Oxalic acid treatment by trickling against *Varroa destructor*: recommendations for use in central Europe and under temperate climate conditions

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Treatment with oxalic acid against *Varroa destructor* in broodless colonies is very effective. The administration of this organic acid by trickling a solution onto the colony is up to now the easiest method of application. The formulation of this solution has however to be optimized to reach maximum efficacy without side effects on the bees. Results for central European conditions are presented in this article together with a small review of the subject.

Introduction

At present, synthetic acaricides are regularly used for the control of *Varroa destructor*. However, due to their lypophilic and persistent nature, they accumulate in wax and, to a lesser extent, in honey[5,36]. Also, acaricide resistant mites have appeared in many countries of the world[12,13,27,28]. These problems have led to the development of natural, non-toxic substances for the control of varroa, such as different organic acids[17,21] and essential oils[15], which are increasingly being used throughout Europe.

In some alternative control strategies[1,17], winter treatment is extremely important against varroa because most of the mites that would form the basis for next year's population are destroyed in this way. These are mites that survived the autumn treatment, or those that have reinvaded the colony.

For the winter treatment, oxalic acid offers a good opportunity. Oxalic acid is a natural constituent of honey. It is allowed for the use in biological beekeeping (EU Council Regulation, No. 1804/1999). Oxalic acid is not registered in most western European countries for general use in beekeeping, although it is widely used because of its high efficiency. Oxalic acid is applied by spraying[18], evaporating[34] and trickling. The trickling method using a sweetened oxalic acid solution has proved successful, as it is simple, quick, cheap and very effective[6,9,26,31]. Under certain conditions, however, weakening of colonies can occur in spring[7,8,22].

Within the framework of a European project, tests were carried out in Switzerland and other countries in order to find a

better treatment solution. Based on our own results and those of the other European institutes, we can now give recommendations for beekeepers in central Europe, which are also most probably valid worldwide for beekeeping with *Apis mellifera* in all temperate climate areas.

Design of the experiment 1999/2000

Oxalic acid solutions tested

Tests carried out in 1998/1999 have shown that the so-called Italian method (a solution of 60 g oxalic acid dihydrate per litre sugar water 1:1) combats varroa effectively, but is not well tolerated by the bees. If the oxalic acid dihydrate content is halved, the effectiveness decreases and varies between colonies. It has also emerged that the addition of sugar in the trickling solution is essential to ensure a good treatment outcome[8].

For the 1999/2000 tests, solutions with varying oxalic acid (OA) content were used, where this was always lower than in the Italian solution, but amounted to at least 30 g OA dihydrate per litre sugar water. A solution with a lower sugar content was also tested because some observers had achieved better bee tolerance with it.

The colonies were treated with one of the following, freshly made solutions:

- 0 g OA dihydrate/litre sugar water 1:1 (by weight).
- 30 g OA dihydrate/litre sugar water 1:1.
- 37 g OA dihydrate/litre sugar water 1:1.
- 45 g OA dihydrate/litre sugar water 1:1.
- 45 g OA dihydrate/litre sugar water 1:2.

The first solution contained no OA and functioned as a control within these tests. The content of 45 g OA per litre sugar water was the formulation we recommended provisionally in 1999[8].

The tests were carried out on 200 colonies in 10 apiaries distributed throughout Switzerland. Four apiaries contained Dadant hives, the others Swiss hives, all of which were fitted with a varroa screen and tray for counting varroa. The test colonies had been treated with formic acid or thymol during August–September. In each occupied bee space, 5–6 ml oxalic acid solution was trickled directly onto the bees. The treatments were carried out between 29 October and 9 December 1999 at a temperature of above 4°C. Since oxalic acid has no effect on varroa in the brood, it is important that the colonies should be broodless.

Effectiveness of the treatment

For estimating the surviving mites, a control treatment was carried out no earlier than three weeks after the oxalic acid treatment. For this either Perizin or a 2.1% oxalic acid spray were used[18]. The cumulative mite-fall after oxalic acid and control treatment was assumed to be 100%.

Overwintering and development of the colonies in Spring

Since bee tolerance represents an important criterion for the evaluation of the trickling treatment, colony strength was determined in five apiaries in the autumn and following spring according to the 'Liebefeld method'[16]. In the Wohlei apiary, colony strength was estimated three times at the beginning of the year in order to follow colony development in spring.

Table 1. Average effectiveness of the trickling treatment with oxalic acid (g oxalic acid dihydrate per 1 litre sugar water 1:1 or 1:2 by weight) 1999/2000.

