Apidologie 32 (2001) 333–340

Original article

Acaricidal effect of oxalic acid in honeybee (*Apis mellifera*) colonies

Aleš GREGORC[a]*, Ivo PLANINC[b]

[a] Veterinary Faculty of the University of Ljubljana, Gerbičeva 60, 1000 Ljubljana, Slovenia
[b] Animal health centre of Slovenia, Dept. of bee health, Slovenia

(Received 30 May 2000; revised 14 March 2001; accepted 2 April 2001)

Abstract – Three oxalic acid (OA) solutions were applied to 24 honeybee colonies to test acaricidal effects on *Varroa destructor*. Daily natural mite drop per colony averaged 0.52. Higher mite mortality (18.33) was found after three August OA treatments. The mean efficacy's of the three water solutions of OA/sucrose (w/w), 3.4%/47.6%, 3.7%/26.1%, and 2.9%/31.9% applied in the presence of brood, was 52.28%, 40.66% and 39.16% respectively. A significantly higher efficacy was recorded when 3.4%/47.6% was applied in comparison to 2.9%/31.9% solution. There was no difference in efficacy between OA solutions administered during a broodless period on October 28. The average efficacy in all colonies was 99.44%. The results suggest that OA has limited acaricidal effect in colonies with brood, but it is highly effective in a broodless period.

***Varroa destructor* (*jacobsoni*) / honeybee / control / oxalic acid /efficacy**

1. INTRODUCTION

Varroa destructor (formerly named *Varroa jacobsoni)* (Anderson and Trueman, 2000) is potentially the main parasite of *Apis mellifera* and it can cause the collapse of untreated colonies in a few years. Colony collapse is due not only to mite infestation, but also to secondary viral infections (Hung et al., 1996). Several chemical substances were used successfully to control mites (Mobus and Bruyn, 1993) and a wide array of chemicals (acrinathrin, flumethrin, fluvalinate, amitraz, coumaphos, cymizole and other) were highly effective, killing more than 99% of the mites present in infested colonies (Ferrer-Dufol et al., 1991). However in consecutive years resistance of mite against these pesticides has been demonstrated (Elzen et al., 1998; Milani, 1995) due to high selection pressure (Milani, 1999). Mite control is imperative in order to maintain the population of honeybee colonies in most beekeeping regions around the world. The use of acaricides should also be minimised in beekeeping because of the

* Correspondence and reprints
E-mail: GregorAl@mail.vf.uni-lj.si

residues and their breakdown products in honey and wax (Wallner, 1999) and because of potential resistance to acaricides (Ruijter, 1994). The selection pressure for resistance against "natural acaricides" such as organic acids and essential oils is presently low (Milani, 1999). Accumulation in wax does not occur and residues in honey are small and toxicologically not important (Imdorf et al., 1996).

Acaricide treatment and counting the mites drop from the colony on the bottom board are reliable diagnostic methods (Ritter, 1981; Fries et al., 1991; Gregorc and Jelenc, 1996; Poklukar, 1999). A relationship was found between mites in the hive debris and mite population in a colony (Liebig et al., 1984). Experiments have been conducted to evaluate oxalic acid (OA) for mite control in colonies with brood and without brood (Imdorf et al., 1996; Brødsgaard et al., 1999; Nanetti and Stradi, 1997; Nanetti, 1999). OA activity tests using a range of OA concentrations from 2 to 5% in 30 and 60% sucrose solutions (w/v) were tested in colonies without or with brood. Achieved efficacy resulted in high levels of mite mortality, estimated as 97.3%, 98.3% and 99.5% respectively during broodless period (Radetzki, 1994; Nanetti et al., 1995; Imdorf et al., 1997). 95% efficacy after three treatments of 5% OA (Mutinelli et al., 1997) and 24% efficacy after one spring treatment (Brødsgaard et al., 1999) administered by trickling was achieved, when the capped brood was present.

This paper presents data on the natural mite fall in non treated and OA treated colonies. The aim was to establish the comparative acaricidal effect of 3.4% OA/47.65% sucrose, 3.73% OA/26% sucrose and 2.9% OA/31.95% sucrose in water solution. The aim of our work was also to establish whether oxalic acid could be successfully used as an alternative to acaricide when brood is present and whether counting of fallen mites on the hive bottom was a reliable method to determine a rate of mite mortality after treatments.

