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Liquid formic acid 60% to control varroa mites (Varroa destructor) in honey bee colonies (Apis mellifera): protocol evaluation

Marco Pietropaoli & Giovanni Formato

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ORIGINAL RESEARCH ARTICLE

Liquid formic acid 60% to control varroa mites (*Varroa destructor*) in honey bee colonies (*Apis mellifera*): protocol evaluation

Marco Pietropaoli and Giovanni Formato*

Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M.Aleandri", Unità Operativa Apicoltura, Rome, Italy

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We verified the acaricide efficacy on *Varroa destructor* and the toxicity on honey bees of summer treatments carried out in three different apiaries in Central Italy with an evaporator filled with 290 ml of formic acid 60%. Acaricide efficacy was evaluated counting the number of mites killed by the application of formic acid in the evaporator according to the indications provided by the producer, while toxicity on honey bees of the treatment was evaluated adopting the Liebefeld method. The mean acaricide efficacy varied from a minimum of 57.0 ± 21.8% to a maximum of 72.7 ± 12.5% and resulted always statistically higher with respect to the natural mite fall observed in the untreated groups. Concerning the toxicity for the honey bees, we observed only a statistically significant reduction of sealed brood with respect to the untreated hives in one of the three apiaries. No other statistically significant effects on the hive population or queen mortality could be observed. Environmental temperatures recorded in the three apiaries during the formic acid administration were within the ranges suggested by the evaporator producer. Considering the efficacy and toxicity obtained with the liquid formic acid treatment, the adopted protocol could represent a new sustainable organic tool to control varroa infestation in temperate areas.

Ácido fórmico líquido al 60% para el control de ácaros varroa (*Varroa destructor*) en colonias de abejas melíferas (*Apis mellifera*): protocolo de evaluación

Se ha verificado la eficacia del acaricida sobre *Varroa destructor* y la toxicidad sobre las abejas melíferas de los tratamientos de verano realizados en tres colmenares diferentes en Italia Central con un evaporador lleno de 290 ml de ácido fórmico al 60%. La eficacia del acaricida se evaluó contando el número de ácaros muertos por la aplicación de ácido fórmico en el evaporador según las indicaciones proporcionadas por el productor, mientras que la toxicidad sobre las abejas melíferas del tratamiento se evaluó mediante el método de Liebefeld. La eficacia media del acaricida varió de un mínimo de 57.0 ± 21.8% a un máximo de 72.7 ± 12.5% y siempre resultó estadísticamente más alta con respecto a la caída natural de ácaros observada en los grupos no tratados. En cuanto a la toxicidad para las abejas melíferas, sólo observamos una reducción estadísticamente significativa de la cría operculada con respecto a las colmenas no tratadas en uno de los tres colmenares. No se pudo observar ningún otro efecto estadísticamente significativo sobre la población de las colmenas o la mortalidad de reinas. Las temperaturas ambientales registradas en los tres colmenares durante la administración del ácido fórmico estuvieron dentro de los rangos sugeridos por el productor del evaporador. Teniendo en cuenta la eficacia y la toxicidad obtenidas con el tratamiento de ácido fórmico líquido, el protocolo adoptado podría representar una nueva herramienta orgánica sostenible para controlar la infestación de varroas en zonas templadas.

Keywords: varroa; formic acid evaporator; efficacy; toxicity; protocol; temperate areas

Introduction

Varroosis is one of the major honey bee diseases responsible for colony losses worldwide (Martin et al., 2012; Nazzi et al., 2012; vanEngelsdorp, Hayes, Underwood, Caron, & Pettis, 2011; Ziegelman, Rosenkranz & Ellis, 2013).

"Soft" acaricides (Rosenkranz, Aumeier, & Ziegelmann, 2010) like formic acid, oxalic acid, lactic acid and thymol are active compounds that present a low risk of residues in hive products. Among them, formic acid is the only one that is effective both against phoretic (on bees) and reproductive phases (in the capped brood) of the mite (Rosenkranz et al., 2010); it has been largely applied by beekeepers and studied since the '80s in European countries (Elzen & Westervelt, 2002; Ritter & Ruttner, 1980; Wachendörfer, Fijalkowski, Kaiser, Seinsche, & Siebentritt, 1985) being administered in beehives using different evaporators and formulations (Bracey & Fisher, 1989; Feldlaufer, Pettis, Kochansky, & Shimanuki, 1997; Fries, 1989; Hoppe, Ritter, & Steven, 1989; Lupo & Gerling, 1995). Efficacy against *Varroa destructor* (Anderson & Trueman, 2000) and the impact of formic acid treatment on honey bee colonies can vary depending on: the commercial products applied, the evaporator chosen, the environmental temperature and humidity, the hive size, the presence of brood, the position of the evaporator in the hive and the area covered by the evaporator (Calderone, 1999; Calis et al., 1998; Eguaras,

