Negative long-term effects on bee colonies treated with oxalic acid against Varroa jacobsoni Oud

Mariano Higes, Aránzazu Meana, Miguel Suárez, Jesús Llorente

Apidologie 30 (1999) 289–292

Original article

Negative long-term effects on bee colonies treated with oxalic acid against *Varroa jacobsoni* Oud.

Mariano Higes[a], Aránzazu Meana[b]*, Miguel Suárez[a], Jesús Llorente[a]

[a] Centro Apícola Regional, Servicio de Investigación Agraria de Castilla-La Mancha, Marchamalo, Guadalajara, Spain
[b] Dpto. Patología Animal I (Sanidad Animal), Facultad de Veterinaria, Universidad Complutense de Madrid, Avda Puerta de Hierro s/n, 28040 Madrid, Spain

(Received 28 March 1998; accepted 4 May 1999)

Abstract – Two oxalic acid treatments were given to five colonies in autumn and five colonies in spring. In each treatment, colonies were treated every 7 days for 4 weeks with a 3 % sprayed oxalic acid. Another five colonies in each season served as controls and were sprayed only with water. Efficacy of oxalic acid in autumn was 94 % and in spring was 73 %. A long-term study of the colonies for 3–4 months after the last application of oxalic acid showed a statistically significant negative effect of the acid on brood development. In addition, three queens died in the treated colonies. © Inra/DIB/AGIB/Elsevier, Paris

***Apis mellifera* / *Varroa jacobsoni* / oxalic acid / brood development**

1. INTRODUCTION

Recent reports on the use of oxalic acid for controlling varroosis seem to prove its high efficacy and a good tolerability by bees [4, 8]. Most tests have been carried out by spraying water-diluted oxalic acid in the colony during a broodless period, and have reported more than 95 % efficacy. Similar results were reported for a topical application of an oxalic acid solution diluted in sugar-water [6]. In countries with warm climates, the broodless period is very short and several treatments are necessary.

Our aims were to evaluate the effect on colony brood development of a 3 % (w/v) water-diluted oxalic acid treatment in colonies of different strengths in autumn and in spring.

* Correspondence and reprints
E-mail: Ameana@eucmax.sim.ucm.es

2. MATERIALS AND METHODS

Two trials were carried out on honey bee colonies naturally infected with *Varroa jacobsoni*. In each trial, ten different colonies were used. The colonies were housed in modified Langstroth hives fitted with a specially made tray on the bottom board for collection and detection of *Varroa*. Trial 1 was performed in hives with 1 557 ± 657 brood cells during September and October. Trial 2 was performed the following spring (March and April) in colonies with 5 144 ± 1 690 brood cells. The number of broodcells was measured using a standard ellipse formula estimating 4.12 cells per cm^2 [5]. Total broodcells (TBC) were calculated as follow: $TBC = \sum (D_1 \times d_1 \times 0.785 \times 4.12)$, where D_1 and d_1 were the longest and smallest diameters of the brood ellipse of one side of each frame of brood.

The treatment was prepared by making a 3 % (weight/vol) oxalic acid solution (30 g of dehydrate oxalic acid, Panreac no. 141041, in 1 000 mL distilled water). About 4 mL per side of each comb occupied by bees was homogeneously distributed with a manual sprayer. The treatment was applied at the same hour of the day (9 a.m.) in autumn and in spring, with about 10 min being spent per hive. In each season, five hives were treated while the other five were sprayed with water only for control. Temperature at the moment of application ranged from 9.7 to 12.3 °C. The treatment was repeated every 7 days for 4 weeks. Mites that fell into the trays were counted weekly.

To determine the number of surviving mites after the oxalic acid treatment, two Apistan® strips were placed in each hive for 5 weeks after the last oxalic acid treatment. Some mites in the experimental site were resistant to fluvalinate [3] (the active ingredient in Apistan). Therefore, at the end of the Apistan treatment period, Perizin® (containing coumaphos) was applied twice at weekly intervals according to the label. The number of fallen mites from both Apistan and Perizin treatments was recorded weekly and summed to give the total number of surviving mites. After each treatment (oxalic, fluvalinate and coumaphos), colonies were tested to verify the presence of queens.

To evaluate the effect of oxalic treatment on colony development, the total number of brood cells in each colony was calculated again 4 weeks after the last coumaphos controlling treatment, about 3–4 months from the beginning of the study.