2. MATERIALS AND METHODS

Twenty-four honeybee *Apis mellifera* L. colonies populated in national standard AŽ "back load" hive type (Zdešar, 1998), with nine combs (41 × 26 cm) in each brood and honey compartments, were located at one site near Vipava, Slovenia with mild Mediterranean climate influence. Colonies were treated with Perizin (Bayer, Germany) the previous winter when no brood was present. In the spring of 1999, metal sheet with dimensions of 38 cm × 29.8 cm were inserted on to the hives floor in order to record natural mite mortality from all bee spaces between combs. Wire screens above the sheet prevent bees from coming in contact with debris. Before the experiment, colonies were equalised to occupy from 5 to 7 brood combs. On sampling dates, mites were recorded and inserts were emptied. Natural mite drop-down was counted in colonies for periods before and after OA treatments and after treatment with Perizin. These periods were from May 22 to June 5, from June 25 to July 12 and from July 31 to August 12 and after August OA treatments. Mite drop was monitored also from September 9 to October 28 and after October 28 OA treatment and after Perizin application on December 29 and January 5 respectively.

Treatments were applied to twenty-four colonies on August 12 as follows: (1) six colonies received a 3.4% OA and 47.6% sucrose in water solution (w/w) using oxalic acid dihydrate (Riedel-de Haën), sucrose (sugar) and de-mineralised water, (2) seven colonies received 3.7% OA and 26.1% sucrose (w/w) (Nanetti, 1999), (3) seven colonies received 2.9% OA and 31.9% sucrose (w/w), (4) four-control colonies received de-mineralised water only. Treatments were repeated on August 21, August 30, when the brood was present, and on October 28 during a broodless period. During the October 28 treatment, colonies previously water treated, received a 3.7% OA and 26.1% sucrose solution, the same concentration previously applied to the treatment 2.

Treatments were applied to each colony by trickling 50 ml of the treatment solution over the combs, in situ, and bees in the brood compartment using a syringe. During the treatment the applicator wore respiration mask, protective glasses and rubber gloves. During the experimental treatments the outside temperature was between 20 and 25 °C, except on the last three treatments dates when the outside temperature were October 28, 15 °C December 29, 7 °C January 5, 7 °C. Oxalic acids and Perizin solutions were approximately 20 °C.

In order to establish the efficacy of different oxalic acid concentrations expressed in mite mortality, two standard Perizin treatments (Mutinelli et al., 1997; Trouiller, 1998) were applied to all colonies on December 29 and January 5. Mite mortality was established by counting mite drop-down four days after each OA and Perizin treatments.

The percent mites killed by treatments when brood was absent (FTNB) was estimated using the formula, FTNB = FOA2/ (FOA2 + FP) × 100, where FOA2 is the number of fallen mites during treatment on October 28 and FP is an estimate of the number of mites not killed by OA treatments and was obtained by counting the mite drop during treatment with Perizin on December 29 and January 5. The percent of mites killed by treatments when brood was present (FTB) was estimated using the formula, FTB = FOA1 / (FOA1 + FOA2 + FP) × 100, where FOA1 is the total number of mites dropping during the treatment periods August 12 to 21, from August 21 to 30 and from August 30 to September 9.

Treatment efficacy was also estimated by comparing mite drop before and after treatments and mite mortality between consecutive OA treatments. Experimental hives were equipped with dead bee traps (Accorti, 1994) to collect and count dead bees five days after each treatment. Colonies strength and behaviour changes in bees were evaluated and compared to untreated colonies visual on the same day of the treatments. Data analyses were carried out by ANOVA (analysis of variance) with the aid of the Statgraphic (1991) programme.

3. RESULTS

3.1. Natural mite mortality

Mite levels in colonies were not different among the treatment groups prior to the application of treatments ($F = 1.89$; $df = 3$; $P > 0.1$) and averaged 0.53 ± 0.45.

3.2. August oxalic acid treatment

Daily mite drop in colonies receiving 3.4% OA/47.6% sucrose solution (group 1) increased from less than 1 mite to 28.11 (± 12.45) in four days after OA treatments conducted on August 12, 21 and 30. The highest daily mite mortality per colony, 11.60 (± 7.05), was also observed in the same treated colonies, during prolonged monitoring time, continuing to September 9. In September and October the mite mortality decreased continuously and the maximal main 1.82 (± 1.09) daily mite drop-down was found in colonies that received 3.7% OA/26.1% sucrose solution. In control colonies, daily mite mortality remained on an average level 0.70 (± 0.68).