*Corresponding author. Email: giovanni.formato@izslt.it

Del Hoyo, Palacio, Ruffinengo, & Bedascarrasbure, 2001; Eischen, 1998; Rosenkranz et al., 2010).

Main disadvantages of formic acid treatments could be: mortality of the queen, depletion of the colony, swarming of the hive and reduction in productivity (Bolli, Bogdanov, Imdorf, & Fluri, 1993).

In this paper, we report the results of a study carried out to evaluate the varroacide efficacy and the honey bee toxicity of a summer protocol performed with Nassenheider Professional® evaporator in temperate areas.

Materials and methods

Nassenheider Professional® (Joachim Weiland Werkzeugbau GmbH & Co. KG Zimmermannsgasse 2, 15366 Hoppegarten, Germany) on the market since 2014, is a new formic acid evaporator for varroa control. It consists of a plastic bottle to be filled with a formic acid solution (at 60 or 85% concentration), a wick and a patented evaporation system that allows a slow release of the acid solution. The temperature range indicated on the label to obtain the best performances with this evaporator without damaging the bees goes from + 8 to + 35 °C.

In August 2014 (Apiary 1 and Apiary 2) and August 2015 (Apiary 3) we tested, in Rome province (Central Italy) (Figure 1), the performances of Nassenheider Professional® evaporator. The evaporator, placed on top of the frames of the brood chamber, is composed of: a tank with a capacity of 290 ml, an evaporation chamber, a wick cover, a tray, a vertical wick and a cloth. The vertical wick sucks the formic acid and transports it downwards into the fleece cloth where it drips off and evaporates.

We used 290 ml of formic acid 60% and the larger U-wick size, according to label instructions for Dadant-Blatt beehives.

Figure 1. Apiary locations for field trials 2014 (markers 1 and 2) and 2015 (marker 3).

Table 1. Protocols adopted in the field trials.

Nassenheider Professional® group

CE			CE OA A		OA	
Nassenheider Professional® Treatment			Queen Caging			
Count of mites killed by formic acid treatment			Count of residual mites			
Day 0	From Day 1 to Day 10	From Day 11 to Day 24	Day 25	From Day 26 to Day 49	Day 49	From Day 25 to Day 56

Control group

CE		CE OA A		OA	
		Queen caging			
Count of natural mite fall		Count of residual mites			
Day 0	From Day 11 to Day 24	Day 25	From Day 26 to Day 49	Day 49	From Day 25 to Day 56

Notes: Legend: CE: Colony strength estimation; OA: Oxalic acid treatment; A: Apistan double dose treatment.

In total, 48 honey bee colonies with a similar strength and housed in 10 frame Dadant-Blatt beehives were monitored. In each of the three apiaries, two homogeneous experimental groups of eight hives were placed. Varroa infestation levels between apiaries were similar. Colonies of one group were treated with formic acid (Nassenheider Professional group), the other group was left untreated (control group). When no more formic acid was present in the bottles (after 10 days from the beginning of the administration) the evaporators were removed.

In order to evaluate the toxicity of the treatment for the honey bees, at the beginning and at the end of the treatment the number of adult bees and the brood coverage observing frame sections (Delaplane et al., 2013) was estimated. In Table 1 it is possible to observe the detailed protocols adopted for each treatment group. The protocols were devised according to the Varroa Control Task Force of the COLOSS association (http://www.coloss.org/taskforces/varroacontrol/protocols-2014–2015). Abnormal behaviour of colonies (e.g., swarming) and mortality of queens were also recorded.

In order to calculate the acaricide efficacy and plot the mite fall dynamics, the amount of mites killed by the formic acid treatments was counted every 2–3 days until 25 days after the beginning of the treatment in order to estimate the mites killed inside the brood cells.