Data in the text are expressed as arithmetic media and standard errors. Differences in control and treated groups 3–4 months after oxalic acid treatment were established using a non-parametric Wilcoxon-Mann Whitney analysis, considering $P \leq 0.05$ as statistically significant [7].

3. RESULTS

Table I shows the total number of sealed brood cells (TBC) for each colony at the beginning of the oxalic acid treatment. The number of brood cells was different in autumn (TBC =1 557 ± 692) and spring (TBC = 5 144 ± 1 690).

In autumn, two of the treated colonies had lost their queens 3–4 months after the last treatment, and one of these colonies did not survive. The remaining queens produced less brood (TBC = 9 263 ± 471) than the controls (TBC = 14 978 ± 1 036). One colony died after the spring treatment, and the remaining four also produced less brood (TBC = 15 266 ± 535) than the control colonies (TBC = 18 795 ± 939).

The differences in the increase in the number of sealed broodcells between all the surviving treated (8 580 ± 1 261) and all the control colonies (13 902 ± 2 612) is significant (U = 40, $P = 0.004$).

In autumn the efficacy of oxalic acid was 94 %, while in spring it was 73 % (*table I*). The average mite population in the ten colonies in autumn was 738 ± 225. In spring the mite population was much higher (3 911 ± 750).

4. DISCUSSION

V. jacobsoni mortality after treatment with oxalic acid seems to be directly influenced by the presence of brood. Recent reports agree on the use of oxalic acid when no brood is present in winter [1]. Its efficacy when brood is present is around 60 % [2]. Thus, the usefulness of the acid seems limited in warm climates with a long brood rearing period. In our experiment in Guadala-

Table I. The number of sealed brood cells at the beginning of oxalic acid treatment, after 3–4 months, and the number of fallen mites and efficacy of oxalic acid.

Hive	Total brood cells Day 0	Total brood cells 3–4 months	% increase in brood cells	Fallen mites		Efficacy
				Oxalic acid	Fluvalinate + coumaphos	
A1	2 101	9 381	347	1 878	237	88.7
A2	1 816	10 014	451	20	4	83.3
A3	0	8 394	–	342	3	99
A4	1 749	queenless	–	255	8	96.9
A5	2 321	dead	–	1 591	6	99.6
CA1	1 816	14 326	689	–	216	no effect
CA2	2 025	17 373	758	–	1 359	no effect
CA3	1 594	12 191	665	–	548	no effect
CA4	801	13 629	1 601	–	161	no effect
CA5	1 349	17 373	1 188	–	753	no effect
S1	5 996	16 300	172	2 215	891	71.3
S2	5 810	14 921	157	952	511	65.0
S3	7 006	13 920	99	3 011	759	79.8
S4	4 294	dead	–	1 298	475	73.2
S5	6 062	15 921	163	1 397	424	74.5
CS1	998	20 203	1 924	–	2 993	no effect
CS2	6 126	21 316	248	–	3 754	no effect
CS3	4 321	16 194	275	–	8 360	no effect
CS4	4 891	19 043	289	–	7 700	no effect
CS5	5 932	17 221	190	–	4 321	no effect

A = colonies treated in autumn; CA = control colonies in autumn; S = colonies treated in spring; CS = control colonies in spring.

jara (close to Madrid), Spain, the efficacy (73 %) of four applications was similar to only one topical application during the broodless period [1, 6]. In autumn, the efficacy (94 %) was similar to treatments when no brood is present in autumn [3] or in spring [1].

Although no effects of the treatment were seen in the colonies during the application period [6, 9], the long-term study of the colonies clearly shows a negative effect of the acid, at least when brood was present.

The most important finding in this study was that the increase in the number of brood cells was significantly higher in the control hives than in the treated colonies. The loss of a queen and two colonies cannot be related to mismanagement by the apiarists, as all colonies were inspected after treatments and all queens were present. This long-term negative effect of oxalic acid on bee colonies has not been reported before.

Although the efficacy of oxalic acid is very high in autumn when there is little brood, the detected negative long-term side-effects on colony development require more thorough studies to determine the reliability of its use.