Mite drop in all OA treated colonies was significantly higher in comparison to natural mite drop-down before treatments ($P < 0.01$) and in comparison to untreated colonies during the experiment ($P < 0.01$). No significant change in mite drop-down was observed in water treated colonies in comparison to pre-treatment, natural mortality ($P > 0.05$).

Mite drop-down after August treatments counted between 9.9 and 28.10 and significant higher ($P < 0.01$) compared to natural mite drop following prior treatments. In the same period, natural mite drop did not been in water treated colonies ($P > 0.05$).

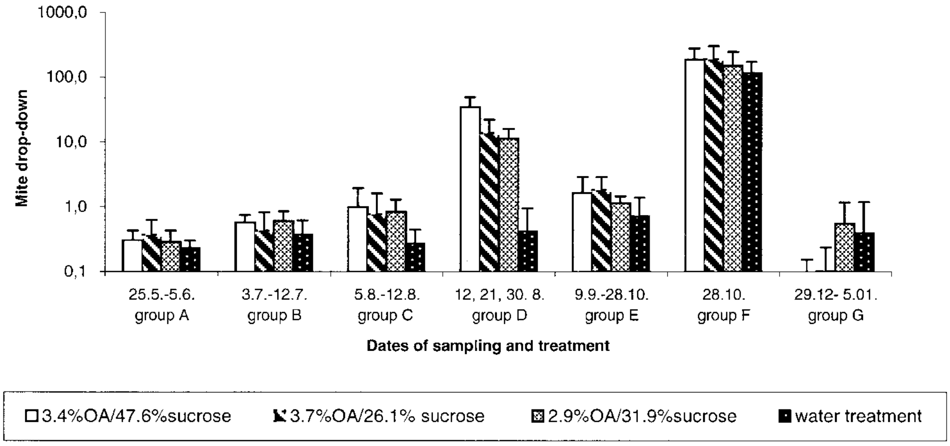

Figure 1. Natural mite mortality per day observed in three pre-treatment periods (groups A, B, C), mite mortality following three oxalic acid (OA) applications in August (group D), post treatment mite mortality per day (group E), one 3.7% OA/26.1% sucrose application on October 28 (group F) and mite mortality following two Perizin applications to all colonies (group G). Groups D and F give the mite mortality per 4 days and group G the total mite mortality. Bars indicate standard deviations.

3.3. October oxalic acid treatment

Mite drop after OA application in the broodless colonies on October 28 was significantly higher ($P < 0.01$) than in the pre-treated period observed between 9.9. and 28.10. One oxalic acid treatment on October 28, also resulted in higher mite mortality ($P < 0.01$) compared to mite mortality triggered by three August OA treatments when the brood was present. Mite mortality during the experiment is shown in Figure 1.

3.4. The efficacy

The sequence of three OA treatments conducted during August resulted in average 44.04% (± 7.19) mite reduction of all counted mites during the season. The relative efficacy of particular OA solution applied is shown in Figure 2. Differences in efficacy between three OA concentrations were significant ($P < 0.001$). The application of 3.4% OA/47.6% sucrose solution was found significantly more effective than 2.9% OA/31.9% sucrose solution applied in colonies with brood ($P < 0.05$). There were no differences in efficacy between 3.7% OA/26.1% sucrose and 2.9% OA/31.9% sucrose ($P > 0.05$).

The analyses of variance show significant higher effectiveness of October OA treatment in comparison to three OA treatments during previous August ($P < 0.001$). The average relative efficacy of final OA application in broodless colonies conducted on October 28, was estimated at 99.44% (± 0.54) and differences between colonies were not significant ($P > 0.1$). The efficacy of OA solution applied to previously untreated colonies was in the same range 99.58% (± 0.81) as colonies of other experimental groups.

