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Original article

Fluvalinate content of Apistan® strips during treatment and efficacy in colonies containing sealed worker brood

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Summary — Over the last few years, some studies were carried out in Sardinia (Italy) on improving varroosis management in colonies containing sealed worker brood using Apistan.
The fluvalinate content in plastic strips remained almost constant during the 10 weeks of treatment. No significant differences between initial (11.5 + 0.6%) and terminal (10.7 + 0.3%) fluvalinate concentration were observed, indicating that removal by the bees was very small and the active ingredient strip dosage more than sufficient to control the varroosis.
Apistan application in apiary confirmed an efficacy of over 99%. Most of the mite mortality (> 97%) occurred during the first 4 weeks.

fluvalinate / persistence / plastic strips / Apistan / chemical control / varroosis / sealed worker brood

INTRODUCTION

Plastic strips impregnated with fluvalinate (Apistan®) are considered to be one of the best means of controlling varroosis. They are generally accepted as being highly efficacious, only slightly toxic, easily employed and long acting. They can, therefore, be used in the presence of the brood – as is usually necessary in Mediterranean regions. However, recent reports have indicated that, due to the phenomenon of resistance (Milani, 1995; Faucon et al, 1995), the efficacy of Apistan® strips has decreased in most Italian regions. Another possible factor of decreased efficacy could be the rapid degradation of the active ingredient in the strips.

The subject of this study was to check the persistence of the fluvalinate in Apistan® strips and their efficacy in the island

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of Sardinia (Italy), where honeybee colonies constantly contain sealed worker brood.

MATERIALS AND METHODS

Description of the field trials

The trial, performed mainly to verify the active ingredient persistence in the strips, began on 24 September 1994 with 12 colonies (*Apis mellifera ligustica* Spin) placed in Dadant-Blatt hives, ten being treated and two left as controls.

The commercial product Apistan® (Sandoz, Orléans, France), consisting of PVC strips (8 g each) impregnated with 10% fluvalinate was used according to the instructions supplied (two strips per hive).

To evaluate fluvalinate content of the strips, disc-shaped portions (ca 5 mm diam; 20 mg wt) were taken weekly from each strip using a punch plier, for 6 weeks from six hives and for 10 weeks from the other four hives. Theses samples were kept in a deep-freeze (–20 °C) until analysed.

To verify the efficacy of the treatment with Apistan®, as in a previous field trial (Floris and Prota, 1993), a white vaseline-covered plastic sheet was inserted in the bottom of each hive for a weekly count of mite mortality before (2 weeks), during (10 weeks) and after (2 weeks) the treatment. We also estimated, before and after treatment, the extent of adult bees and sealed brood using one sixth of a Dadant-Blatt frame (188 cm^2) as a unit of measure (Marchetti, 1985); the infestation level of the adult bees by sampling 500 bees from three combs per hive (Ritter and Ruttner, 1980), and the infestation of the sealed worker brood (female adult mites per cell) by sampling 300 cells chosen from three combs per hive and opened in crossway manner (Pappas and Thrasyvoulou, 1988) to overcome the problem of the aggregated distribution of mites within the brood nest (Floris, 1991). To avoid data distortion from reinfestation (due to drift or robbery), the trial was conducted in a low colony-density area (Ritter and Leclerq, 1987), without other apiaries within a range of a least 3 km.

Active ingredient extraction procedure

After weighing, each disc-shape sample was placed in a 30 mL screw-capped test-tube, to which 10 mL methylene chloride was added; this mixture was rotary-shaken (GFL, Germany) for 30 min. A 50 mL aliquot of the solvent was transferred into a 10 mL beaker and evaporated to dryness under a nitrogen stream. The residue was recovered with 5 mL n-hexane containing an internal standard (is) and injected into the chromatograph. To verify the efficacy of the extraction method, five disc-shaped portions were taken from each of two Apistan® unused strips from the same stock along the median line of each strip at 2 cm intervals. These samples were analyzed using the above procedure. The experimental data were 10.35 ± 0.41% and 10.39 ± 0.57% (p/p), respectively (minimum value on label = 10%).