Apiary	*n*	ml*	0 g oxalic acid (1:1)		30 g oxalic acid (1:1)		37 g oxalic acid (1:1)		45 g oxalic acid (1:1)		45 g oxalic acid (1:2)	
			mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
Boden[1]	6, 7, 7, 7	39	3.4 a**	0.1	97.0 b	0.3	98.0 b	0.2	98.9 b	0.5		
Chaux de Fds[1]	5, 5	38							97.4 a	0.1	96.7 a	0.6
Frümsen[1]	10, 9	33							91.5 a	0.9	89.1 a	1.4
Grangeneuve[1]	12, 10	35							93.9 a	3.3	88.0 a	3.8
Hofen[2]	8, 8	45			96.8 a	0.4	97.7 a	0.9				
Landikon[2]	8, 8, 8	49			95.2 a	1.4	97.8 a,b	0.4	99.0 b	0.2		
Rüteli[2]	6, 6, 6	45			98.3 a	0.7	99.7 a	0.3	99.8 a	0.9		
Schwand[1]	6, 6, 6, 6	41	9.2 a	1.3	93.4 b	1.6	97.8 b	0.5	97.3 b	1.1		
Wohlei[1]	5, 5, 6, 5	46	1.9 a	0.3	96.8 b	0.5	98.3 b	0.3	98.4 b	0.2		
Zweisimmen[2]	10, 9	44					97.7 a	2.3	98.8 a	0.2		

*average dosage per colony
**Means within a line followed by different letters are significantly different ($P < 0.05$). Multiple *t* test (Tukey) after an angular transformation (Snedecor & Cochran, 1980)
Control treatment: [1]Perizin treatment, [2]Spray treatment with oxalic acid

Results

Effectiveness against varroa

The average effectiveness of the trickling treatment with the different solutions is given in table 1.

Caution must be exercised when evaluating the results obtained from the Grangeneuve apiary as the number of mites counted after the control treatment suggests a re-invasion in the six weeks between the trickling treatment (15 November 1999) and the control treatment (26 December 1999) (table 2).

Oxalic acid solutions with 30 g, 37 g and 45 g OA dihydrate per litre sugar water, resulted in an effectiveness of above 90% in all apiaries. Only four out of 146 colonies treated with one of these solutions (except Grangeneuve) showed more than 50 surviving mites. This number should not be exceeded if a rapid increase in the varroa population is to be avoided before the next treatment opportunity in August of the following year. It can be assumed that in the four colonies with more surviving mites small amounts of remaining brood were present.

The effectiveness of the three solutions was not significantly different, while the effect of the 30-g solution was slightly less than that of the other two. This suggests that this concentration lies at the lower limit and that therefore the dosage cannot be further reduced without interfering with effectiveness. These observations confirm the results of 1998 with this same solution[8] (decrease and greater scatter of the effectiveness compared with higher dosages), as well as those of Büchler[6].

The lower sugar content of the solution with the ratio 1:2 seems to have a negative influence on the effectiveness (table 1), but the difference is not significant. The 1998/1999 tests had already demonstrated the importance of the sugar content. Since

Table 2. Number of dead mites after control treatment. OA (g oxalic acid dihydrate per 1 litre sugar water 1:1 or 1:2)

Apiary	0 g OA (1:1)		30 g OA (1:1)		37 g OA (1:1)		45 g OA (1:1)		45 g OA (1:2)	
	av.	max.	av.	max.	av.	max.	av.	max.	av.	max.
Boden	532	1523	9	40	11	16	4	8		
Chaux de Fds							11	55	12	57
Frümsen							7	19	11	85
Grangeneuve							66	572	132	971
Hofen			12	19	6	34				
Landikon			17	29	8	18	2	7		
Pfeffikon			3	6	1	6	1	2		
Schwand	368	955	30	149	9	21	11	197		
Wohlei	447	1708	15	21	8	24	10	30		
Zweisimmen					6	34	4	15		

the solutions are not taken up orally by the bees (Ritter, personal communication), but act by contact[25], the role of the sugar remains unclear. Perhaps the solution adheres better and increases effectiveness by contact with the bees or, the sucrose makes the solution more hygroscopic[29].

Bee tolerance

The four 1:1 sugar water solutions were tested in three apiaries. The colonies in the Wohlei and Boden apiaries were clearly weakened during the winter, independent of the OA concentration of the solution used (fig. 1). Even control colonies treated with plain sugar water lost up to 40% of wintering bees, which indicates unfavourable overwintering conditions. In the Boden apiary, losses may be traced to a late harvest of honeydew. The solution without oxalic acid gave the best overwintering results in all cases. Losses increased with increasing OA content of the solution.

In the Schwand apiary, the loss ratios of the different variations were not significantly different. Only colonies treated with the 45 g OA solution showed a slight weakening.

Overwintering losses by themselves are not serious when the colonies have recovered by the spring harvest. In the Wohlei apiary, where colony development was measured in spring, there was still a shortfall of between 1000 and 2000 bees in oxalic-acid treated colonies at the end of April (fig. 2).