3.5. Other observations

In OA treated colonies no queenlessness was recorded and worker bee mortality (3.25 ± 1.25 bees/day) five days after August treatments were not significantly different from control colonies (2.5 ± 0.58 bees/day, $P > 0.1$). Mean bee mortality after October

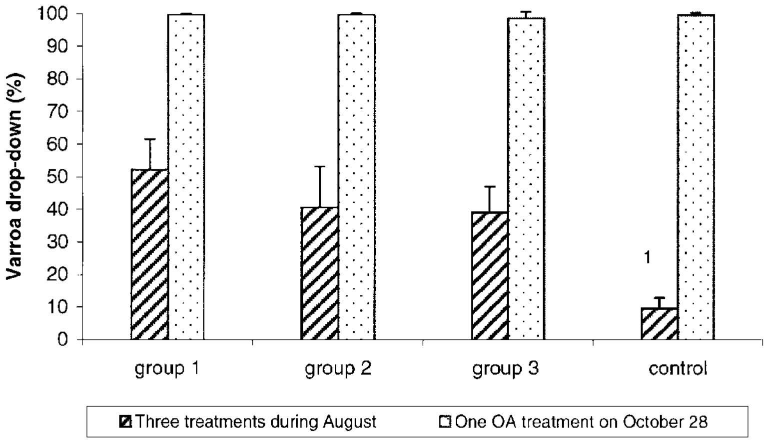

Figure 2. Efficacy of OA treatments expressed in % of drop mites compared to all drope mites during the experiment. Treatments:
Group 1: 3.4% OA / 47.6% sucrose.
Group 2: 3.7% OA / 26.1% sucrose.
Group 3: 2.9% OA / 31.9% sucrose.
Control: 1 = water treatment; 2 = 3.7% OA/ 26.1% sucrose.

OA treatment was on average 1.63 (± 1.32) bees/day and was significantly lower than that observed after Perizin treatments (11.54 ± 3.49 bees/day). Over-wintering colonies and early spring development in the year 2000 were normal without visible changes.

4. DISCUSSION

Daily natural mite mortality indicating infestation (under 1 mite/day) and colony development was average for the local natural conditions during the season 1999. Monitoring the natural mite mortality during the spring and summer time is an essential diagnostic method to obtain estimation of mite population development (Liebig, 1994; Martin, 1998). Three OA trickling, within the period when the brood is present, showed an effectiveness of approximately 44% significant difference in mite mortality compared to the control. Relatively low efficacy of OA is due to mite survival in the sealed brood (Radetzky, 1994). In colonies with capped brood cells, three OA treatments resulted in 95% mite population reduction (Mutinelli et al., 1997). Under our experimental conditions, OA treatments showed a significant influence on the final reduction of infestation level. The reduction of mite population with OA when the brood is present and after honey extraction could be a positive experience in highly infested colonies. A reduction by about half of the mite population during August seems to be important for survival of colonies. OA treatments when the brood was present did not affect the colony development compared to the control colonies. The results of this study also indicate that 3.4% OA/47.6% sucrose is significantly more effective than 2.9% OA/31.9% sucrose and is of practical importance for mite control.

Mite drop after August treatments (observed during September and October) was significant higher compared to pre-treatment natural mite mortality estimated from May to August. Reasons for this could include a reinvasion from controls and from colonies in the neighbourhood (Imdorf et al., 1999), and an yet unstudied residual effect of OA on mites in the colony. Autumn natural mite mortality is normally high and thus the efficacy of acaricide is often higher than expected (Trouiller, 1998). OA efficacy was underestimated, when the brood is present, because surviving reproductive mites were killed and finally counted after October OA and December/January Perizin treatments.

Effectiveness of OA solutions trickled in broodlees period was over 99% in our studies. Other OA and sucrose concentrations also resulted in very high efficacy, in the range of 95% (Imdorf et al., 1999), up to 98.6% (Nanetti et al., 1995; Nanetti, 1999). In a broodless period all applied concentrations of OA demonstrated effectiveness against mites. Remarkably, OA effectiveness against mites in the autumn and winter treatment is comparable to Perizin effectiveness which is used as a second – control acaricide (Mutinelli et al., 1997; Trouiller, 1998). The finding of this study was, that OA solution concentrations are highly effective in broodless period without visible side effects. Adverse effects on bee colonies after OA spraying and trickling were also not observed when used in March (Brødsgaard et al., 1999). OA side effects have been reported by Higes et al. (1999) and additional field and lab analyses need to be conducted using different OA/sucrose concentrations in varied climatic and year-season conditions. Good efficacy of OA and as yet undeveloped resistance against acaricides of natural origin (Milani, 1999) suggest that application of OA can be effectively used as an "alternative *Varroa destructor* control". At a colony infestation expressed in less than 1 fallen mite/day, only autumn OA application into broodless colonies ensures normal colony development next spring.

