Available online at www.sciencedirect.com
ScienceDirect
Veterinary Parasitology 148 (2007) 174–178
veterinary parasitology
www.elsevier.com/locate/vetpar

Short communication

# Efficacy of repeated trickle applications of oxalic acid in syrup for varroosis control in *Apis mellifera*: Influence of meteorological conditions and presence of brood

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Received 3 January 2007; received in revised form 30 May 2007; accepted 1 June 2007

**Abstract**

Oxalic acid field trails for the control of varroosis (*Varroa destructor*) were carried out in an apiary located on the Mt. Imittos (Attica, Greece). The colonies received four successive applications (approximately one every 16 days) with 4.2% oxalic acid (OA) and 60% sugar solution by trickling method with two alternative types of syringes (an automatic self-filling dosing and a single-use) from the broodright to broodless period. The results indicate that the first three applications (from 6th October to 25th November—broodright period) resulted in 65.3% cumulative mite mortality, while only the last application (after the 26th November—broodless period) resulted in 77.3% mite mortality. Very low outern temperatures reduce to the minimum the bee movability, which may result into a slower development of the OA efficacy. No poor colony growth or queen loss were observed even if the bee colonies were received the four successive OA applications with the last one taken place at a very low outern temperature (6.2 °C). The trickling method using an automatic-filling syringe seems to be a very quick way for applying oxalic acid in large apiaries (approximately 150 hives/h).


*Keywords:* *Varroa destructor*; *Apis mellifera*; Oxalic acid; Sucrose; Trickling; Syringe

## 1. Introduction

The intensive utilization of many chemical substances (acrinathrin, amitraz, coumaphos, cymizole, flumetrine, fluvalinate, etc.) against *Varroa destructor* resulted in the development of resistance and the reduction of their efficacy (Floris et al., 2001). In addition these substances accumulate in bee products (wax and honey) due to their lipophilic and persistent nature (Wallner, 1999). To limit the toxicological and biological risks the application of oxalic acid (OA), a widespread organic acid with low toxicity, has been developed (Nanetti and Stradi, 1997). Its acaricidal properties are known from the last decades of the 20th century (Koeniger, 1984) but the mechanism of its acaricidal action against *V. destructor* has not been investigated in detail and it is partly attributed to a sensitivity of this species to acid pH (European Agency for the Evaluation of Medicinal Products, 2004).

The acid is applied by spraying or by trickling a water solution of OA without or with sugar, respectively, or by sublimating crystals with heat. To date, the

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doi:10.1016/j.vetpar.2007.06.001

trickling method is probably the most common way to apply OA solutions, thanks to quick, cheap, safe and simple application (Rademacher and Harz, 2006). An efficacy greater than 90% has been reported when broodless or almost broodless colonies have been treated with the trickling method (Gregorc and Planinc, 2001; Nanetti et al., 2003). On the contrary, the efficacy in colonies with brood had been less than 60% (Gregorc and Poklukar, 2003; Gregorc and Planinc, 2004).

The aim of this experiment was to evaluate the acaricidal effect and to assess the tolerability of bees when the OA solution was repeatedly given to the colonies by trickling in the autumn period (colder weather). The treatments were made in bee colonies during the broodright and the broodless period as well.

## 2. Materials and methods

The experiment was conducted in an apiary located on the Mt. Imittos (Attica, Greece) from October to December (weeks 43rd to 54th) of the year 2004. Honey bee colonies (*Apis mellifera* macedonica), naturally infested with *V. destructor* hosted in standard ten frame Langstroth hive bodies, were used. The hives were equipped with removable bottom boards allowing the trapping of falling mites on a sticky surface. Wire screens prevented the contact of the bees with the debris.

Twenty-four colonies were divided into two groups. The first group (called ''O'') consisted of 16 colonies that received 50 mL each of freshly made 4.2% (w/v) OA ($C_2H_2O_4{\cdot}2H_2O$, Cod.: 1.00495.1000, Merck) and 60% sucrose (table sugar) solution in double-distilled water (Nanetti et al., 2003) while the second group (called ''C'') consisted of 8 colonies that served as a negative control. The OA solution was applied by the trickling method directly onto the bees between the frame spaces, using, for the one-half of the experimental colonies, an automatic self-filling dosing (Hsw Drench-Matic®, Henke-Sass Wolf, Germany) and, for the other half, a single-use syringe of 50 mL capacity. The colonies received four successive OA applications starting from the middle to the end of the autumn (6/10, 22/10, 8/11 and 26/11). During the first two applications the colonies were occupied from 3 to 4 brood combs. In the third application the brood combs were fewer (1–2) while in the fourth one the capped brood cells were absent. In order to establish the efficacy, in terms of the mite mortality (%), of the different OA applications, a standard Perizin (3.2, w/v cumafos) treatment (Mutinelli et al., 1997) was applied to all groups on 14 December. The counting of the trapped mites was done every 2 or 3 days.

The evaluation of the efficacy (%) for each experimental group was based on the reliable method of counting mite drop-down, after each OA and Perizin treatment (Fries et al., 1991), and calculated according to the equation:

$$E\,(\%) = \frac{V_{OA}}{V_{OA} + V_C} \times 100,$$

where $E$ (%) is the efficacy; $V_{OA}$ the trapped mites after the use of oxalic acid solution; $V_C$ is the trapped mites after the use of controlling medicine (Perizin).

In order to evaluate short-term side effects on the bees (behavioral changes, colony strength and queen losses) the experimental group was compared (visual observations) to the control group for 3 days post each OA application. During the experimental period the number of the dead bees in front of the colonies was recorded. The outern temperature and the relative humidity were recorded on an hourly base with HOBO H8 equipment. Additionally the time required using each type of syringe was estimated.

Analysis of variance test was applied to the experimental data using STATGRAPHICS Plus Version 2.1. Before ANOVA, efficacy percentages were submitted to angular transformation according to the algorithm $x_1 = \text{arcsine}(x)^{0.5}$ to fit the normal distribution of the data. The level of significance was $\alpha = 0.05$.

## 3. Results and discussion

The daily minimum, maximum and mean values of the outern temperature (°C) and the relative humidity (%) occurred over the entire experimental period are presented in Fig. 1. On the time of the first three OA applications the outern temperature was 19.4, 22.5 and 17.5 °C, respectively, while on the fourth application it was very low (6.2 °C). Extremely low temperature (−2.0 °C) was recorded a few days before the fourth application. At the end of the experiment the Perizin application was applied at 10.8 °C. The average value of the relative humidity during the experimental period ranged from 68.7 to 79.8% and the lowest value (40.4%) was observed during the first application.

As expected, the time needed to trickle the solution by the single-use syringe, was significantly longer ($0.7 \pm 0.1$ min per application per colony) in comparison to the automatic device ($0.4 \pm 0.1$ min per application per colony) ($p < 0.0001$). The higher practicability of the quicker method did not seem to be associated to the treatment performance ($p = 0.8617$).

The 6-days pretreatment period revealed a daily natural mite mortality (mean ± S.E.) of $4.8 \pm 0.39$