EVALUATION OF TWO METHODS FOR APPLYING APIGUARD® IN AN AREA WITH CONTINUOUS NECTAR FLOWS AND BROOD REARING

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SUMMARY

The results of a trial conducted in Southern Italy to evaluate the effectiveness of Apiguard® treatments against Varroa destructor Anderson and Trueman are reported. Twenty one colonies of Apis mellifera L. in Dadant-Blatt hives were used. Two groups of seven colonies each were treated and one group was left as untreated controls. Two aluminium trays of Apiguard® were installed in the hives with a two week interval between treatments. The trays of one of the treatments (group 1) were covered with a plastic mesh which only allowed the bees' legs and mouthparts to contact the product. The plastic mesh allowed evaporation but reduced bee contact and product removal. The other Apiguard® treated group (group 2) received uncovered trays as recommended by the manufacturer. Apiguard® trays were present in the hives for 30 days. Two methods were used to evaluate the treatment efficacy: the percent effectiveness (E%) and the degree of efficacy (DE%). Mean E% (± s.e.) was significantly higher in the uncovered trays (93.34 ± 1.18%) than in the covered trays (87.23 ± 1.80%) ($F= 8.80$, d.f.= 1, $P= 0.012$). Similarly DE% also was significantly different between the 2 groups (Covered trays = 84.24 ± 2.23%; Uncovered trays = 91.78 ± 1.45%) ($F= 8.781$, d.f.= 1, $P= 0.012$).

INTRODUCTION

The use of synthetic acaricide molecules in the control of Varroa destructor Anderson and Trueman has caused the development of resistance to these compounds (Lodesani et al., 1995; Milani, 1995; Elzen et al., 2000; Spreafico et al., 2001) and problems related to the contamination of hive products (Gamber, 1990; Lodesani et al., 1992; De Greef et al., 1994; Fernandez Muino et al., 1997; Wallner, 1999). As a consequence, alternative control strategies, mainly based on the use of natural and eco-sustainable compounds, have been investigated and tested. In the warm climate of Southern Italy, mite population control is made more complex by the almost constant presence of bee brood in the hives, due to the continuous presence of nectar and pollen sources. In addition, the warm climate results in rapid evaporation of volatile acaricides. Thymol has strong acaricidal activity and leaves low residues in honey that are considered toxicologically harmless to man (Bogdanov et al., 1998). According to the World Health Organization, the residues of thymol in food are risk-free up to 50 mg/kg (Imdorf et al., 1994). The goal of this study was to evaluate the efficacy of a commercial preparation, (Apiguard®, Vita – Europe Ltd, United Kingdom) under conditions found in Southern Italy. Apiguard® (50g of the product contains 12.5g of the active ingredient) was recently registered in Italy (Decreto del Ministero della Sanità N. 103567018). It also received approval from the USA's Environmental Protection Agency in January 2006. This study examined the efficacy of Apiguard® under optimal conditions for varroa population growth. We also examined the value of covering Apiguard® trays with plastic mesh to reduce product removal.

MATERIALS AND METHODS

The trial was conducted in an apiary of 21 colonies during autumn (Sept. – Nov., 2002) in the town of Catona (province of Reggio Calabria, Italy). The colonies had not been treated with chemicals during the three years prior to the trial. The bees had access to multifloral pasture mainly consisting of Inula viscosa (L.) Ait. and Metcalfa pruinosa (Say) honeydew.