ACKNOWLEDGEMENTS

The authors thank Jasna Kralj (Bekeeping Association) for her comments and helpful discussion and J. Verbic (Agricultural Institute of Slovenia) for validating some of the statistics. We thank Prof. I.D. Bowen for reading and correcting the manuscript. We also thank anonymous reviewers for useful suggestions substantially improving the manuscript. This work was founded by the Ministry of Science and Technology (Research group No. 502) and the Ministry of Agriculture Forestry and Food.

Résumé – Action acaricide de l'acide oxalique dans les colonies d'abeilles (*Apis mellifera*). Le but de l'étude était de déterminer si l'acide oxalique (AO) pouvait être utilisé comme moyen de lutte alternatif contre *Varroa destructor* lorsqu'il y a ou non du couvain dans la colonie. Vingt-quatre colonies d'abeilles, situées en climat méditerranéen doux (Slovénie) ont été utilisées pour étudier l'action acaricide des trois solutions aqueuses suivantes : (i) 3,4 % d'AO et 47,65 % de saccharose (solution A), (ii) 3,73 % d'AO et 26,1 % de saccharose (solution B), (iii) 2,9 % d'AO et 31,95 % de saccharose (solution C).

La mortalité de l'acarien a été enregistrée avant et après les traitements à l'AO et après un traitement final au Perizin. Les solutions d'AO ont été administrées à trois groupes de colonies le 12 août, avec répétition les 21 et 30 août en présence de couvain et le 28 octobre en l'absence de couvain. Le traitement a consisté à faire couler goutte à goutte à l'aide d'une seringue 50 mL de solution sur les rayons et les abeilles du nid à couvain. La température extérieure lors des traitements était entre 20 et 25 °C en août, de 15 °C le 28 octobre et de 7 °C les 29 décembre et 7 janvier.

Le pourcentage d'acariens tués a été estimé en présence (FTB) et en l'absence (FTNB) de couvain. La mortalité totale des acariens de toutes les colonies traitées a été significativement plus élevée que la mortalité naturelle avant traitements ($P < 0,01$). La mortalité suite aux trois traitements du mois d'août a atteint 44,04 % (± 7,19). La solution A a été significativement plus efficace que la solution C ($P < 0,05$) ; il n'y a pas eu de différence entre les solutions B et C ($P > 0,05$). Un traitement à l'AO dans les colonies sans couvain en octobre a provoqué une mortalité plus élevée ($P < 0,01$) que celle due aux

trois traitements d'août (Fig. 1). L'efficacité relative moyenne du traitement final à l'AO dans les colonies sans couvain a été estimée à 99,44 % (± 0,54). Les différences entre colonies n'étaient pas significatives (Fig. 2). Aucune perte de reine n'a été notée et la mortalité des ouvrières (3,25 ± 1,25/jour) cinq jours après les traitements à l'AO n'a pas été significativement différente de celle des colonies témoins. L'hivernage des colonies et le développement printanier l'année suivante se sont déroulés normalement sans changements apparents.

L'efficacité relativement faible de l'AO est due à la survie des acariens dans le couvain operculé. Le développement des colonies avec couvain n'a pas été affecté par les traitements à l'AO comparé aux colonies témoins. Les résultats de cette étude montrent aussi que la solution A est significativement plus efficace que la C, ce qui est important du point de vue pratique pour la lutte contre l'acarien. L'efficacité de l'AO en présence de couvain a été sous-estimée parce que les acariens survivants capables de se reproduire ont été tués et finalement comptés après le traitement à l'AO d'octobre et ceux au Perizin de décembre et janvier. En conclusion, l'acide oxalique peut être utilisé efficacement comme moyen de lutte alternatif contre *V. destructor*.