A comparison of bee tolerance between the 45 g OA in 1:1 sugar water and 1:2 solution shows that the mixture with the lower sugar content was better tolerated (fig. 3).

Residues and human toxicity issues

Oxalic acid is a natural constituent of honey and values between eight and 300 mg/kg have been found for different honeys[2,11,19,30]. Oxalic acid is a natural constituent of most

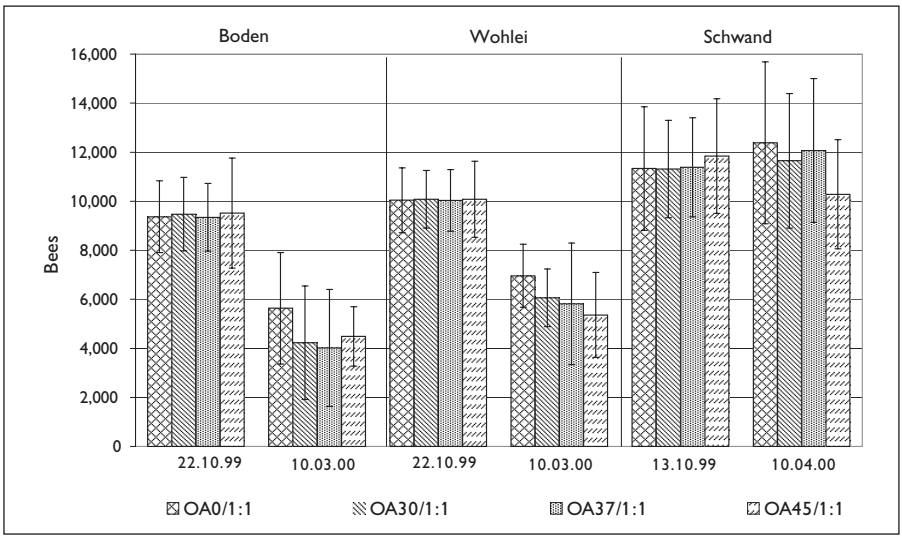

FIG. 1. Overwintering of colonies subjected to trickling treatment with oxalic acid, average and standard deviation, Boden, Wohlei, Schwand, 1999/2000.

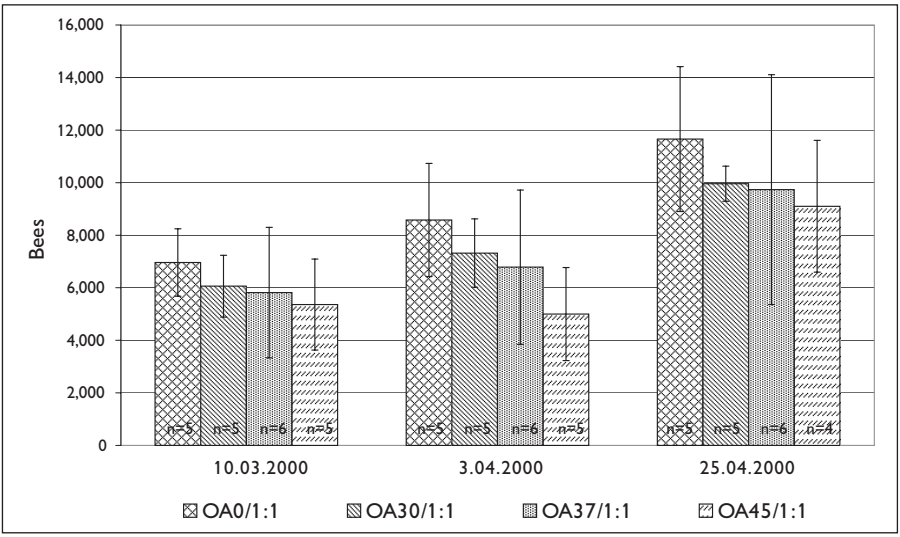

FIG. 2. Spring development of colonies subjected to trickling treatment with oxalic acid, average and strandard deviation, Wohlei, 1999/2000.