The colonies were maintained in a single Dadant-Blatt hives with 10 frames and were provided with anti-varroa bottom boards. These are prepared by substituting a 3 mm screen mesh for the standard hive floor and placing a drawer underneath covered with paraffined paper with a thin layer of vaseline oil to capture the fallen varroa mites. The colony populations were similar and occupied seven to eight frames. Colony strength was evaluated using the method described by Accorti (1985) and Marchetti (1985). A few weeks before the experiment began, the colonies were divided into three randomized groups of seven colonies and the three groups were placed 500 m apart. The first two groups were treated by placing the Apiguard® trays on the top bars directly above the brood, and in one of the groups, the tray was covered with a 3 mm plastic screen mesh. The control group colonies were left untreated. Temperature and relative humidity were monitored and recorded during the whole experiment using a data-logger (Testo® 175) placed in one colony of each group. The mean temperature and relative humidity recorded inside the hives were 20.89°C (min 10.81°C; max 36.31°C) and 75.12% R.H. (min 39.50%; max 95.70%) respectively. To evaluate the mite's population density before and after the treatment, a portion of sealed brood containing at least 100 cells was inspected for each colony. Paraffined paper, covered with a thin layer of vaseline oil was placed under the anti-varroa bottom boards one week before applying treatments, to determine the natural mortality for all the groups. The bottom boards were checked daily during the trial, and the fallen mites were recorded.

The following week the trial began by placing one Apiguard® tray in each treated hive as indicated by the manufacturer. In group 1 the trays were covered with a 3mm plastic mesh whic let in only the mouthparts and the legs, but not the rest of the body, to reduce Apiguard® removal by the bees. In the second treated group (group 2) the trays were placed following the procedure described for group 1, but they were not covered with plastic mesh. The trays were placed directly above the brood, and any upper entrances were closed. The Apiguard® trays were replaced after 2 weeks, and they were removed at the end of 4 weeks. The residual product was weighed each time trays were removed. During the 6th week, the efficacy of the treatments was determined by counting the number of fallen mites. Finally, on the 7th week all the hives were treated with coumaphos (Perizin®) one week later with cymiazole hydrochloride (Apitol®) and then after one week the Perizin® treatment was repeated according to the recommendations from the European working group (2001). The fallen mites were counted daily during the 6 weeks of treatments with Apiguard® and weekly during the Perizin® an Apitol® treatments. Total mite fall was summarized on day 70.

Efficacy evaluation

The efficacy of the treatments was determined using two parameters (Floris et al., 2001): the percent effectiveness (E%) (Bornek & Merle, 1989) and the degree of efficacy (DE%) (Henderson & Tilton's formula, 1955). This method was preferred to Abbott's formula (1925), that is usually employed in similar trials, because it is more appropriate to field experiments and to data for which the distribution of animal populations is not sufficiently homogeneous (De Maeyer et al., 2002).

The percentage of fallen and/or dead mites on the total number of individuals present (E%) was calculated as:-

$$E\% = 100 \times [Ntr/(Ntr + Nafter)]$$

where Ntr and Nafter are the number of mites collected on the bottom of the hives treated with the tested acaricide and the number of mites fallen after the control treatments, respectively. The degree of efficacy was calculated as:-

DE% = 100 x [1-(t x a1)/(t1 x a)] where t and t1 are respectively the mites present before and after the treatment on the untreated group; a and a1 are the mites present before and after the treatment in the treated colonies.

Data analysis

The data on mite population density collected in sealed brood cells were small numbers, and in some cases, no mites were found; therefore, they were square-root transformed ( ) (Landi, 1987) before analysis. Subsequently, they were subjected to a two-way analysis of variance (ANOVA) (treatment x time), and means were separated by applying the Least Significant Difference (LSD) test. The percentage of fallen mites was also transformed using the arcsine of the square root to reduce the heterogeneity of the variances. The data were then analyzed using a one-way analysis of variance. When the F test was significant, means were separated by applying the LSD test. The analysis was conducted using STATISTICA 6.0 (StatSoft Inc., 2003).

RESULTS

The mean number of mites found in sealed cells (n = 100) before the treatment (Table 1) was 6.14 ± 2.52 in group 1, 4.71 ± 2.17 in group 2 and 3.71 ± 1.64 in the untreated control group. After the treatment the mean number of mites/cell decreased in the two treated groups and increased in the untreated control (Table 1). The variable “before and after the treatment” had a significant effect (F= 4.62, d.f.= 1, P= 0.038) as well as an interaction between the two variables (F= 4.13, d.f.= 2, P= 0.024). The LSD test showed significant differences (P≤ 0.05) before and after the treatment in groups 1 (0.014) and 2 (0.031), whereas on the untreated group no significant differences were found (0.28) (Table 1). There were no significant differences in the 2 treatment methods on the number of mites in brood

(F= 1.86, d.f.= 2, P= 0.17).