***Varroa destructor* / lutte chimique / acide oxalique / efficacité**

Zusammenfassung – Milbengiftigkeit von Oxalsäure in Honigbienenvölkern (*Apis mellifera*). Eine Untersuchung der Milbengiftigkeit Oxalsäure (OA) in den Formulierungen von 3,4 % OA/47,65 % Zucker, 3,73 % OA/26,1 % Zucker und 2,9 % OA/31,95 % Zucker in Wasser wurde an 24 Bienenvölkern (*Apis mellifera*) in einem milden mediterranen Klima durchgeführt. Hierbei sollte festgestellt werden, ob Oxalsäure bei Anwesenheit oder Abwesenheit von Brut eine Alternative zu anderen Akariziden sein könnte. Die Mortalität der Milbe *Varroa destructor* wurde vor und während der Behandlung mit OA und nach einer anschlieβenden Behandlung mit Perizin erfasst. OA-Lösungen kamen zunächst am 12. August bei drei Gruppen von Bienenvölkern zur Anwendung. Die Behandlung wurde am 21. und 30. August wiederholt, zu diesen Zeitpunkten war in den Völkern Brut vorhanden. Eine weitere Behandlung wurde am 28. Oktober an brutlosen Völkern durchgeführt.

Für die Behandlung wurden unter Verwendung einer Pipette 50 mL der OA-Lösungen auf die Waben und Bienen des Brutnests geträufelt. Die Auβentemperaturen an den Behandlungstagen lagen zwischen 20 und 25 °C, am 28. Oktober betrugen sie 15 °C und am 29. Dezember sowie am 5. Januar 7 °C. Der Prozentsatz getöteter Milben bei Anwesenheit von Brut (FTB) und bei Abwesenheit von Brut (FTNB) wurde abgeschätzt. Der Milbentotenfall war in allen OA-behandelten Völkern signifikant höher als vor der Behandlung ($P < 0,01$). Eine OA-Behandlung in brutlosen Völkern hatte eine höhere Milbensterblichkeit zur Folge als alle 3 Behandlungen im August (Abb. 1). Diese brachten eine Verminderung der Milbenpopulation um 44,04 % (± 7,19) während der Saison. Eine 3,4 % OA/47,6 % Zuckerlösung war signifikant wirksamer als 2,9 % OA/31,9 % Zuckerlösung ($P < 0,05$). Zwischen der Wirksamkeit einer 3,7 % OA/26,1 % Zuckerlösung und einer 2,9 % OA/31,9 % Zuckerlösung gab es keinen Unterschied ($P > 0,05$). Die Behandlung im Oktober war signifikant wirksamer als die drei Behandlungen im August. Im Mittel wurde die Wirkung der letzten OA-Behandlung in brutfreien Völkern als 99,44 % (± 0,54) abgeschätzt; Unterschiede zwischen Völkern waren hierbei nicht signifikant (Abb. 2). Es wurden keine Königinnenverluste festgestellt, die Bienensterblichkeit von (3,25 ± 1,25 Bienen/Tag) war 5 Tage nach der Behandlung von der in den Kontrollvölkern nicht unterschiedlich. Überwinterung der Völker und die Frühjahrsentwicklung im Jahr 2000 waren normal und ohne sichtbare Unterschiede.

Die geringe Wirkung von OA im Sommer ist auf das Überleben der Milben in den

Brutzellen zurückzuführen, die Behandlung während der Brutperiode hatte im Vergleich zu den Kontrollen keine Auswirkungen auf die Völkerentwicklung. Die Ergebnisse der Studie weisen darauf hin, dass 3,4 % OA/47,6 % Zuckerlösung eine signifikant höhere Wirksamkeit hat als 2,9 % OA/31,9 % Zuckerlösung, dieses Ergebnis ist für die Milbenbekämpfung von praktischer Bedeutung. Die Wirksamkeit von OA bei Anwesenheit von Brut wurde unterschätzt, da reproduzierende überlebende Milben bei der OA-Behandlung im Oktober und in den Perizinbehandlungen im Dezember und Januar getötet und gezählt wurden. OA kann als eine "alternative Varroa Behandlung" eingesetzt werden.

***Varroa destructor* / Honigbienen / Behandlung / Oxalsäure / Wirksamkeit**

REFERENCES

Accorti M. (1994) Le api e il monitoraggio ambientale. Valutazioni a lungo termine sulle gabbie per la raccolta delle api morte, Apicoltura 9, 19–29.

Anderson D.L., Trueman J.W.H. (2000) *Varroa jacobsoni* (Acari: Varroidae) is more than one species, Exp. Appl. Acarol. 24, 165–189.

Brødsgaard C.J., Jensen S.E., Hansen C.W., Hansen H. (1999) Spring treatment with oxalic acid in honeybee colonies as varroa control, Danish Inst. Agric. Sci. report No. 6 Horticulture, 16 p.