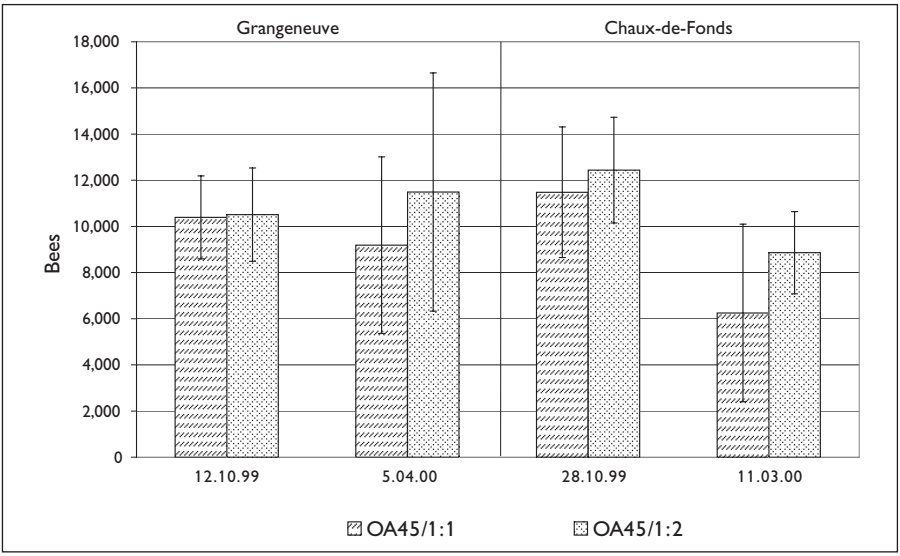

FIG. 3. Overwintering of colonies subjected to trickling treatment with oxalic acid, average and standard deviation, Grangeneuve, La Chauxde-Fonds, 1999/2000.

FIG. 4. Winter control of varroa, either by spray treatment or, as here, by a trickling treatment with an oxalic acid solution, is extremely important in alternative control strategies. It keeps the initial mite population for the following season very low.

vegetables and its content lies between 300 and 17 000 mg/kg. Thus, most vegetables contain much higher amounts of oxalic acid than honey. If we consider the small daily intake of honey, its contribution to the total daily intake of oxalic acid is negligible. Thus, from a nutritional point of view, oxalic acid should, like formic acid, also have a GRAS (Generally Recognized As Safe) status. Moreover, no significant residues are expected after oxalic acid treatments. Indeed, there is no risk of honey residues after all types of oxalic acid treatments[2,3,11,30,33,34].

To our knowledge spraying and trickling of oxalic acid is accepted for use against *V. destructor* in only three western European countries (Switzerland, Austria and Finland), but is widely used by beekeepers throughout Europe as the treatments are very efficient. The main registration problem of these treatments seems to lie in the toxicity of oxalic acid to the person applying the treatment. If the necessary safety precautions are taken, the spraying and trickling methods for using this acid are not dangerous for the user[20].

As no oxalic acid residues are to be expected after repeated field spraying and trickling use of this acid, there are no objective arguments against the registration of these treatments for the control of *V. destructor*.

Conclusions

In determining the ideal formulation for a trickling treatment with oxalic acid, effectiveness as well as bee tolerance must be taken into account. Numerous experiments carried out in Switzerland[8,9,10] and elsewhere[6,7,14,22] have demonstrated that it is difficult to do justice to both parameters. This is indicated by the following facts:

● The solutions with 37 g and 45 g OA dihydrate permit an effective varroa control. But, under certain conditions the 45 g solution is less well tolerated by the bees.

● The solution with 30 g OA dihydrate has only a slight influence on the overwintering success of bees, but shows slightly less effectiveness compared with the solutions containing 37 g and 45 g OA dihydrate.

● A lower sugar content increases bee tolerance, but decreases effectiveness.

● Under certain, undefinable, overwintering conditions, higher bee losses may occur through trickling treatment. This does not always apply, as the example of the Schwand apiary shows.

In a future experiment, bee tolerance to a trickling application of 35 g OA dihydrate will be compared with that of the OA spray and evaporation methods[35], the application of Perizin and an untreated control.

Based on our tests over the last years, the results of which have been mainly confirmed by research institutes in central Europe[7,24], we are able to recommend the following for a trickling treatment with oxalic acid:

Recommendations for the trickling treatment with oxalic acid (Switzerland and central Europe).

Composition of the solution:
35 g oxalic acid dihydrate in 1 litre sugar water 1:1

Amount of solution:
● 30 ml for a small colony

● 40 ml for a medium-sized colony

● 50 ml for a large colony

This amount corresponds with 5–6 ml per occupied bee space in a Dadant or Swiss hive

Timing of the treatment:
in broodless colonies (November–December)

Remarks:

● Carry out one treatment only in autumn

● Trickle the solution directly onto the bees between the frames

● Treat with lukewarm solution

● Carry out treatment at an outdoor temperature of above 0°C

● Use only freshly made-up solutions, or those stored for no more than 6 months at a maximum of 15°C[4,32]

● Under certain conditions a trickling treatment with oxalic acid can lead to a slight weakening of colonies in spring

● The spray treatment with oxalic acid is well tolerated by bees (30 g OA dihydrate/litre water, 3–4 ml per occupied frame side)[9] and represents an alternative to the trickling method

● Wear gloves and safety goggles during treatment[23]

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