The residual amount of mites was evaluated with a follow-up treatment with:

- two doses of oxalic acid solution (Apibioxal®, Chemicals Laif, SpA, Vigonza, PD, Italy) trickled, one at the beginning of a queen caging period of 24 days and one at the end of the queen caging period (Table 1);
- a double dose (4 strips/hive) of Apistan® (tau-fluvalinate; Vita Europe Ltd, Basingstoke, Hants, United Kingdom) administered during all the queen caging period until 7 days after the queen release (Table 1). The double dosage was preferred considering the possibility of resistance mechanisms (Milani, 1995) reducing the risk on an overestimate of the efficacy of formic acid treatment.

The untreated colonies of the control group, used to verify the natural mite mortality, received the same follow-up treatments (Table 1).

To calculate the acaricide efficacy (AE) obtained in each hive, the following formula was used: AE = (VF/V(F + FOLLOW-UP)) *100 where VF is the number of mites killed with the formic acid treatment with Nassenheider Professional® and V(F + FOLLOW-UP) represents the sum of the above-mentioned mites with those killed by the follow-up treatments.

Mean, maximum and minimum environmental temperatures were collected from data available by the meteorological stations nearest to the apiaries (https://www.wunderground.com).

Statistical analysis was performed to verify if acaricide efficacy and variations in adult bee populations, sealed and unsealed brood of treated groups were significantly different, in each apiary separately, with respect to the control group. Mann-Whitney test (Mann & Whitney, 1947) was applied using XLSTAT™ software (Addinsoft, 2010).

Results

Natural mite falls observed in the control groups were, respectively, 25.5 ± 7.9% (*n* = 8) in apiary 1, 18.9 ± 3.0% (*n* = 7) in apiary 2 and 27.5 ± 12.1% (*n* = 8) in apiary 3. Nassenheider Professional® evaporator recorded a mean acaricide efficacy of 60.2 ± 16.5% (*n* = 6) in apiary 1, 57.0 ± 21.8% (*n* = 8) in apiary 2 and 72.7 ± 12.5% (*n* = 8) in apiary 3 (Figure 2 and Table 2). In Figure 2 are shown boxplots and mite fall dynamics observed in the three field trials. In Table 2 there is reported a descriptive analysis of the acaricide efficacies.

The efficacies obtained with the formic acid treatments resulted always statistically higher ($p < 0.0001$) with respect to the corresponding control groups (Table 3).

In Table 4 the variations with respect to the beginning of the treatments concerning the adult bee population and the brood distribution observed in the groups of the three apiaries are expressed. The amount of adult bees recorded after the formic acid treatment with Nassenheider Professional® evaporator was higher with respect to the control group in apiary 1 and 2 and lower in apiary 3 but those differences were not statistically significant (Table 4). The amount of brood, in the Nassenheider® group, was significantly lower with respect to the control group after the treatment (U = 2.000; Expected value = 20.000; Variance (U) = 46.667; $p = 0.010$) only in apiary 2.

No queen mortality or abnormal behaviours were observed after the formic acid application.

In Figure 3 the minimum, mean and maximum environmental temperatures recorded during the 10 days of formic acid evaporation that never exceeded the range suggested for Nassenheider Professional® application (from + 8 °C to + 35 °C) are summarized.