Elzen P.J., Eischen F.A., Baxter J.B., Pettis J., Elzen G.W., Wilson W.T. (1998) Fluvalinate resistance in *Varroa jacobsoni* from several geographic locations, Am. Bee J. 138, 674–676.

Ferrer-Dufol M., Martinez-Vinuales A.I., Sanchez-Acedo C. (1991) Comparative tests of fluvalinate and flumethrin to control *Varroa jacobsoni* Oudemans, J. Apic. Res. 30, 103–106.

Fries I., Aarhus A., Hansen H., Korpela S. (1991) Comparison of diagnostic methods for detection of low infestation levels of *Varroa jacobsoni* in honey-bee (*Apis mellifera*) colonies, Exp. Appl. Acarol. 10, 279–287.

Gregorc A., Jelenc J. (1996) Control of *Varroa Jacobsoni* Oud. in honeybee colonies using Apilife-Var, Zb. Vet. Fak. Univ. Ljubljana 33, 231–235.

Higes M., Meana A., Suarez M., Llorente J. (1999) Negative long-term effects on bee colonies treated with oxalic acid against *Varroa jacobsoni* Oud., Apidologie 30, 289–292.

Hung A.C.F., Shimanuki H., Knox D.A. (1996) The role of viruses in Bee Parasitic Mite Syndrome, Am. Bee J. 136, 731–732.

Imdorf A., Charriere J.D., Bachofen B. (1997) Efficiency checking of the *Varroa jacobsoni* control methods by means of oxalic acid, Apiacta 32, 89–91.

Imdorf A., Charriere J.D., Fluri P. (1999) Apimondia congress 36, Vancouver, Proceedings, 143.

Imdorf A., Charriere J.D., Maquelin C., Kilchenmann V., Bachofen B. (1996) Alternative Varroa control, Am. Bee J. 136, 189–193.

Liebig G. (1994) Bedeutung des natürlichen Milbenfalls, Dtsch. Bienen J. 1, 14–15.

Liebig G., Schlipf U., Fremuth W., Ludwig W. (1984) Ergebnisse der untersuchungen über die befallsentwicklung der Varroa-Milbe in Stuttgart-Hohenheim 1983, Allg. Imkerz. 18, 185–190.

Martin S. (1998) A population model for the ectoparasitic mite *Varroa jacobsoni* in honey bee (*Apis mellifera*) colonies, Ecol. Model. 109, 267–281.

Milani N. (1995) The resistance of *Varroa jacobsoni* Oud. To pyrethroids: a laboratory assay, Apidologie 26, 415–429.

Milani N. (1999) The resistance of Varroa jacobsoni Oud. to acaricides, Apidologie 30, 229–234.

Mobus B., Bruyn C. (1993) The new *Varroa* handbook, Northern Bee Books.

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

Nanetti A., Stradi G. (1997) Oxalsäure: Zuckerlösung zur Varroabehandlung, Allg. Dtsch. Imkerztg. 11, 9–11.

Nanetti A., Stradi G. (1997) Varroasi: trattamento chimico con acido ossalico in sciroppo zucherino, Ape Nostra Amica 19, 6–14.

Nanetti A., Massi A., Mutinelli F., Cremasco S. (1995) L'acido ossalico nel controllo della varroasi: note preliminari, Apitalia 22, 29–32.

Poklukar J. (1999) Influence of queen mothers on the honeybee hive *Varroa* mite population in the year 1998, Zb. Bioteh. Fak., Univ. v Ljubljani 74, 57–63.

Radetzki T. (1994) Oxalsäure eine weitere organische Säure zur Varroabekämpfung, Allg. Dtsch. Imkerztg. 12, 11–15.

Ritter W. (1981) Varroa disease of the honeybee *Apis mellifera*, Bee World 62, 141–153.

Ruijter A. (1994) Issues in the control of *Varroa* infestation. New perspectives on *Varroa*, Matheson A. (Ed.), IBRA Cardiff.

Statgraphics (1991) Statistical graphic system, STSC, Rockville.

Trouiller J. (1998) Monitoring *Varroa jacobsoni* resistance to pyrethroids in western Europe, Apidologie 29, 537–546.

Zdešar P. (1998) Izdelava AŽ-panja, in: Poklukar J. (Ed.), Od čebele do medu, Ljubljana, 193–200.

Wallner K. (1999) Varroacides and their residues in bee products, Apidologie 30, 235–248.