A gas chromatograph HRGC Mega serie 2 (Carlo Erba, Milan, Italy) was used. It was fitted with an ECD 400 detector, AS 800 autosampler (Carlo Erba), and a split-splitness injector; connection was made to an HP 3396-II reporting integrator (Hewlett-Packard, Avondale, Pennsylvania, USA). A fused silica column (15 m × 0.32 mm id) (Chrompack, Middelburg, The Netherlands) was employed, with CP-Sil 8 CB liquid phase (95% methylpolysiloxane) (film thickness 0.25). The injector and detector were operated at 240 and 340 °C, respectively. A sample (1 mL) was injected in the split mode (1:60), and the oven temperature was programmed as follows: 150 °C for 1 min, raised to 300 °C (20 °C/min) and held for 7 min. The carrier and make-up gases were helium (50 kPa) and nitrogen (150 kPa), respectively. Good linearities were achieved in the range 2.0-3.5 ppm with correlation coefficient of 0.9996.

Fluvalinate and myclobutanil (analytical standard; Eherenstorfer, Augsburg, Germany), methylene chloride and n-hexane (HPLC solvent grade; Carlo Erba, Milan, Italy) were used for standard solutions: the stock solutions of the acaricide (ca 500 ppm each) were prepared in n-hexane; the working solutions were obtained by dilution with n-hexane containing the internal standard myclobutanil at 2.0 ppm.

RESULTS AND DISCUSSION

Apart from a slight variation attributable to analytical and/or sampling limitations, no statistical significant differences between initial (11.5 ± 0.6%) and terminal (10.7 ±

0.3%) concentration of the fluvalinate in the strips was observed (table I). The constant active ingredient strips concentration during the 10 weeks of the experiment indicated that removal by the bees was insignificant and the active ingredient dosage more than sufficient to control the varroosis.

Efficacy was over 99%. During the treatment, 3 615 ± 1 187 fallen mites per treated hive were recorded. Most of the mite mortality occurred during the first 4 weeks (Floris et al, 1995). Post-treatment examination revealed an additional mite mortality of 0.6 ± 0.2% per hive per day in comparison with a pre-treatment natural mite mortality of 11.5 ± 2.9% per hive per day. Extent of adult bees and sealed brood, their infestation levels, before and after treatment, are reported in table II. In treated hives, worker pupae and adult bee infestations decreased from 14.2 ± 7.3% to zero and from 15.7 ± 7.3% to zero, respectively. Whereas, in the two control hives, during the first 6 weeks, the average worker pupae infestation increased from 15.9 ± 2.9% to 19.7 ± 3.5% and the natural mite mortality increased from 11.5 ± 2.9% to 50.0 ± 13.1%.

Based on these results, where fluvalinate is still effective, the re-use of Apistan® strips would be justified (Pechhacker, 1991). However, the assessment that the fluvalinate content of the strips does not decrease over a few weeks is not enough to justify the re-use of Apistan® strips: in particular, one should check that the release of the active ingredient does not change over time. Application period (strips permanence in hive) must be restricted to the minimum necessary (4 weeks) to reduce excessive toxicological and biological risks from residual accumulation (Moosbeckhofer, 1991), diminution of natural bee immunity (Stark et al, 1993) and selection of resistant mite strains (Milani, 1995).

Table I. Content (%) of fluvalinate in the strips during the treatment.

Hive	*Week*										
	0	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
1	11.9	11.1	11.5	11.1	12.7	10.8	11.6				
2	10.1	11.0	11.1	10.5	12.3	11.9	11.0				
3	11.7	10.6	11.0	10.4	11.3	11.3	10.8				
4	12.1	11.3	11.2	10.2	11.6	11.3	10.7				
5	10.7	10.2	11.0	10.0	12.2	11.1	11.7				
6	11.8	11.0	11.2	10.7	11.5	11.1	10.8				
7	11.6	11.3	10.8	10.6	10.8	11.5	10.8	10.7	9.7	10.2	10.5
8	11.2	11.5	11.0	10.6	11.4	11.1	11.1	11.4	10.3	10.6	10.3
9	11.8	11.3	11.1	10.1	11.7	11.1	11.2	10.5	11.1	10.7	11.1
10	11.9	10.6	11.1	10.3	11.8	11.6	11.0	10.8	10.9	10.6	10.7
Mean	11.5	11.0	11.1	10.5	11.7	11.3	11.1	10.9	10.5	10.5	10.7
± sd	0.6	0.4	0.2	0.3	0.5	0.3	0.3	0.4	0.6	0.2	0.3

Table II. Population estimate and infestations of adult and pupal bees in the treated hives.

