the highest presence of brood, even in late October-early November (T1-T11). Red line indicates the presence of brood.

At the beginning of the trial (T1), four hives (ID: 3a, 4a, 1b and 2b) showed the presence of brood cells, which still

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remained in hive 1b until T20. This resulted in a reduced efficacy of the trickling Api-Bioxal® application compared to hives without brood. Therefore, the first Api-Bioxal® sublimation elicited a greater mite drop in hives 3a, 4a, 1b and 2b compared to the others (Figure 4). The second sublimation treatment was performed in all hives in broodless conditions achieving a better overall efficacy, considering the total amount of fallen *Varroa* per hive. In late December (T65), sealed immature stages reappeared in eight hives (ID: 1a, 3a, 4a, 5a, 6a, 1b, 2b, 4b) until the end of the trial, therefore, the Apitraz® strips were administered in the presence of capped brood. Conversely the opposite, hives 2a and 3b exhibited sealed cells again during the amitraz treatment, at T101 and T87, respectively. Notably, hive 2a had the lowest overall presence of brood during the study period. In contrast, hive 1b had the longest duration of capped cells presence and consistently showed reduced treatment efficacy compared to other hives (Figure 4). From T20 to T57, in conformity with brood absence (T20-T65), no importation of fresh pollen was recorded.

Recorded temperature

Average maximum temperatures remained close to 10 °C, and minimum temperatures fell below 0 °C on only six occasions (Figure 5).