Résumé – Effets négatifs à long terme sur les colonies d'abeilles du traitement à l'acide oxalique contre *Varroa jacobsoni*. Des travaux récents [4, 8] semblent montrer que l'acide oxalique (A.O.), utilisé en vaporisation est efficace à 95 % contre l'acarien *Varroa jacobsoni* Oud. et bien toléré par les abeilles (*Apis mellifera* L.). Des résultats semblables ont été obtenus avec une application topique d'A.O. dilué dans du sirop [6]. Notre étude vise à évaluer les effets de l'A.O. sur le développement du couvain à l'automne et au printemps. Cinq colonies ont été traitées à l'automne et cinq autres au printemps, une fois par semaine durant quatre semaines avec une solution d'A.O. à 3 %, vaporisée sur les cadres. À chaque saison cinq autres colonies n'ont reçu que de l'eau pour servir de témoins. Le nombre d'acariens encore en vie à la fin du traitement a été déterminé par un traitement à l'Apistan, puis au Perizin. Afin d'évaluer les effets du traitement à l'A.O. sur le développement des colonies, le nombre total de cellules de couvain a été compté dans chaque colonie 3 à 4 mois après le début de l'expérience. L'efficacité a été de 94 % à l'automne et de 73 % au printemps (*tableau I*). L'A.O. a eu un effet négatif statistiquement significatif sur le développement du couvain (*tableau I*). En outre trois reines des colonies traitées sont mortes. © Inra/DIB/AGIB/Elsevier, Paris

Varroa jacobsoni* / acide oxalique / développement couvain / *Apis mellifera

Zusammenfassung – Negative Langzeiteffekte bei Bienenvölkern nach der Behandlung mit Oxalsäure gegen *Varroa jacobsoni*. In zwei Versuchen wurden je 5 Bienenvölker mit Oxalsäure im Herbst und im Frühjahr behandelt. Bei jeder Behandlung wurden die Völker 4 Wochen lang jeden 7. Tag mit 3 %iger Oxalsäure gesprüht. Als Kontrolle dienten jeweils 5 Völker in jeder Saison, die mit Wasser gesprüht wurden. Die Wirksamkeit der Oxalsäure wurde mit Apistan Streifen und Perizin überprüft und betrug im Herbst 94 %, im Frühjahr 73 %. Drei bis 4 Monate nach der letzten Säurebehandlung zeigten sich statistisch gesicherte negative Nebenwirkungen auf die Brutentwicklung. Zusätzlich starben in 3 der behandelten Völkern die Königinnen (*Tabelle I*). © Inra/DIB/AGIB/Elsevier, Paris

***Apis mellifera* / *Varroa jacobsoni* / Oxalsäure / Brutentwicklung**

REFERENCES

[1] Barbero R., Panella F., Bonizzoni L., Ácido oxálico y el tratamiento de limpieza radical de otoño-invierno, Vida Apíc. 85 (1997) 8–13.

[2] Charrière J.D., Potentiel et limites de l'emploi des acides organiques, Sanitaire 161 (1997) 219–227.

[3] Higes M., Llorente J., Sanz A., Meana A., Calonge R., Varroa Sensibilidad al fluvalinate, Vida Apic. 89 (1998) 41–45

[4] Imdorf A., Charriere J.D., Bachofen B., De l'utilisation de l'acide oxalique comme varroacide, Apiacta 32 (1997) 89–91.

[5] Llorente J., Robles E., Salvachúa C., Investigaciones sobre procedimientos de lucha química contra *Varroa jacobsoni* Oud. Investigación Agraria en Castilla-La Mancha. Área de Producción Animal, no. 1, Edita Consejería de Agricultura y Medio Ambiente de la Junta de Comunidades de Castilla-La Mancha, 1991, 72 pp.

[6] Mutinelli F., Baggio A., Capolongo F., Piro R., Prandin L., Biasion L., A scientific note on oxalic acid by topical application for the control of varroosis, Apidologie 28 (1997) 461–462.

[7] Siegel S., Castellan N.J., Estadística no paramétrica aplicada a las ciencias de la conducta, Editoral Trillas, México, 1995.

[8] Radezki T., Reiter M., von Negeelein B., Oxalsäure zur Varroabekämpfung, Schweiz. Bienenztg. 117 (1994) 263–267.

[9] Takeuchi K., Harada K., Control of *Varroa jacobsoni* mites with oxalic acid spray, Honeybee Sci. 4 (1983) 113–116 (in Japanese).