Hive	*Comb area (adult) (cm^2)*	*Infestation (adult) (%)*	*Comb area (pupae) (cm^2)*	*Infestation (pupae) (%)*
Before treatment				
1	7 330	4.5	1 690	12.0
2	8 180	22.7	2 070	25.4
3	10 720	12.4	3 380	10.3
4	5 830	29.1	1 460	31.2
5	9 120	7.7	1 830	11.2
6	10 060	13.5	1 270	11.3
7	6 200	16.6	1 500	18.6
8	6 580	11.2	1 970	8.2
9	6 060	9.0	1 460	14.4
10	6 770	14.8	1 690	14.2
Mean	7 690	14.2	1 830	15.7
± sd	± 1 750	± 7.3	± 600	± 7.3
After treatment				
1	10 060	0.0	1 970	0.0
2	8 370	0.0	1 500	0.0
3	8 840	0.0	3 200	0.0
4	4 610	0.0	752	0.0
5	9 780	0.0	2 440	0.0
6	10 810	0.0	2 630	0.0
7	6 580	0.0	1 410	0.0
8	9 490	0.0	1 170	0.0
9	9 310	0.0	990	0.0
10	8 370	0.0	890	0.0
Mean	8 630	0.0	1 700	0.0
± sd	± 1 820		± 830	

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Résumé — Teneur en fluvalinate des lanières Apistan® pendant le traitement et efficacité dans des colonies ayant du couvain operculé d'ouvrières. Ces dernières années, des études ont été faites en Sardaigne pour améliorer le traitement de la varroose par l'Apistan® dans des colonies renfermant du couvain operculé d'ouvrières. Notre expérimentation, visant principalement à vérifier la persistance de la matière active dans les lanières Apistan®, a été réalisée en septembre 1994 dans une région ayant une faible densité de colonies d'abeilles et a porté sur 12 colonies d'*Apis mellifera ligustica* dans des ruches Dadant-Blatt. L'efficacité du traitement a été estimée en comptant les acariens tombés sur une

feuille de plastique blanche enduite de vaseline et placée sur le fond de chaque ruche avant (2 semaines), pendant (10 semaines) et après (4 semaines) le traitement. Avant et après le traitement, nous avons aussi estimé : i) la quantité d'abeilles adultes et le couvain operculé, utilisant pour cela un sixième (188 cm^2) d'un cadre Dadant-Blatt comme unité de mesure (Marchetti, 1985), ii) l'infestation des abeilles adultes en prélevant 500 abeilles/ruche réparties sur trois cadres (Ritter et Ruttner, 1980) et iii) l'infestation du couvain operculé d'ouvrières en inspectant 300 cellules/ruche réparties sur trois cadres et ouvertes de manière croisée pour surmonter le problème de la distribution irrégulière de l'acarien dans le nid à couvain (Pappas et Thrasyvoulou, 1988). Pour évaluer la teneur en fluvalinate des lanières, de petits disques (5 mm de diamètre et 20 mg) ont été prélevés chaque semaine dans chaque lanière à l'aide d'un poinçon. La teneur en fluvalinate est restée pratiquement constante tout au long des 10 semaines de traitement. On n'a observé aucune différence significative entre les concentrations initiale (11,5 ± 0,6 %) et finale (10,7 ± 0,3 %) en fluvalinate (tableau I), ce qui indique que le prélèvement par les abeilles est insignifiant et que le dosage en matière active de la lanière est plus que suffisant pour contrôler la varroose. L'efficacité au champ a été > 99 %. Au cours du traitement, on a dénombré 3 615 ± 1 187 acariens/ruche tombés sur le fond. La mortalité des acariens est survenue en majorité (> 97 %) au cours des 4 premières semaines. L'examen après le traitement a montré une mortalité naturelle supplémentaire de 0,6 ± 0,2 % acarien/ruche/jour comparée à une mortalité naturelle avant traitement de 11,5 ± 2,9 % acarien/ruche/jour. Les infestations moyennes des nymphes et des adultes sont passées de 15,7 ± 7,3 % à zéro et de 14,2 ± 7,3 % à zéro, respectivement. Sur la base de ces résultats, la réutilisation des lanières Apistan® serait justifiée (Pechhacker, 1991). Néanmoins, le maintien des lanières dans la ruche doit être limité au minimum nécessaire (4 semaines) afin de réduire les risques toxicologiques et biologiques.