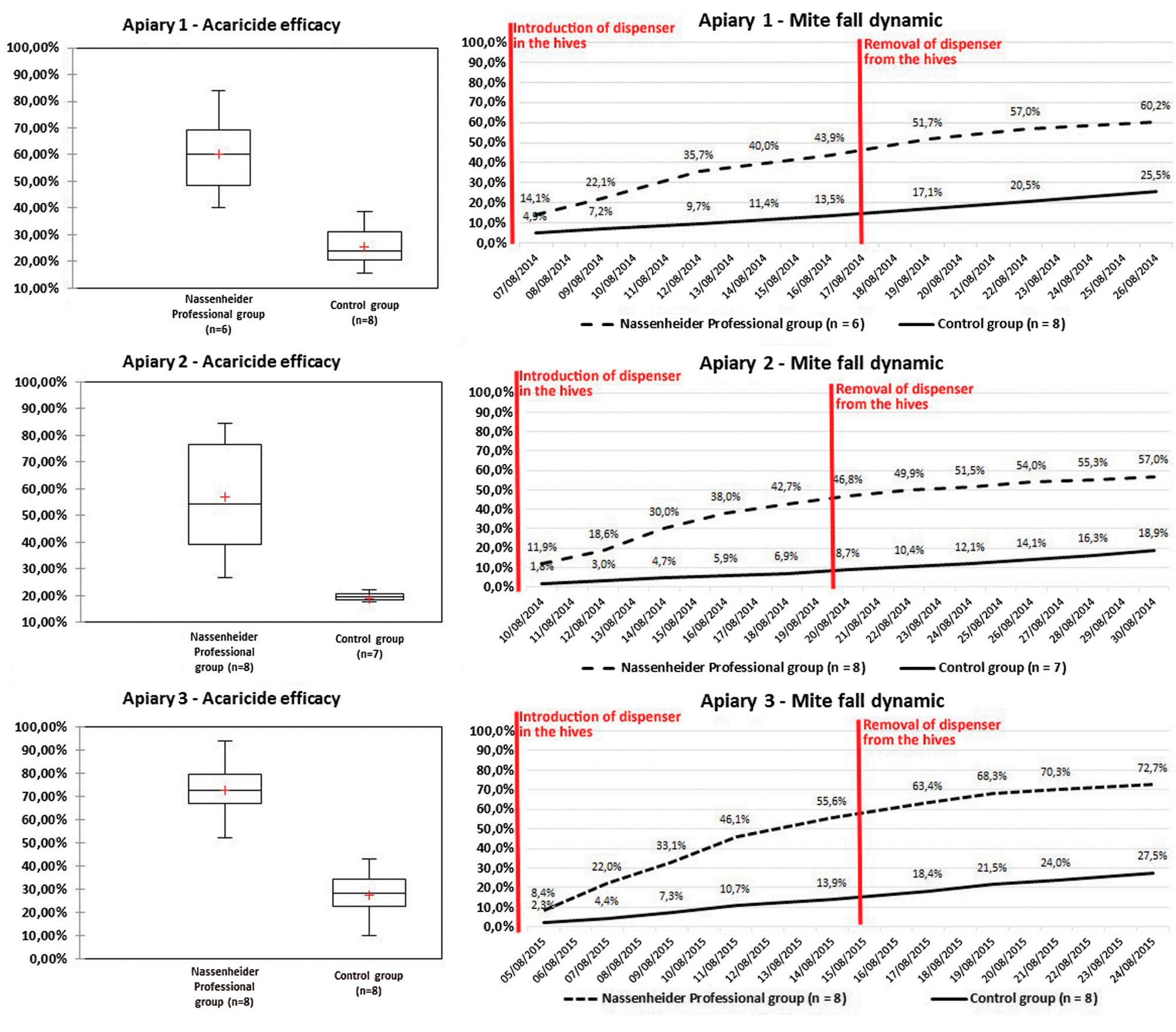

Figure 2. Acaricide efficacy (boxplots and mite fall dynamics) of 2014 and 2015 field trials.

Table 2. Descriptive analysis of acaricide efficacy obtained in the three apiaries.

Treatment group	Apiary No. 1 Nassenheider professional®	Apiary No. 1 Control	Apiary No. 2 Nassenheider professional®	Apiary No. 2 Control	Apiary No. 3 Nassenheider professional®	Apiary No. 3 Control
Minimum	40.3%	15.6%	26.9%	12.9%	51.9%	10.0%
Maximum	84.0%	38.5%	84.4%	22.1%	93.8%	43.2%
1st Quartile	48.3%	20.4%	39.1%	18.4%	66.8%	22.7%
Median	60.0%	23.7%	54.5%	19.4%	72.7%	28.4%
3rd Quartile	69.3%	30.9%	76.6%	20.5%	79.3%	34.4%
Mean	60.2%	25.5%	57.0%	18.9%	72.7%	27.5%
Variance (*n*−1)	2.7%	0.6%	4.8%	0.1%	1.6%	1.5%
Standard deviation (*n*−1)	16.5%	7.9%	21.8%	3.0%	12.5%	12.1%

Table 3. Statistical analysis results of comparison of acaricide efficacies between treated and control group in each apiary.

	Apiary No. 1	Apiary No. 2	Apiary No. 3
U	48.000	56.000	64.000
Expected value	24.000	28.000	32.000
Variance (U)	60.000	74.667	90.667
P	0.001	< 0.001	< 0.001

Discussion

There is a general lack of data concerning the performances of formic acid summer treatments for varroa control in temperate areas. This could probably be linked to the fact that, in this area and time of the year, high formic acid evaporation rates could cause mortality of queens and adult bees, or interruption of reared brood (Satta et al., 2005).