Table 1. Mean number of mites (± s.e.) found in brood cells before and after the treatment (groups 1, 2) and untreated colonies.

Group	Before treatment	After treatment
1	6.14 ± 2.52a	0.17 ± 0.42bc
2	4.71 ± 2.17ab	0.14 ± 0.14c
Control	3.71 ± 1.64abc	5.08 ± 1.06a

Values followed by the same letter were not statistically different (ANOVA, LSD, P <0.05).

The total number of mites recovered was 6,135 ± 173.73 in the first treated group, 5,067 ± 132.76 in the second one and 7,388 ± 356.26 in the untreated control. No statistically significant differences were found among the three groups (F= 0.473, d.f.= 2, P= 0.63).

In the first group the percent efficacy (E) was 87.23% ± 1.80 and the degree of efficacy (DE) was 84.23% ± 2.23 (Table 2). In the second group both measures of efficacy were significantly higher (E = 93.34% ± 1.18 and DE = 91.78% ± 1.45) (F= 8.8, d.f.= 1, P= 0.01 and F= 8.78, d.f.= 1, P= 0.01). In the two Apiguard® treated groups the highest number of fallen mites was recorded during the first week of treatment, and thereafter it progressively decreased until the end of the treatment period (Table 3).

Table 2. Efficacy of Apiguard® treatment expressed as percent effectiveness (E%) and degree of efficacy (DE%).

Group	Mean initial number of mites (± s.e.)	Mean number of fallen or dead mites after control treatments (± s.e.) (*)	Mean E% (± s.e.)	Mean DE% (± s.e.)
1	876.43 ± 173.73 a	96.00 ± 18.07 a	87.23 ± 1.80 a	84.23 ± 2.23
2	723.85 ± 132.76 a	45.57 ± 7.31 a	93.34 ± 1.18 b	91.78 ± 1.45
Control	1055.43 ± 356.26 a	732.71 ± 244.27 b	–	–

(*) In Groups 1 and 2 this value represents the mean number of mites surviving Apiguard® treatments. Values followed by the same letter were not statistically different
(ANOVA, LSD, P <0.05).

Table 3. Percentage of mites recovered during the Apiguard® treatment period (mean ± s.e.)(*).

Group	Before treatment	Apiguard® 2nd week	3rd week	Apiguard®	5th week
1	12.14 ± 3.56a	30.54 ± 2.98 a	20.28 ± 1.40 a	13.50 ± 1.50 a	8.71 ± 1.92 a
2	10.42 ± 2.10 a	39.26 ± 7.43 a	23.13 ± 2.78 a	10.71 ± 1.83 a	6.60 ± 1.84 a
Control	5.84 ± 1.37 a	7.00 ± 0.90 b	6.29 ± 1.16 b	6.02 ± 1.13 b	7.58 ± 0.76 a

(*) Apiguard® treatments were applied at the beginning of the 2nd and 4th weeks. Values followed by the same letter in the same time interval were not significantly
different (ANOVA, LSD, P <0.05)

Table 4. Percentage of mites recovered during the Perizin® and Apitol® clean up treatments (mean ± s.e.) (*).