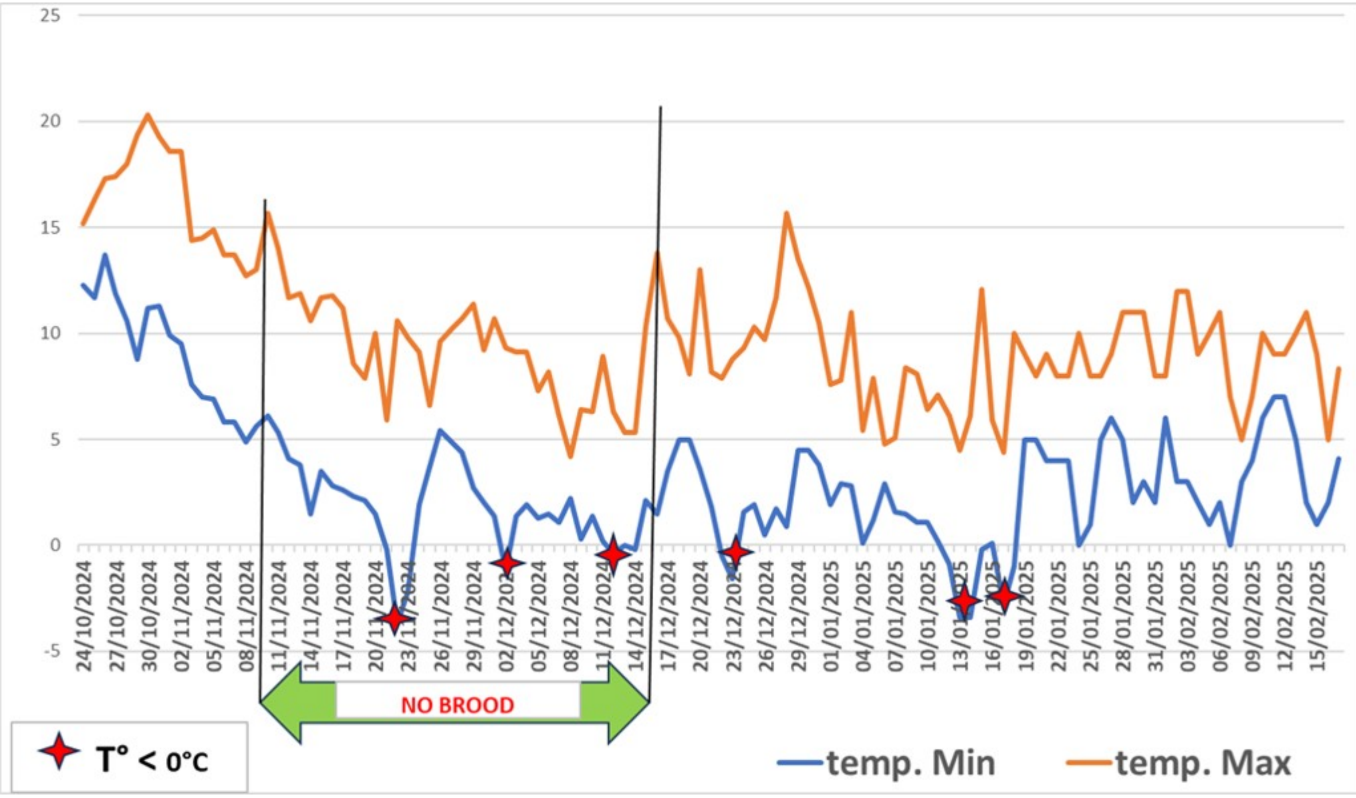

Figure 5. Minimum and maximum temperature recorded during the period of the study.

Discussion

In this pilot study, repeated administrations of Api-Bioxal® through trickling and sublimation were investigated for their impact on *Varroa* mite drop and honey bee colony strength under field conditions. According to the Italian Ministry of Health guidelines, all colonies had been treated in July, in broodless conditions, and subsequently in October using trickled Api-Bioxal®. Furthermore, two additional experimental sublimations of the same OA formulation and one application of Apitraz® strips were performed to verify the potential increase in the efficacy of the Api-Bioxal®. The summer treatment is assumed to have achieved an estimated efficacy of approximately 90%, due to its application during the induced brood interruption (Adjlane et al., 2016; Maggi et al., 2016; Berry et al., 2023; Prouty et al., 2023). Oxalic acid showed the highest efficacy during the late fall treatment; however, the two subsequent sublimations of OA also proved to be important for a further significant drop of *Varroa* mites (Figure 2 and Table I). The presence of brood limits the acaricidal efficacy of oxalic acid, representing a perfect refuge for mites (Berry et al., 2023); indeed, *Varroa* drop was significantly reduced after the reappearance of new capped brood. The first trickled administration of Api-Bioxal® showed high acaricide efficacy in hives 1a, 2a, 5a, 6a, 3b, and 4b, where brood was absent. As a

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consequence, the following sublimations and the control treatment with Apitraz® strips recorded low mite drops (Figure 2 and Table I). In contrast, hives 3a, 4a, 1b, and 2b showed a reduced efficacy of the drug due to the presence of brood. The following sublimation of Api-Bioxal® showed less efficacy in hive 1b due to the residual presence of brood cells (Figure 4c).

After each drug administration, the number of residual *Varroa* mites and, consequently, the potential risk of each family being highly parasitized before the summer treatment was calculated, considering the hypothetical number of *Varroa* that could be found in summer if 50, 100 or 200 mites still remained after the winter treatment (Istituto Zooprofilattico delle Venezie, 2025). Therefore, residual *Varroa* levels should be kept below 25 mites per hive to allow colonies to enter the summer season with minimal damage and a lower risk of viral transmission (Plamondon et al., 2024). Recent climate change, particularly the increase in average temperatures and the shift in seasonal trend, may lead to an earlier and prolonged egg-laying period by queen honey bees. This extended brood-rearing window can facilitate the reproductive cycle of *Varroa*, which relies on the presence of brood to reproduce. As a result, climate change may indirectly contribute to higher *Varroa* infestation rates and increase pressure on colony health throughout the year. During the study period, brood was still present in 4 out of 10 hives in late autumn and already present in 8 out of 10 hives by mid-December, approximately one month earlier than in previous years. A prolonged similar situation may lead to a no-stop egg-laying even in winter and, subsequently, to an incomplete efficacy of the treatment due to the lasting presence of broods. As a consequence, the legally mandated treatments alone may not be sufficient, and many beekeepers resort to additional interventions beyond those required by regulation; in addition, beekeepers can no longer carry out treatments at the same time every year as they used to, but they must first verify the absence of brood. Moreover, sublimated oxalic acid may enhance the overall efficacy of the treatment but cannot replace the trickling method. Unfortunately, repeated treatments applied in broodless conditions and at short intervals may elicit damaging effects on bees (Jack et al., 2020). On the contrary, this study suggests that the combination of trickled and sublimated Api-Bioxal® in a proper time interval may result in a significant reduction of mite units without any negative effect on colonies. Indeed, according to the Liebefeld method adapted for the Dadant-Blatt hive and to the statistical analyses, the strength of honey bee colonies generally increased (Figure 3 and table II).