The mean acaricide efficacy observed in the three field trials we carried out in summer in Central Italy with a single treatment with Nassenheider Professional® and formic acid 60% ranged from a minimum of 57.0 ± 21.8% to a maximum of 72.7 ± 12.5%. These values were lower than those obtained by Marinelli (Marinelli et al., 2007), who used in the same area and period of treatment a higher concentration of formic acid. In fact, he found efficacies that ranged from 89.5% ± 11.3 to 97.2% ± 4.1 with four consecutive administrations of a total of 100–120 ml of formic acid 85% using different evaporators (Er Form®, petri plate and hive feeder). In another study Mutinelli too (Mutinelli, Cremasco, & Irsara, 1994), with the use of a soaked pad with 40 ml of 60% formic acid solution, recorded an acaricide efficacy similar to Marinelli (from 89.6 to 98.8%) even though he carried out four administrations/week and observed a much higher mortality. Indeed, the efficacy of the treatment with Nassenheider Professional® we experienced was similar to another study of Marinelli (Marinelli et al., 2008) where he applied a single treatment with 120 ml of formic acid 65% smeared on Mitegone® pads (efficacy of 70.9 ± 16.3%) and three weekly treatments with 120 ml of formic acid 65% placed in the hive feeder (efficacy of 55.98 ± 24.2%).

In our field trials, with a single low concentration treatment, as Mitegone®, we had no adverse effects on adult bees and queens. On the contrary, the other treatments carried out by Marinelli in 2007 recorded higher queen losses (Er Form® 67%; petri plate 28%; hive feeder 14%) than ours (0%), probably linked to the high formic acid concentration. In fact, the more irregular the evaporation rates of formic acid are, the higher is the toxicity of the treatment (Elzen, Westervelt, & Lucas, 2004). Indeed, the Nassenheider Professional®

Table 4. Variations in adult bee population, sealed brood and unsealed brood at the end of the treatment period in the two groups.

	Apiary No. 1 Nassenheider Professional® group	Apiary No. 1 Control group	Apiary No. 2 Nassenheider Professional® group	Apiary No. 2 Control group	Apiary No. 3 Nassenheider Professional® group	Apiary No. 3 Control group
Variation in number of adult bees (mean ± s.e.)	+ 6.9% ± 13.3%	−5.9% ± 18.6%	+63.5% ± 88.4%	+35.1% ± 17.0%	−23.3% ± 24.4%	−8.4% ± 18.4%
Variation in number of cells of sealed and unsealed brood (mean ± s.e.)	−16.7% ± 16.7%	−14.4% ± 3.7%	−50.8% ± 21.8%*	−18.0% ± 12.7%	+64.9% ± 75.0%	+35.2% ± 104.5%

*Significant variation after treatment respect control group (U = 2.000; Expected value = 20.000; Variance (U) = 46.667; *p* = 0.010).