varroose / lutte chimique / fluvalinate / persistance / Apistan®

Zusammenfassung — Fluvalinatgehalt von Apistanstreifen während der Behandlung und ihre Wirksamkeit in Völkern mit Brut. In den letzten Jahren wurden auf Sardinien (Italien) Studien zur Verbesserung der Varroabehandlung von Völkern mit Brut mit Apistan® durchgeführt. Die letzte Untersuchung sollte vor allem die Dauerhaftigkeit des aktiven Bestandteils der Apistanstreifen prüfen und begann am 24. September 1994 in einem Gebiet mit niedriger Volksdichte. Sie umfaßte 12 *Apis mellifera ligustica* Völker in Dadant-Blatt Beuten. Zur Abschätzung der Wirksamkeit der Behandlung wurder eine mit Vaseline eingestrichene Plastikeinlage auf den Boden jeder Beute eingeschoben und die auf sie gefallenen Milben gezählt. Die Zählungen erfolgten 2 Wochen vor, 10 Wochen während und 2 Wochen nach der Behandlung. Vor und nach der Behandlung schätzten wir außderdem die Anzahl der adulten Bienen und der verdeckelkten Brut, indem 1/6 des Dadantblatträhmchens (188 cm^2) als Meßeinheit benutzt wurde (Marchetti, 1985). Für die Bestimmung des Befalls der adulten Bienen wurden 500 Bienen von 3 Waben gesammelt (Ritter und Ruttner 1980). Für die Schätzung des Befalls der Brutzellen wurden 300 Zellen von 3 Waben pro Volk in sich kreuzenden Reihen geöffnet, um das Problem der aggregierten Verteilung der Milben in der Brut zu vermeiden (Pappas und Thrasyvoulou, 1988). Um den Fluvalinatgehalt der Streifen zu bestimmen, wurden scheibenförmige Teile (etwa 5 mm im Durchmesser mit einem Gewicht von 20 mg) wöchentlich mit einem Locher ausgestanzt. Der Fluvalinatgehalt der Streifen blieb während der 10 wöchigen Behand-

lung nahezu konstant. Es wurden keine signifikanten Unterschiede zwischen der Anfangs-(11,5 ± 0,6%) und der Endkonzentration (10,7 ± 0,3%) von Fluvalinat in den Streifen (Tabelle I) beobachtet. Das zeigt, daß die Abgabe von Fluvalinat aus den Streifen unbedeutend ist und daß die Dosierung der aktiven Substanz mehr als ausreichend ist, um die Varroosis zu kontrollieren. Die Wirksamkeit der Apistan® - Applikation im Feldversuch war höher als 99%. Während der Behandlung fielen 3.615 ± 1.187 Milben pro Volk. Die stärkste Milbenmortalität (mehr als 97%) trat in den ersten 4 Wochen auf. Die Prüfung nach der Behandlung zeigte eine zusätzliche natürliche Milbenmortalität von 0,6 ± 0,2 pro Volk und Tag im Vergleich zu der natürlichen Mortalität vor der Behandlung von 11,5 ± 2,9 pro Volk und Tag (Tabelle II). Der durchschnittliche Befall der verdeckelten Arbeiterinnenbrut und der adulten Bienen ging von 15,7 ± 7,3% bzw von 14,2 ± 7,3% auf Null zurück. Auf Grund dieser Ergebnisse ist die mehrfache Nutzung von Apistanstreifen gerechtfertigt (Pechhacker 1991). Der Verbleib der Streifen im Volk sollte jedoch auf das notwendige Minimum (4 Wochen) begrenzt sein, um übermässige toxikologische und biologische Risiken zu vermeiden.

Fluvalinat / Dauerhaftigkeit / Plastikstreifen / Apistan® / chemische Kontrolle / Varroosis / verdeckelte Arbeiterinnenbrut

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