Summary (p- values):		
Variable\Subsamples	Wilcoxon signed-rank test	p.value
N° of "sixths" T1 - N° of "sixths" T117	0,109	**0,012**

Table II. Statistical analyses and the p value confirm the increasing of the strength of honey bee colonies at the end of the trial compared to T1.

The possibility that early egg-laying was facilitated by Api-Bioxal® treatments was also considered. However, this hypothesis was rejected as sublimated treatments only slightly raise the cluster temperature and do not induce egg-laying by the queen (Besomi, 2022). The early onset of egg-laying was more likely influenced by the relatively mild climatic conditions during the study period; indeed, minimum temperatures fell below 0 °C on only six occasions, while average maximum temperatures remained close to 10 °C (Figure 5). Such conditions likely promoted early floral blooming and facilitated pollen foraging, which may have stimulated earlier egg-laying. Families 2a and 3b showed a trend inversion due to the delay in egg-laying, resulting in a reduction of colony strength. Importantly, both families showed the lowest strength at the beginning of the trial and a delayed egg-laying pattern compared to the other families (Table III). Considering how climate change can modify the natural behaviour of bee colonies, effective scientific communication regarding the One Health concept, as well as interdisciplinary collaboration, becomes essential (Bellucci et al., 2019; Berke, 2024).

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Beehives	N° of " sixths " T1	N° of " sixths " T117	Variation of N ° of "sixths"
1a	51	65	14
2a	25	24	**-1**
3a	52	61	9
4a	45	59	14
5a	31	37	6
6a	43	54	11
1b	48	57	9
2b	33	39	6
3b	24	23	**-1**
4b	58	69	11

Table III. Number of “sixths” at the beginning and at the end of the trial for each hive.

Conclusion

This pilot study indicates that *Varroa* remains sensitive to Api-Bioxal®, as long as it is administrated in broodless conditions. To maximise treatement efficacy, it could be useful to uncap the residual brood before treatment and to induce a brood interruption during fall. These strategies could also prove relevant for the management of emerging mite species, such as *Tropilelaps* spp. which may pose future threats to European and Italianhoney bee colonies (World Animal Health Information System).

The results suggest that a single winter trickling treatment may not be sufficient to reduce the number of mites in order to enter the summer with a reduced *Varroa* infestation. However, at least two treatments with oxalic acid, spaced appropriately and applied during broodless periods, were associated with significant reductions in mite drop without detectable adverse effects on colony strength. These findings underscore the importance of timing and sequence in oxalic acid applications to maintain colony health and effective mite control.

However, the study is limited by the small numbers of hives and the absence of a fully controlled comparison group treated strictly according to the Italian Ministry of Health guidelines. Further, large-scale, multi-enviroment studies are needed to confirm these preliminary observations and to evaluate whether these results may support a revision of standard treatment protocol. Such investigation may suggest for the development of evidence-based strategies that ensure optimal *Varroa* control under variable environmental and management conditions.

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Conflict of interest

The authors declare no conflicts of interest.

Author Contributions

Conceptualization: G.L.; Methodology: GL, R.S, G.G., L.R, S.B.; Formal analysis: G.L.; Data curation: R.S., G.L; Writing original draft preparation: G.L.; Writing, review and editing: G.L., S.B.; Supervision: G.L., L.R., G.G., R.S., S.B.

All authors have read and agreed to the published version of the manuscript. Data from the present study are kept confidential but can be provided upon reasonable request to the authors.

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