Group	After Apiguard® treatment	Perizin® 7th week	Apitol® 8th week	Perizin® 9th week
1	4.43 ± 0.76 a	5.46 ± 1.35 a	3.20 ± 0.62 a	1.73 ± 0.37 a
2	4.25 ± 1.37 a	1.97 ± 0.51 a	2.39 ± 0.61 a	1.27 ± 0.20 a
Control	8.30 ± 1.01 b	18.84 ± 2.07 b	25.87 ± 1.94 b	14.27 ± 1.22 b

(*) Perizin® treatment was applied at the beginning of the 7th and 9th week, while Apitol® treatment was applied at the beginning of the 8th week. Values followed by the same letter in the same time interval were not significantly different (ANOVA, LSD, P <0.05)

During the first week of the study the number of fallen mites was not significantly different within the 3 groups (F= 2.302, d.f.= 2, P= 0.129). After the first Apiguard® treatment (week 2) the percentage of the mites recovered increased in the two treated groups (30.54% in the first group, 39.26% in the second one) while in the untreated group the percentage of fallen mites recovered was 7.0% (Table 3). Analysis of variance and the LSD test revealed significant differences between the two treated groups and the untreated one (F= 18.16, d.f.= 2, P= 0.00005). The effect of the treatment with Apiguard® was also positive during the following week (week 3), and the differences betwee the two treated groups and the untreated one were still significant (F= 26.39, d.f.= 2, P= 0.000004). The percentages o mites recovered on the paraffined bottom boards during the third week were 20.28% ± 1.40 in the first group, 23.13% ± 2.7 in the second one and 6.29% ± 1.16 in the untreated grou (Table 3). During the second treatment (week 4), the percentage of fallen mites was 13.50% ± 1.5 in group 1 and 10.71% ± 1.83 in group 2; in the untreated control the percentage of fallen mites was 6.02% ± 1.13. The differences between the treated groups and the untreated one were statistically significant (F= 5.80, d.f.= 2, P= 0.011) (Table 3). At the end of the last week of Apiguard® treatment (week 5) the percentage of fallen mites was similar in the three groups (8.71% ± 1.92 in group 1, 6.60% ± 1.84 in group 2 and 7.58% ± 0.76 in the untreated group). The analysis of variance did not reveal any significant difference (F= 0.32, d.f.= 2, P= 0.73) (Table 3). During the weeks after

the removal of the trays containing Apiguard®, the percentage mite fall was significantly higher in the untreated control hives (8.30% ± 1.01) than in the two treated groups (respectively 4.43% ± 0.76 and 4.25% ± 1.37; F= 4.49, d.f.= 2, P= 0.03) (Table 4). The results of an analysis of variance conducted on the percentage of fallen mites before and after the treatment (1st and 6th week), show statistical significance (LSD, P=0.03) only for the treated groups (F= 6.06, d.f.= 1, P= 0.03.). The percentage of fallen mites in th two treated groups was always lower than the value recorded for the untreated group with statistically significant differences during all the three weeks (F= 37.69, d.f.=2, P=0.0000004 after the 1st control treatment; F= 84.73, d.f.= 2, P= 0.0000000007 after the 2nd control treatment; F= 95.02, d.f.= 2, P= 0.0000000003 after the 3rd control treatment). The amount of residual product recovered when replacing the trays was different in the two treated groups. On average 7.68g ± 0.60 and 9.53g ± 0.38 of unconsumed product were found on the 1st and 2nd replacement dates in group 1; whereas 4.9g ± 1.12 and 6.67g ± 0.43 remained on the two dates in group 2. In both treated groups the average amount of residual product was significantly higher at the end of the second treatment (F= 4.70, d.f.= 1, P= 0.039).

DISCUSSION

Our results demonstrate that Apiguard® is effective in Southern Italy where brood is present throughout the year. The two parameters (E% and DE%) used to evaluate the efficacy gave results that were not significantly different (F= 0.72, d.f.= 1, P= 0.41). Therefore, although the level of efficacy calculated applying Henderson and Tilton's correction formula might be more rigorous from a methodological viewpoint because it takes the mite natural mortality into account, the "percent of effectiveness" method (E%) is sufficient for practical purposes.We also demonstrated that bee contact with Apiguard® provides greater efficacy than covered treatments designed to reduce product removal. Although covering trays conserved product and delayed product removal, it resulted in significantly less mite control.

In conclusion, our results support recommending Apiguard® for the control of *V. destructor* in warm climates with continuous nectar flows and continuous brood rearing. Our results do not support covering Apiguard® with plastic mesh to reduce product removal.