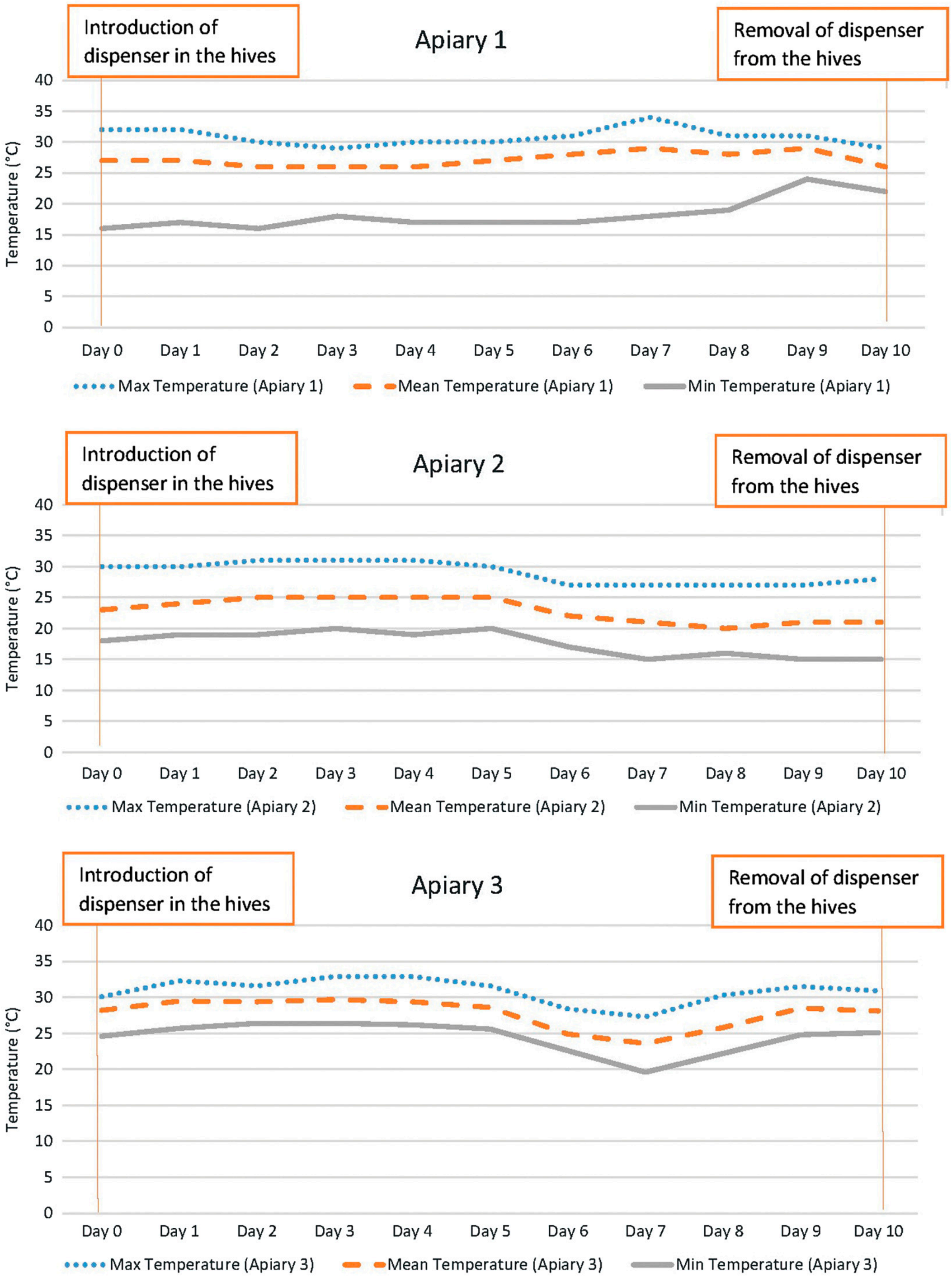

Figure 3. Environmental temperature recorded during the field trials.

evaporator showed regular evaporation rates as we could observe through the mite fall dynamics (Figure 2) that is characterised by a gradual and constant increase of the varroa mortality. The absence of peaks in mite mortality could be related also to the environmental temperatures observed in our field trials that were always quite homogenous in the three apiaries and never exceeded the ranges of temperature indicated for the treatment (Figure 3).

Considering the handling aspects related to the use of formic acid in the Nassenheider Professional® evaporator, a certain risk for the beekeeper's health could be linked to the management of the formic acid liquid. This risk could be reduced, for example, by adopting disposable containers already filled and ready for the treatment. Moreover, other references (Avila-Ramos, Otero-Colina, Sánchez-Arroyo, Santillán-Galicia, & Tecante, 2010; Eguaras et al., 2001, 2003; Feldlaufer et al., 1997;

Satta et al., 2005) identified possible solutions able to minimize the risks of handling this organic acid by mixing it with a gel matrix.

Finally, evaluating the logistic aspects, the Nassenheider Professional® evaporator requires for its application the use of empty supers to gain an additional volume to contain the evaporator in the hive. This requires beekeepers to have empty supers ready to be installed for the treatment or specific shims able to increase the internal volume of the hive.

The tested varroa control protocol with Nassenheider Professional® evaporator filled with 290 ml of formic acid 60% could be useful for beekeepers in temperate areas considering that a single treatment application is needed and it is well tolerated by both the adult bees and the honey bee queens. Even though its efficacy was not so high, considering the constant evaporation rate of the formic acid, further studies could be carried out in order to increase the efficacy prolonging the duration of the treatment (e.g., increasing the container volume of the dispenser) or associating it with other beekeeping techniques (like the brood removal/interruption) or active compounds (like oxalic acid considering its high efficacy on phoretic mites (Giacomelli et al., 2011).

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Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Marco Pietropaoli http://orcid.org/0000-0002-9073-909X
Giovanni Formato http://orcid.org/0000-0002-1202-5745

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