This paper first appeared in *Journal of Apicultural Research* 46(2): 105-109 (2007)

REFERENCES

ABBOTT, W S (1925) A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology* 1: 265–267.

ACCORTI, M (1985) Valutazione numerica degli adulti di *Apis mellifera* L.: variazioni e modifiche al metodo dei sesti. *Apicoltura* 1: 63–74.

BOGDANOV, S; IMDORF, A; KILCHENMANN, V (1998) Residues in wax and honey after Apilife Var® treatment. *Apidologie* 29: 513–524.

BORNEK, R; MERLE, B (1989) New test for Varroa control with Apistan (Fluvalinate). *Proceedings of the Meeting of EC Expert's Group* Udine, 28–30 November 1988: 315–330.

DE GREEF, M; DE WAEL, L; VAN LAERE, O (1994) The determination of the fluvalinate residues in the Belgian honey and beeswax. *Apiacta* 29: 83–87.

DE MAEYER, L; SCHMIDT, H W; PEETERS D (2002) Envidor – a new acaricide for IPM in pomefruit orchards. *Pflanzenschutz-Nachrichten* Bayer 55(2-3): 211–236.

ELZEN, J P; BAXTER, R J; SPIVAK, M; WILSON, T W (2000) Control of *Varroa jacobsoni* Oud. resistant to fluvalinate and amitraz using coumaphos. *Apidologie* 31: 437–441.

EUROPEAN WORKING GROUP CA 3686 (2001) Evaluation of treatments for control of varroa mites in honey bee colonies. I. Standards for experimental protocols. [http://www.apis.admin.ch/host/doc/pdfvarroa/Guidelines.pdf].

FLORIS, I; SATTA, A; GARAU, V L; MELIS, M; CABRAS, P; ALOUL, N (2001) Effectiveness, persistence, and residue of amitraz plastic strips in the apiary control of *Varroa destructor*. *Apidologie* 32: 577–585.

FERNANDEZ MUINO, M A; SANCHO, M T; SIMAL-GANDARA, J; CREUS-VIDAL, J M; HUIDOBRO, J F; SIMAL-LOZANO, J (1997) Acaricide residues in honeys from Galicia (N.W. Spain). *Journal of Food Protection* 60(1):78–80.

GAMBER, W R (1990) Fluvalinate scare should serve as warning. *American Bee Journal* 130: 629.

HENDERSON, C F; TILTON, W (1955) Acaricides against the Brown Wheat Mite. *Journal of Economic Entomology* 48(2): 157–161.

IMDORF, A; BOGDANOV, V; KILCHENMAN, V; MAQUELIN, C (1994) 'Apilife Var' – Un prodotto per la lotta contro la varroa la cui sostanza attiva principale è il timolo. *Centro Svizzero di Ricerche Apicole*; pp 1–9.

LANDI, R (1987) *Metodologia sperimentale in agricoltura*. CEDAM ed., Padova, 428 pp.

LODESANI, M; COLOMBO, M; SPREAFICO, M (1995) Ineffectiveness of Apistan® treatment against the mite *Varroa jacobsoni* Oud. in several districts of Lombardy (Italy). *Apidologie* 26: 67–72.

LODESANI, M; PELLACANI, A; BERGOMI, S; CARPANA, E; RABITTI, E; LASAGNI, P (1992) Residue determination for some products used against Varroa infestation in bees. *Apidologie* 23: 257–272.

MARCHETTI, S (1985) Il 'Metodo dei sesti' per la valutazione numerica degli adulti in famiglie di *Apis mellifera* L.. *Apicoltura* 1: 41–61.

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

SPREAFICO, M; EÖRDERGH, R F; BERNARDELLI, I; COLOMBO, M (2001) First detection of strains of *Varroa destructor* resistant to coumaphos. Results of laboratory tests and field trials. *Apidologie* 32: 49–55.

STATSOFT INC. (2003) Statistica (data analysis software system) version 6. Tulsa, OK [http://www.statsoft.com].

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